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

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- Appendix 2. Published refereed journal article. "The stored grain ecosystem: a global perspective." *Journal of Stored Product Research*. 28:73-87.
- Appendix 3. In press refereed journal article. "Oviposition patterns in two species of bruchids (Coleoptera: Bruchidae) as influenced by the dried leaves of Tetradenia riparia, a perennial mint (Lamiales: Lamiaceae) that suppresses population size." *Environ. Entom.* in Press (anticipated issue October 1992).
- Appendix 4. Refereed journal article in review. "Toxicity and protectant potential of the essential oil of Tetradenia riparia (Lamiales: Lamiaceae) against Zabrotes subfasciatus (Coleoptera: Bruchidae) infesting dried Pinto beans (Fabales: Leguminosae)." to be submitted to the *Journal of Applied Entomology*. in USDA-ARS and MSU review.
- Appendix 5. Refereed journal article in review. "Contact and fumigant toxicity of milled Ocimum canum Sims to Zabrotes subfasciatus (Boheman) adults." to be submitted to *J. Stored Prod. Res.* in USDA-ARS and MSU review.
- Appendix 6. Published article from proceedings of international conference. "Toxicity of R,S-linalool to four species of storage Coleoptera as influenced by volatilization and degradation." *Proc. Fifth Internat. Conf on Stored Product Protection*. Sept. 1990. Vol III, pp. 1609-1617.
- Appendix 7. Published article from proceedings of international conference. "Growth regulatory effects of a Rwandan medicinal plant Tetradenia riparia (Lamiaceae) on stored grain and bean insects." *Proc. Fifth Internat. Conf on Stored Product Protection*. Sept. 1990. Vol III, pp. 1609-1617.
- Appendix 8. Manuscript drafted for submission to a refereed journal. "The imboho: a traditional structure for storage of dry pulses in East Africa." for submission to *J. Stored Product Res.* drafted.
- Appendix 9. Published refereed journal article. "Evaluation de la toxicite et de l'effet repulsif de certaines plantes du Rwanda contre les bruches du haricot: Acanthoscelides obtectus Say et Zabrotes subfasciatus Boheman" *Insect Sci. Applic.* 12:695-697.

1.0. EXECUTIVE SUMMARY

The purpose of this project was to test the hypothesis that plants used by Rwandan for traditional pest management and for traditional medicine, has measurable insecticidal properties. As a result of this project, those involved in plant protection in Rwanda, particularly in the postharvest area, are seeking and finding alternatives to synthetic chemicals. These alternatives are coming at a time when Rwandan leaders, such as those in charge of the research and management of OPROVIA, are realizing that their destructive insect populations are becoming resistant to yet another imported chemical. At the close of this project, the Director of OPROVIA has requested that full scale experiments begin with an extract of Chrysanthemum cinerarifolium. This plant has been grown for decades in Rwanda, but always exported, generally by non-Rwandans. This project has contributed to the agricultural leaders of the country seeing value for Rwanda in such plant products.

With such value placed on traditional practices, Rwanda can now search for economical and environmentally sound answers to its pest management. Rwanda can be a model in balancing technological development with traditional wisdom. Overdependence on synthetic chemical pesticides is a global problem which involves developing as well as developed countries. For countries that see progress as only technological, it is easy to lose sight of traditional practices or plant species until their utility is no longer remembered by any of the living generations.

Also as a result of the training component of this project, the skills to design, conduct, analyze, and interpret the results of insect bioassays to test efficacy of these plants has been developed in Rwandan research laboratories at OPROVIA/ GREARWA II - Recherches and CURPHAMETRA. Scientifically, we have discovered the fumigative action and determined the exact time of action of the traditional insecticidal and medicinal plant, the annual mint, Ocimum canum Sims. We have determined the spectrum of efficacy of the most common Rwandan medicinal plant, the perennial mint, Tetradenia riparia (Hochst.) CODD. We have discovered the strong residual nature of the essential oil and its safety with respect to germination. This preparation is produced in a large scale steam distillery by Rwandans as a by-product of a medicinal production process, without using toxic or expensive chemicals that must be imported. To make these discoveries, detailed bioassay protocols had to be developed.

The success of these studies in Rwanda has been observed by scientists in other countries. The search for new botanicals and new applications of botanicals has been encouraged by our results. Specifically, when the Moroccan and US scientists (including Dr. F. Dunkel) determined that the traditional underground sealed storage technique (matmora) was in jeopardy because of insects resistant to low oxygen atmospheres, botanicals already in use by traditional farmers were sought as an answer. At the International Institute of Tropical Agriculture (IITA) in Nigeria (Kano Station), the results of mint studies in Rwanda and marigold studies in companion studies in the labs of the P.I. and collaborators, have encouraged these scientists. In 1992, at IITA, experiments were begun in the field with intercropping Tagetes erecta for preharvest bean insects and growing a crop of Ocimum basilicum that can

be harvested with the cowpeas and incorporated into sealed storages for postharvest protection. And finally, the laboratory of the P.I. , Dr. F. Dunkel, has now identified the pesticidal properties of preparations of leaves of four local Rocky Mountain plant species.

Seven Rwandan scientists have received training funded by the project and seven U.S., Canadian, and Pakistani scientists have received training with funding provided totally or in part by other, parallel projects. Six refereed journal articles have been published or are in review. Two international proceedings articles have been published. One abstract has been published. Two additional manuscripts have been drafted for refereed journals. Two Rwandans have received the Master of Science degree from the National University of Rwanda with research undertaken by them within the project. Nineteen presentations have been made at professional meetings. The private sector in Montana has become a collaborator during the latter part of the project. As a result of this project, \$310,500 of new funding has been obtained for parallel projects. The P.I. has received one of the 1992 Lindbergh awards for this work and its relation to the balance between technology and traditional wisdom.

2.0. RESEARCH OBJECTIVES

2.1. Purpose of Project

The purpose of the grant was for the investigation of the feasibility of using four indigenous plants in Rwanda, i.e., Ocimum kilamansharcum (= Ocimum canum in Rwanda) (essential oil), Capsicum frutescens (powdered plant), Iboza (= Tetradenia riparia (powdered plant), and Chenopodium schraderanum (essential oil) to prevent loss of both stored and growing kidney beans, Phaseolus vulgaris L. mainly to the bean weevil, Acanthoscelides obtectus and the Mexican bean weevil, Zabrotes subfasciatus.

2.2. Hypotheses Tested

The main hypothesis tested was: Traditional natural materials of plant origin used by farmers for postharvest protection of dry edible beans, Phaseolus vulgaris L., in Rwanda can be adapted for use as protection against insects in large scale storage within the same country.

The following specific research hypotheses tested were:

1. Indigenous plant preparations vary in their ability to protect beans from storage loss due to insects depending on ambient temperature and equilibrium relative humidity.
2. The four indigenous plant preparations do not affect a) consumer preference measured by sensory panels or b) cooking time measured by instrumental hardness.

3. Some of these indigenous plants provide insect protection for stored beans because of their repellent properties. Part of these repellency properties are exhibited by the senescing plant in the field and may have a protective action against field infestation of bruchids. They can then be harvested along with the beans and provide continuous protection.
4. Plant preparations developed for protection of stored beans in Rwanda are more toxic to the insect at certain periods of the insect's life cycle.
5. In a comparison of both labor costs for the Rwandan plants and an equally effective commercial dose of imported insecticides, the natural plant preparations will provide a savings.
6. An active extract from appropriate parts of the plant, Ocimum kilimansharicum (= O. canum) can be obtained and the components which cause the action identified.
7. Indigenous plant preparations used by Rwandan farmers are also effective insect protection for dry edible beans in long term, large scale storage in Rwanda.

2.3. Importance of the Hypotheses to Development

Development of sustainable pest management techniques means that the techniques are environmentally compatible and economically feasible. The hypotheses tested in this project provide the basis for developing a set of environmentally sound pest management materials. These plant derived material, moreover, can be efficacious without importation of any additional solvent or reagent chemicals or equipment.

It is also important that these hypotheses utilized plants that already were part of the Rwandan household, at least the farm household. Subsistence farmers constitute 95% of the population. A country's development is ultimately tied to the skills and development of the women of the country. The "new" pest management strategies suggested by the results of testing these hypotheses, are already familiar products to the women and their cultivation is traditionally part of their responsibility. Involving the women of Rwanda in this "new" technology should be feasible.

2.4. Summary of Related Research

The literature prior to the initiation of this proposal is summarized in the proposal. Since the commencement of our research in this project, several related research projects had contributed to our own interpretations. Throne (1990) and Baker et al. (1991) have demonstrated that non-lethal inhibition of populations may result in prolonged significant reductions in stored product insect populations. This underscores the possibility that simple addition of anti-insecticidal plant material to stored foodstuffs such as leaves of Tetradenia

riparia (Hochst) CODD. may have value even though our data indicate the mortality was not remarkable.

Other studies that aided in the interpretation of our results were the hormoligosis (Luckey 1968) observed in A. obtectus emergence by Lambert et al. (1985) in response to low dosages of the traditional protectant derived from Hyptis spicigera Vahl. We also observed an enhancement of oviposition and longevity with increased dose in T. riparia to a certain point, creating a U-shaped dose response curve. The literature contains numerous "screening" studies done with protectant properties of plants. Variability in comparisons made between these tests with slightly different dosages have given botanicals an "unreliable" reputation. Hormoligosis may be responsible for part of this variability. In our experiments, we learned to overcome the variable reputation of plant pesticides. We took special care to control as many variables as possible, and by being aware of the fragility (volatilization, light sensitivity) of some of the bioactive chemical components.

2.5. Innovative Aspects of Project

The innovative aspect that now is part of a patent pending is the discovery of how to release the fumigative properties of the leaves of Ocimum canum Sims. The original project was suggested by observations made of several plant components used in an unusual traditional storage structure (the imboho). We proposed that we could determine, by our experimentation, why the structure works. Our research has suggested how one of the components, the mint leaves, O. canum can provide good insect control in certain structures. The overall innovation of this project is to ascribe value to traditional practices and actually use these practices as a source of new technologies.

2.6. Skills/Awareness remaining in Rwanda as a Result of Project

There are several levels of skills/awareness that seem to have remained in Rwanda as a result of the project. There is now evidence that traditional practices are perceived to have value. Rwandan plants are viewed as a particularly good source of pesticides. The urgency to find alternatives to e.g. the prophylactically used residual insecticide, Actellic, is understood. There is an understanding of how to develop and rear mass cultures of insects to use for bioassays. The understanding of how precise conditions must be controlled during the bioassay, how exact the observations must be, and how well replicated all tests must be remains, now, in Rwanda with those trained in the project. Some understanding exists of the fragility some of the active chemicals in these plants.

3.0. MATERIALS AND METHODS

3.1. Insect Rearing

All mass cultures were maintained in incubators at $27 \pm 1^{\circ}$ C, $65 \pm 5\%$ r.h. (relative humidity) with a 12:12 light:dark photoperiod. Each mass culture was maintained in 0.95 liter glass jars containing 0.5 liter of equilibrated beans or grain, depending on species of insect cultured. This culture medium was inoculated with approximately 300 adults. Culture diet was discarded after emergence of two successive generations.

The four insects chosen for research were the four main destructive insect species infesting stored beans, sorghum, and corn in Rwanda. These species were the Mexican bean weevil, Zabrotes subfasciatus (Bohem); the bean weevil, Acanthoscelides obtectus Say; the rice weevil, Sitophilus oryzae (L.); and the lesser grain borer, Rhyzopertha dominica (F.). Dried Pinto beans (Phaseolus vulgaris L.) were used for rearing Z. subfasciatus, and dried red kidney beans (P. vulgaris) were used for A. obtectus. Equilibrium moisture content of the beans were 13.7%. Because organic sorghum was not available in Montana, R. dominica and S. oryzae were reared on a diet of 96:2:2 w/w (weight per weight) soft white wheat: whole wheat flour: brewer's yeast. Equilibrium moisture content of this diet was 17.1%.

The lesser grain borer culture was developed from a field collection made in Yellowstone County, Montana (Huntley Ag Experiment Station) in 1989. The other insects were obtained from stock cultures in the USDA-ARS Stored Product Insect Laboratory, Department of Entomology, University of Wisconsin, Madison WI. This stock had been laboratory maintained for many years. Because Z. subfasciatus does not occur in the USA, this species is kept in laboratory quarantine.

3.2. Plant Culture and Preparation of Plant Products

3.2.1. Tetradenia riparia Hochst. CODD.

Fresh leaves of the perennial medicinal mint, Tetradenia riparia Hochst. CODD. were collected by the staff of CURPHAMETRA (Centre Universitaire de Recherche sur la Pharmacopée et la Médecine Traditionnelle) in Butare, Rwanda ($2^{\circ}35'S$, $29^{\circ}44'E$). Plants used were located in the area surrounding Butare. The habitat of this area is relatively level, well-drained and is located at approximately 1,330 meters above sea level. A voucher specimen of the plants collected has been deposited by Dr. F. Dunkel in the Montana State University Herbarium (Voucher F.V.Dunkel 1 (MONT; MSU Herbarium)). The collection consists of the floescence plus an apical whorl of leaves collected by F.V.D. in the Arboretum of the National University of Rwanda, Butare, Rwanda on November 14, 1986. It also consisted of basal leaves collected March 10, 1991 by L.V.P. at the same location. Additional voucher specimens were submitted by Dr. L. Van Puyvelde to the Rwandan National Herbarium.

Leaves were dried in a 40° C oven for 24 hr. Dried leaves were then crushed (mode particle size 3-5 mm, particle size range 0.75-15.0 mm) and shipped via courier to Montana State University. Upon receipt, the plant material was stored at -20° C until used. Two collections of plant material were used and were collected from the same sites. The first was collected and shipped in February, 1989 (=Lot A) and the second in June, 1990 (=Lot B). All experiments were begun within 14 days of the receipt of the plant material.

To produce the milled preparation, intact leaves or the crushed preparation were removed from the freezer and milled for two minutes in a glass Waring Blender. Resulting particles had a mode particle size of 0.1-0.4 mm. These particles were then mixed with the beans or grain and insects were immediately added to begin the bioassay.

The essential oil was prepared at CURPHAMETRA on a pilot-scale Clevenger-type hydrodistillation apparatus (yield- 0.07% w/w). The oil was express shipped to Montana State University and stored at -20° C under N₂ (nitrogen) gas until use. A large composite sample (40 ml) was prepared to eliminate potential variability among experiments. The density of this essential oil was 0.92 g/ml. A second major shipment of 40 ml was sent toward the close of the project. This is being used for follow-on studies.

3.2.2. Ocimum canum Sims

The annual medicinal mint, O. canum, was grown in the plant nursery adjacent to the pilot plant of CURPHAMETRA in Butare, Rwanda. Leaves of O. canum were harvested in August of 1988, 1989 and 1990. Leaves were oven dried at 40°C and express shipped intact to Montana State University (MSU), Bozeman MT, U.S.A. There, the leaves were stored in glass jars under N₂ in a -20°C freezer until chemical analysis and bioassay.

3.3. Quantification of Secondary Plant Compounds

Essential oil of T. riparia obtained by steam distillation as described above was subjected to gas chromatography-mass spectroscopy (GC-MS) analysis at the initiation and termination of the experiments described in this report. GC-MS was primarily done at Montana State University (MSU) with some studies completed at the University of Wisconsin, Department of Entomology. At MSU, the GC was conducted on a Varian Model 3700 equipped with a 30m X 0.25mm (i.d.) DB-5 column with a 0.25 um film thickness. GC conditions were: He carrier gas velocity- 30 cm³/s (220°C); temperature programming- initial temperature- 50°C, initial hold- 4.0 min, temperature increase- 5°C/min, final temperature- 280°C, final hold- 10 min, injection port temperature- 260°C, detector temperature-290°C. Electron impact MS were obtained on a VG Analytical VG 70EHF operating at 70 eV with a source temperature of 200°C.

To quantify the secondary plant compounds of O. canum, leaves (1.0 g in each of four replicates) were milled in a Waring blender for 2 min and extracted immediately in 60 ml of 1:4 isopropanol:hexane (containing 40 ng/ul decane as an internal standard) in a 125 ml Erlenmeyer flask for 24 hr. Flasks were covered to prevent photodegradation and occasionally agitated.

The resulting supernatant solution was directly injected into a gas chromatograph for quantitative analysis. All solvents were purchased from the Fisher Chemical Co., FairLawn, N.J., U.S.A. GC was performed on the same model as above, but with a flame-ionization detector containing a 50m x 0.25 mm i.d. HP-1 column with 0.11 μ film thickness; He carrier gas velocity was 31 cm³/s (220°C). The temperature program was as follows: initial temperature 60°C, initial hold 8 min, temperature increase 4°C/min, final temperature 260°C.

The linalool peak in the *Q. canum* studies was identified using narrow-bore capillary GC-Mass Spectrometer (MS). The GC was the same as described above with a 30.0m x 0.25 mm i.d. DB5 column with 0.25 μ film thickness. Column conditions used were: He carrier gas velocity of 30 cm³/s (220°C); temperature programming of an initial temperature 50°C, initial hold 4.0 min, temperature increase 5.0°C, final temperature 280°C, final hold 10 min, injector temperature 260°C, detector temperature 290°. The electron impact mass spectra were obtained on a VG Analytical model VG 70 EHF MS operating at 70 eV with a source temperature of 200°C.

3.4. Contact Toxicity, Adult Insects

Contact toxicity of leaf extracts of *Q. canum*, essential oil of *T. riparia*, and of pure linalool was tested with adult insects in glass Petri dishes. The basic procedure is as follows. A Whatman No. 1 filter paper was placed in the bottom of the Corning Petri dish so that the edge of the filter paper met the sides of the dish. An aliquot of the appropriate dilution of the solution to be tested was delivered by pipette to the filter paper in absolute ethanol (Quantum Chemical Co., Tuscola, Ill.). The ethanol was allowed to evaporate prior to the addition of insects. For each dose, ten replicates were used. Ten replicates of an ethanol control were conducted simultaneously for each trial. Ten adult insects at a specific time after adult emergence from the bean were used. Specific captions of data tables or curves should be consulted for this information. Generally, the timing was 0-1 days after adult emergence.

Specific conditions of the bioassay container were as follows. For the linalool efficacy study (Weaver et al. 1990, 1991), 9 cm diam filter paper was used in 10 cm diameter Petri dishes, 1.0 ml aliquot of the appropriate dilution was applied, and ethanol evaporation allowed was 20 min. For the essential oil studies with *T. riparia* (Weaver et al. in review 1992a; Dunkel et al. 1990), 5.5 cm diam filter paper was placed in the inverted lid of a 5.0 cm (internal diameter) glass Petri dish, 0.5 ml aliquot of the appropriate dilution was applied, and evaporation was allowed for 20 min.

Mortality was evaluated if the insect was immobile and did not react to three probings with a blunt dissecting probe. Moribundity was assessed by selecting those insects that were on their backs and ambulating very weakly. These insects were subsequently righted and viewed carefully. Those that immediately fell onto their backs again as a result of intoxication were classified as moribund. With higher doses of linalool, all moribund insects subsequently dies. Recovery occurred occasionally at lower dosages. Mortality / moribundity were evaluated 24 hr after the introduction of insects into each trial.

The bioassay conditions for all bioassays were the same as the insect rearing conditions with the following exception. Linalool studies (Weaver et al. 1991) were conducted at $27 \pm 2^{\circ}\text{C}$, $65 \pm 8\%$ r.h., and 12:12 light:dark photoperiod.

3.5. Growth and Development Bioassays

3.5.1. With whole, crushed, or milled leaves

For bioassays with whole, crushed, or milled leaves, leaf preparations were added to ten replicates of 20 g dried Pinto beans (approximately 50 beans; moisture content for each test was determined by a 24 hr oven drying at 110°C .) All doses, including the control were replicated 10 times. Plant materials were mixed by shaking with the beans and were then placed with the beans into 10 dram plastic shell vials.

Ten adult *Z. subfasciatus* (5 male and 5 female, 0-1 da post-adult emergence) were then added. Females were allowed to oviposit until death, which occurred within 17 da for all individuals. The fecundity of females and fertility of eggs laid was assessed at 25 da and the number of emergent F_1 adult progeny determined at 55 da. At 55 da, both live and dead emerged adults were counted. Live insects were returned to the vials and the experiment was continued another 30 da, at which time all vials were frozen and the emerged adults were again counted. An earlier study (Howe and Currie 1964) and our previous experience (D.K.W. and F.V.D. unpublished data) suggested that counts should be conducted at these intervals to maximize the accuracy of counts for both eggs and adults. These count dates are also necessary for accuracy if developmental delay occurs as a function of sublethal treatment as reported by Su (1977). The bioassay conditions were standard as described above and ten replicated of all concentrations and controls were used.

3.5.2. With essential oil

For treatment of beans with the essential oil of *T. riparia*, first the bean surface was determined. Pinto bean masses were determined on a Mettler AT-250 Digital Balance (sensitivity- 0.1 mg). Subsequently, beans were soaked in reagent grade acetone for 24 hrs. The resultant endosperm mass was reduced so that the testa (seed envelope) was loose and readily removed using a sharp scalpel. The removed fragments of the testa were pressed flat between two microscope slides that were taped tightly together using transparent tape. The surface areas of the testa for individual beans was determined three times using a Li-Cor Model LI-3100 Area Meter (Li-Cor, Inc., Lincoln, Nebraska, U.S.A.) set at 0.001 cm^2 sensitivity. The predictive equation derived provided a theoretical surface area estimate of 127.08 cm^3 for each 20 g replicate of beans (Weaver et al. in review 1992a). This procedure aided in the conservation of the oil by facilitating the calculation of concentrations to be tested in beans based on the same units as those used in the initial acute toxicity study on filter paper. This reduced the number of experiments needed to yield data that indicated partial inhibition of the insect population.

The essential oil of T. riparia is insoluble in water. Emulsions for application on the beans were prepared in distilled, de-ionized water using 0.5% Triton X-100^R to better approximate actual field usage, rather than using solutions prepared in a volatile organic solvent. Five experiments were conducted which used 1.0 ml aliquots of these emulsions to deliver the appropriate concentration of essential oil to 20 g Pinto beans (14.1% eMC (= equilibrium moisture content)) in a glass Petri dish lid. The liquid was uniformly applied to the surface of the beans by gentle agitation using 20 semi-circular arm motions. The water was then evaporated for 2 hr in the open container, after which the bioassay was initiated. Dilutions used are described in Weaver et al. 1992a in review.

Treated beans were transferred to a 10 dram (2.5 cm internal diameter X 8.0 cm height) plastic snap-cap vial immediately after H₂O evaporation. For evaluation of protection potential of oil versus adult insects, insects added and observation periods were the same as for leaf material bioassays described above.

For the ovicidal activity of freshly applied oil, cohorts of 10 male and 10 female adult Z. subfasciatus (0-1 da post-adult emergence) were added to 20 g of equilibrated (as in section 3.1 above) Pinto beans. These insects were allowed to mate and oviposit for 24 hrs prior to removal. Eggs were allowed to harden for 24 hrs after which beans and eggs attached to them were treated. Beans were treated with seven concentrations of T. riparia essential oil (Weaver et al. 1992a in review). A second experiment was conducted with six concentrations over the portion of the treatments that caused partial suppression of the insect population. Disturbance of the beans during treatment resulted in mortality due to egg destruction, so an additional undisturbed control was included to measure this. Egg destruction during treatment made it impossible to do accurate counts of the number of treated eggs and determinations of fertility. Even careful attempts to pre-count eggs with minimal handling were found to significantly alter fertility). Therefore, number of F₁ adult progeny at 55 da was used to measure ovicidal activity of the essential oil relative to that of the disturbed controls (H₂O + emulsifier only and H₂O only). The bioassay was consisted at standard conditions as described above in section 3.1. The bioassay was conducted in plastic, 12 dram (2.9 cm internal diameter X 7.0 cm height) snap-cap vials in which treated material was placed immediately after H₂O evaporation. There were ten replicates of all concentrations and controls.

For the larvicidal activity of freshly applied oil, cohorts of 10 male and 10 female adult Z. subfasciatus (0-1 da post-adult emergence) were added to 20 g of equilibrated (as above) Pinto beans. These adults were allowed to mate and oviposit for 24 hr prior to removal. The eggs were allowed to mature with subsequent larval penetration under standard bioassay /mass culture conditions (described in 3.1.) for a period of seven additional days. At this point the beans were treated with seven concentrations of T. riparia essential oil (Weaver et al. 1992a in review). This procedure made impossible an accurate assessment of initial egg number and associated fertility. F₁ adult progeny were counted at 55 da. Adult emergence was again determined at 85 da. The bioassay was conducted at standard bioassay /mass culture conditions (described in 3.1.). After H₂O evaporation, treated beans were placed in plastic 12 dram (2.9

cm internal diameter X 7.0 cm height) snap-cap vials. There were ten replications of all concentrations and controls.

3.6. Oviposition, Choice and No-Choice Bioassays

The bruchid oviposition tests are described in detail in section 3.8. below. In addition, some studies utilized the rice weevil, *Sitophilus oryzae* (L.), one of the major destructive stored grain insects in Rwanda (Dunkel et al. 1986). Because this species oviposits inside the grain and then secretes a mucilaginous plug over the egg for protection, a staining procedure was developed to reveal this (Appendix 7, 1990 Ann. Rept. this project). Following the staining, the actual growth and development information was obtained by dissecting, or finely slicing, the grain kernel. To perform the dissection, the grain kernel must be sufficiently soft. An optimal soaking regime was, thus developed (Appendix 7, 1990 Ann. Rept. this project).

3.7. Fumigation Bioassays

Glass screw cap vials (42.5 ml capacity) with teflon cap liners were used as fumigation chambers. Inside each chamber, a 7.2 ml glass vial was supported on the head of a 2.5 cm stainless steel screw standing obliquely at the bottom of the vial. Individual female *Z. subfasciatus* (0-18 hr post-adult emergence) were placed in the small vial and sealed with nylon mesh secured by a rubber band. The insect was thus exposed to the volatile components of the system only. Test material and test insects were deposited into the fumigation chambers in the standard bioassay conditions as described above in 3.1. This procedure ensured that the initial atmospheric humidity within the fumigation chambers was equivalent to that within the rearing incubator.

Three such experiments were conducted. The first experiment used stock synthetic linalool (Sigma Chemical Co., St. Louis MO, U.S.A.). Volumes of 0.5, 1, 2, 5, and 10 ul per vial were used (1 ul /vial = 20.7 mg/l assuming all of the material volatilized). Ten replicates of each treatment and ten replicates of a no linalool control were prepared. Mortality counts were made 15 times during the next 188 hours. The second experiment utilized finely-milled leaves of *Q. canum* at 1 and 1.5 g per vial (1 g leaves of *Q. canum* = 205 mg/l linalool assuming complete volatilization or 10 ul of pure linalool per vial). Ten replicates of each treatment and ten replicates of a no linalool control were prepared. Mortality counts were made 6 times during the next 120 hours. The third experiment used linalool sorbed onto a proteinaceous oat microparticle (Nurture^R- sequestering grade, Basic Bio Systems, Inc., Missoula MT, U.S.A.) at 0.85% w/w. Treatments of 1 and 1.5 g per vial of this preparation were used (1 g/vial = 205 mg/liter linalool assuming complete volatilization or 10ul of pure linalool per vial). There were two types of controls, one consisting of untreated particles and the other of no material added to the vial containing the insect. Ten replicates of each treatment and controls were prepared. Mortality counts were made 6 times during the next 120 hours.

3.8. Residual Activity Bioassays

Beans treated with 0, 5.4, 26.8, 53.6, 267.8, 535.5, and 1071 ug essential oil of T. riparia per cm² of surface were stored in an incubator under standard mass culture conditions as described above (3.1.). This storage was maintained for 210 da. At 210 da, three treated from each concentration and three control beans were mixed and placed in a 20 dram plastic snap-cap vial (4.8 cm internal diameter X 6.2 cm height). Treated and control beans were 14.2% equilibrium moisture content. Beans were identified using a small colored ink dot on both treated and control beans (colors were exchanged among replicates, red and green were used).

The experiment was conducted as a two choice test. Two male and two female adult Z. subfasciatus (0-1 da post-adult emergence from the bean) were added and allowed to oviposit until death. At 25 da, the number of eggs laid and eggs hatched on the treatments and the control were determined. No accurate counts could be conducted subsequently due to the potential for cannibalism among conspecifics within the beans. However, the relative activity of the material to prevent oviposition could be readily assessed. The bioassay was conducted at standard mass culture conditions as described above (3.1.). There were four replications of each concentration.

The experiment was repeated as a no-choice test. Conditions were exactly as in the two choice test. The only exception was that only two beans were used per replicate. Both beans had been treated 210 days previously. There were four replications of each concentrations.

3.9. Germination Studies

Pinto beans treated with 0, 5.4, 26.8, 53.6, 267.8, 535.5, and 1071 ug essential oil of T. riparia per cm² of surface were stored in an incubator under bioassay conditions for 180 da. At this time (180 da after treatment), five beans for each concentration were placed in equally spaced, randomized locations on each of four isocratic (27.0 ± 0.6°C) thermal gradient bars (15.2 cm wide by 106.7 cm long). The bar was covered with Whatman #1 chromatography paper that was thoroughly wetted with distilled H₂O. The paper was maintained moistened by wicking from reservoirs containing distilled H₂O at the ends of the bar. Humidity was controlled by covering the apparatus with acrylic boxes to maintain near 100% r.h. Germination was evaluated at 24 hr intervals and the natural photoperiod was approximately 13L:11D. The experiment was replicated twice.

3.10. Scanning Electron Microscopy

The following specimens were prepared for observation with scanning electron microscopy (SEM):

- Z. subfasciatus and Q. obtectus exposed to freshly milled dried leaves of Q. canum.
- Z. subfasciatus and Q. obtectus exposed to dried leaves of Q. canum 2 months after milling.

- residue from ethanol extraction of milled leaves of Q. canum (extraction occurred 2 months after milling).
- residue from methanol extraction of milled leaves of Q. canum (extraction occurred 2 months after milling).
- freshly milled dried leaves of Q. canum.
- dried leaves of Q. canum milled 2 months prior to SEM preparation.
- unmilled leaves of Q. canum, T. riparia, Chenopodium schraderanum.

Specimens were mounted on stubs and gold coated with a sputter coater for 90 seconds to a thickness of 200-300 angstroms.

SEM utilized for the study was a Hitachi S570. Photos were taken with Polaroid^R film.

3.11. Large Scale (Mini-bin) Trial

For the cookability (= instrumental hardness) and human sensory preference analysis, a lab scale experiment was constructed. The scale was similar to that amount of beans stored after one harvest by a producer (Dunkel et al. 1986). 50 kg beans were obtained from OPROVIA warehouse at Kicukiro before the routine (prophylactic) treatment with Actellic (pirimiphos methyl). This lot of beans was divided into six equal portions. Each portion was given one of the following six treatments: crushed, dried leaves of T. riparia 1% w/w (wet weight/wet weight); powdered Actellic, 1% w/w; water dilution of neem seed extract (Margosan-O W.R. Grace & Co. Baltimore, Maryland) 0.08% w/w (wet weight/wet weight) and triton, a surfactant; crude pyrethrin extract (produced in Ruhengeri Prefecture, Rwanda by OPYRA) 5% w/w (wet weight/wet weight); control, nothing added; and control, triton (same concentration as the triton added with the neem treatment) + H₂O. The Actellic treatment also provided control information, since that treatment was made with the same formulation and concentration as that used prophylactically by the OPROVIA warehouses prior to storage and sale to consumers.

Beans with these six treatments were placed in locally manufactured plastic pails with snap tops (4 cm height 25X25 cm). Entire set of mini-bins were placed in the incubator at OPROVIA/ GRENDARWA II - Recherches Laboratory. Mini-bins were maintained at standard bioassay conditions as described above in 3.1. General quality analyses were conducted at 15 months after treatment of beans. Moisture content was determined with a Motomco moisture meter and by the oven dry method. Test weight (g/1.1 liter), % insect bored kernels, and % broken kernels were determined on 100 g subsamples. Number of insects (live /dead)/kg was determined by sieving (30 level strokes with a 10 mesh sieve) and counting insects observed in the bottom pan.

3.11.1. Cookability /Instrumental Hardness Testing

Cookability analysis was conducted in the food science laboratory of OPROVIA-GRENDARWA II - Recherches. From the test storage containers, 75 g Rwandan beans, variety

Rubona 5, were randomly taken. Beans were rinsed and particles of pyrethrum extract and *T. riparia* leaves were removed. To these beans, 4 liters of tap water and 4 g of NaCl were added. This mixture was brought to a boil over high heat on an electric Thermolyne hotplate (model HPA2230M, Thermolyne Corp., 2555 Kerper Blvd., Dubuque Iowa 52001 U.S.A.). When the beans began to boil (at ca. 96°C), the heat was reduced to maintain a moderate boil with the saucepan covered. Cooking time of 3 hrs is measured from the start of boiling. (Benchtop sensory tests of beans stored 1-2 months after harvest and cooked in this laboratory in Rwanda for different lengths of time indicated that a cooking time of three hours was adequate to cook the beans to a sensory acceptable level of doneness (Edmister et al. 1986).) Hot water was added slowly as necessary during cooking to maintain a liquid condition and in such a way that the boiling rate did not change appreciably.

Following the cooking preparation, beans were drained and transferred to small enamel dishes for cooling. An inverted plate was placed over the beans to avoid changes in hardness due to drying. Beans were cooled for 3 hrs, or to room temperature, before instrumental hardness testing.

One hundred randomly chosen beans were selected and tested for gram force with a Chatillon dial push/pull gauge (model DPP-500G) mounted on a test stand (model LTS; John Chatillon and sons, Inc., 83-30 Kew Gardens Rd., Kew Gardens, NY 11415 U.S.A.). This model is relatively low in cost, easy to operate, small in size, and requires no electricity. The push/pull gauge is equipped with a 0.318 cm diameter stainless steel probe mounted on the test stand. As the test stand arm is raised, the probe pierces the bean. The gauge dial registers the grams force necessary to completely pierce the bean on a scale from 0 to 500 g. Readings are to the nearest 5 g.

In previous studies (Edmister et al. 1986), it was determined at what point the gram force becomes detectable as "hard to cook" by a trained Rwandan sensory panel and by untrained consumer panels. Hard-to-cook beans are those registering ≥ 450 g force on the Chatillon tester. The percent hard to cook (=DAC, Durete a cuire) is the number of beans in each 100 bean sample ≥ 450 g.. Instrumental hardness and hard-to-cook determinations were made on the beans stored in the mini-bins 0.5, 4.0, 8.0, and 12.0 months after treatment.

3.11.2. Human Sensory Preference for Treated Beans

To conduct the sensory preference and acceptability analysis, a trained sensory panel of 20 Rwandans (10 males and 10 females) ages 24 to 47 years. Panel members were OPROVIA staff members. They were asked to participate based on their availability and interest in the project. The subjects had been previously trained (Edmister et al. 1986). During this training, the panel met with a member of the research staff weekly over a 6 week period. Definition of hardness was explained and samples were tested on their ability to detect hardness. Subjects were considered to be "trained" after six weeks if they were able to put samples differing greatly in hardness in the correct order and if their responses for the same samples were generally consistent over time.

For this study, the panel members were presented with beans cooked in the traditional Rwandan manner. An electric plate was substituted for the wood fire and three stone support of the metal cooking pot. Preparation of the beans was as described for the cookability /instrumental hardness testing. Sensory tests were held within a 7-day period of instrumental tests. At the end of the cooking time, bean samples were drained and served to the panel of judges.

Panel members were seated in the sensory evaluation area adjacent to the food science laboratory and given a pencil and score sheet. The panel was presented with samples in private booths with equivalent amounts of lighting. Each presentation consisted of two samples with equal amounts of beans. All samples were presented to subjects at the same time, each in a separate coded enamel bowl. The order in which samples were tasted was 16-25 randomized for each panel member. Panelists were instructed to taste each sample and then answer the following questions: 1) Which of the two samples of cooked beans do you prefer?; 2) Which of these samples are you more likely to choose to eat?; and 3) For each sample, what are the positive and negative properties of each sample that you consider important? Sensory preference and acceptability determinations were made on the beans stored in the mini-bins 0.75, 4.0, and 8.0 months after treatment. Tests involving human evaluation were discontinued after 8 months due to the development of insect populations in the control.

3.12. Statistical Analysis

LC₅₀ (concentration which is lethal for 50% of the test population) and LT₅₀ (time required, at a fixed dose, for mortality to occur in 50% of the test population) were estimated on all appropriate data. To obtain these values, dose-response bioassay data were subjected to probit analysis (Finney 1971). Probit-transformed percent mortality was regressed over time and against log₁₀-transformed concentration using PROC PROBIT (SAS 1988). Abbott's formula (Abbott 1925) was used to adjust for control mortality when necessary. Further details of the statistical analyses are available in the published articles from this project and our journal manuscripts (see Appendix of this report). A summary of specific analyses follows.

Initial volatilization and efficacy studies of linalool, a major component of Q. canum

- The dominant response of moribund insects was to die after prolonged exposure (more than 24 hrs). Therefore, moribundity and mortality were pooled for analysis in LC₅₀ determinations. LT₅₀ values were calculated for 500 ug/cm² with all species using knockdown data prior to 18 hr and pooled moribundity and mortality data for 24 hrs. Percent mortality data were normalized by arcsine transformation and were subjected to analysis of variance (Sokal and Rohlf 1981). If significant interaction occurred between species (or sex) and dosage, then LSD multiple comparisons were conducted with $\alpha = 0.05$ (Sokal and Rohlf 1981).

Contact and fumigant toxicity of milled Q. canum leaves - Dose response data were subjected to regression analysis. All models were evaluated for validity using lack-of-fit tests because the percent variation explained (r^2) values were very low for certain regressions used (Draper and Smith 1981). Some data for intact leaves were subjected to linear regression using

PROC REG (SAS 1988). Other data for intact leaves were subjected to non-linear regression using either a two-parameter positive asymptotic regression equation ($y = a + be^{-x}$) or a two parameter negative exponential decay equation, ($y = a + be^x$) (PROC NLIN in SAS 1988). The data for finely-milled leaves suggested an asymmetric transition and was evaluated using the logistic dose response transition equation $y = a + b / (1 + (x/c)^d)$ where $x = \log_{10}(\text{concentration} + 1)$ and $y = a$ a measure of biological productivity (eggs per female, hatched eggs per female or F_1 adult progeny at 55 days per female) transformed using $(y + 0.375)^{0.5}$ (Anscombe 1948). This procedure was conducted using PROC NLIN (SAS 1988).

Contact toxicity data were transformed using arcsine (proportion killed)^{0.5} to stabilize variances. A one-way analysis of variance (ANOVA) was used to determine if treatment had a significant effect on transformed proportion killed. If treatment was significant in the ANOVA, individual treatments were compared to controls with Dunnett's one-tailed tests to determine if transformed proportion killed for treatments was higher than for the controls ($\alpha = 0.01$) (PROC GLM in SAS 1988). Untransformed means were tabulated with the differences between the transformed treatment and transformed control means. The 99% confidence limits for the differences between transformed means were also reported.

Cookability /Instrumental Hardness of treated beans - Mean grams force, percent hard-to-cook beans, standard deviation, standard error of the estimate, and coefficients of skewness and kurtosis (descriptors of the shape of the sample distribution) were determined for each bean sample. To determine if there is a difference in mean hardness (MGF) between samples an ANOVA or T test was used. Multiple comparisons test were conducted to determine which samples were significantly different from each other. If 100 beans are tested, the 95% confidence interval (C.I.) is about 24 grams force. This value is based on an observed variance of 15,000 and calculated according to the formula $(1.96 \text{ sigma} / \text{square root of } n = L)$ (Snedecor and Cochran, 1967) where sigma is the square root of the variance, $n =$ the number of beans tested and $\pm L$ is the 95% C.I.). For this equation to be valid, bean hardness values must be normally distributed. Observations of some histograms of their hardness data indicated to Edmister et al. (1986) that this assumption probably does not hold for Rwandan bean mixtures.

The % hard-to-cook beans were analyzed with a Chi Square test to determine if there was a difference in % hard-to-cook beans between samples, and if so, what the difference was.

4.0. RESULTS

4.1. With the annual mint, Ocimum canum Sims

Gas chromatography of these leaves indicate that there are 25 to 30 compounds. Linalool was, by far, the most predominant compound. It is apparent that the milled leaves of Q. canum could serve as both contact and fumigant preparations to control bruchids in stored beans. The fumigative properties of Q. canum leaves is due to a loss (volatilization) of linalool (Weaver et al. 1991). The increase in loss of volatiles that occurs after milling also makes it imperative that this preparation be used immediately, or the results may again be less reliable.

Milled Q. canum leaves killed all adult Z. subfasciatus at 48 hrs for both the 1% and 2% w/w preparation (Weaver et al. in review). The 5% oat microparticle w/w formulation caused only 44% mortality in females and 56% mortality in males at 48 hrs (Weaver et al. in review). The oat particles contained linalool at the concentration extractable from milled Q. canum leaves, 0.85%).

Extracted compounds or essential oils frequently show great differences in activity for difference stored product insect species. It is exceedingly important that concentrations required for control be determined for each insect species prior to use. There are several chemotypes of Q. canum reported from Rwanda. These are characterized by the concentration of linalool present in their essential oil (Ntezurubanza 1987). The Q. canum used in these experiments would be classified as low linalool content-type (about 60%). Extraction and quantitative gas chromatography indicated that linalool was present in milled, air-dried leaves of Q. canum at 8.59 ± 0.92 s.d. (standard deviation) mg/g. The proportion of alpha-bergamotene extracted (about 25%) is greater than the expected (10%). The amount of beta-caryophyllene (about 6%) agrees with the amount found for low-linalool content Q. canum (Ntezurubanza 1987). It appears that the combination of these three secondary plant metabolites is more effective than linalool alone in a topical treatment.

The linalool dose-response bioassays indicated that the LC_{50} values for the four main storage insect species in Rwanda were similar : Z. subfasciatus 429.3 ug/cm²; A. obtectus 412.1 ug/cm²; R. dominica 430.2 ug/cm²; and S. oryzae 426.7 ug/cm². The amount of linalool in the stock solution decreases most dramatically when the solution is delivered to filter paper. After the introduction to filter paper, the next major decrease occurs 15 min after evaporation of the ethanol solvent is begun. No further changes in quantity of linalool on the filter papers occurs after 6, 12, or 18 hrs of air- exposure, either qualitatively or quantitatively.

The freshly milled leaves of Q. canum are quite toxic to Z. subfasciatus males. Equivalent amounts of linalool delivered to the insects as nearly pure linalool applied to a filter paper surface or as a component of freshly milled leaves does not correlate. One reason for this, we hypothesize, is that the insects become coated with the milled leaves and are essentially coated with this fumigative component, linalool. Whereas, in the filter paper dose-response trial, the insects only contact the linalool through their tarsi and in the volatiles. If the leaves are milled and the insects are not exposed immediately, the leaves neither adhere to the insect or cause any mortality.

In the fumigation experiments with freshly milled leaves of Q. canum there was a significant concentration-dependant toxicity. The LT_{50} for the 1 g treatment was 3.5 times greater than that for synthetic linalool at the amount present in 1.0 g milled Q. canum (Appendix 5, table 2). At 48 hrs after exposure, 100% of the insects, both female or male Z. subfasciatus, were dead when exposed to the vapors of milled leaves of Q. canum. However, if the same amount of linalool was sorbed (adsorbed and absorbed) onto oat microparticles, after 48 hrs, only 44% of the females and 56% of the males were dead (Appendix 5 Table 3).

For the intact, dried leaves, a weak, but significant linear concentration-dependant increase in fecundity and fertility occurred (Appendix 5 Figure 1a,b). Scanning electron microscopy studies of the surface of intact leaves and after milling indicate that the glandular (Annual Rev. July 1 to December 31, 1988) hairs or trichomes are broken in milling. This process releases more of the linalool and other toxic volatile substances.

We have concluded that in the traditional storage structure, the imboho, some protection is provided by the undisturbed, intact leaf of Q. canum. This is a non-catastrophic effect (Throne 1990). Everyday, however, the family generally removes from the imboho, approximately 1 kg of beans for the meal. The imboho is opened in a very small slit to allow several beans at one time to drop out. The imboho is then resealed. We have followed this process in the laboratory and observe that the fresh Q. canum leaves dry around the beans surrounding them. When the beans are withdrawn each day, the leaves are slowly broken into finer and finer particles. Thus, the Q. canum leaves in the imboho create a slow release of the fumigant, linalool, and probably other compounds.

4.2. With the perennial medicinal mint, Tetradenia riparia (Hochst.) CODD

Gas chromatography of the essential oil of T. riparia leaves indicate there are 206 distinct peaks, with no particularly dominant compound. Linalool was present but not in concentrations strong enough to cause significant mortality (Appendix 7).

The efficacy of the dried leaves of T. riparia is governed primarily by decreases in the fecundity and, to a lesser extent, the fertility of the parental females with high concentrations of the plant material. F₁ larval mortality was also increased at higher concentrations. Bean protection was more likely to be due to behavioral effects induced by physical and/or chemical properties of the odorous leaves of this perennial mint. In replicates that contained greater quantities of leaves, oviposition by Z. subfasciatus occurred on fewer beans. Any physiological effects on fecundity or fertility of individual females is less likely, since the parental adults were removed directly from the stock culture and added to the experiment without any prior exposure to T. riparia volatiles.

In an earlier study (Dunkel et al. 1986), it was found that beans with bruchid emergence holes were unacceptable for either planting or food. These were identified and discarded by Rwandan consumers. At the 4% wet weight/ wet weight (w/w) crushed concentration, in our experiments, there was a nearly 50% reduction in the number of beans oviposited upon by Z. subfasciatus. At the 10% (w/w) crushed concentration, there was a 70% reduction in F₁ emergence by A. obtectus. This indicates that there will be fewer emergence holes. The net losses, therefore, to Rwandan consumers may be considerably reduced by using crushed dried leaves of T. riparia at these levels in their storage containers.

The germination of Pinto beans that had been treated with the essential oil of T. riparia and stored for 180 da under bioassay conditions was rapid and no concentration dependant effects were evident (Appendix 4). Evaluation of behavioral activity insects exposed to beans

treated with the essential oil of T. riparia and incubated 210 da in a choice test indicated that the 53.5 ug/cm² treatment reduced the oviposition on treated beans to nearly zero with an EC₅₀ of 10 ug/cm₂ (Appendix 4 Figure 8a). In contrast, the EC₅₀ for adults emerging after 55 days after treatment as larvae within the bean at the time of treatment was 3980 ug/cm₂. One to two day old eggs attached to the bean surface were slightly more sensitive to the mixing treatment with the oil than were the adults to the presence of freshly-applied oil on the bean surface. The suppression began at 27 ug/cm² and was complete at 125 ug/cm². It is important to note that the process of agitation itself reduced F₁ adult progeny to 42% of that for an undisturbed control. The freshly applied T. riparia oil was active at a concentration range beginnings at 55 ug/cm² of bean surface and caused complete suppression at approximately 250 ug/cm² of bean surface (Appendix 4, figure 4).

In human sensory evaluation of beans stored for two weeks with the same preparation of T. riparia, but at a lower concentration, 55% of the panel preferred these beans than beans stored the conventional way with actellic (Appendix 7 Table 3). When asked to evaluate properties that contributed to positive and negative aspects of the samples, the scores were similar the conventional insecticide. Texture and odor were more positive than the conventional beans.

Therefore, studies on leaves of T. riparia indicate that the crushed leaves would be appropriate for reducing loss in beans stored in the most common on-farm structure in Rwanda, the open, dung-lined basket. The essential oil, after efficacy and non-target testing, may be appropriate for use on seeds in long-term storage at the national warehouses.

4.3. With Chenopodium schraderanum

Since the availability of T. riparia products and Q. canum leaves was so much greater than that for C. schraderanum, only preliminary studies were completed in the US with this plant. These studies indicated that the leaves may be more effective than the flowers. Leaves caused 60% of the mortality and flowers caused a mortality of 35% (Ann. Rept. July 1-Dec. 31, 1988). Larger scale tests were undertaken as part of a Masters Memoir by Mr. Nizeyimana in Rwanda.

4.4. With red pepper, Capsicum frutescens

Since the availability of T. riparia products and Q. canum leaves was so much greater than that for C. schraderanum, only preliminary studies were completed in the US with this plant. These studies indicated that the leaves may be more effective than the flowers. Leaves caused 60% of the mortality and flowers caused Larger scale tests were undertaken as part of a Masters Memoir by Mr. Nizeyimana in Rwanda.

5.0. IMPACT, RELEVANCE AND TECHNOLOGY TRANSFER

5.1. Recommendations and plans for utilization of results

The Lindbergh award had been received by Dr. F. Dunkel to provide support for returning the traditional practice of using Q. canum in sealed containers back to the subsistence farmers where the "discovery" was made in 1984 (Dunkel et al. 1986). This process will involve reintroducing the hand milling stones abandoned in the past ca. 20 years for local commercial mills. This process will improve the efficacy of the leaves. Dr. Dunkel and the Rwandan scientists trained in the present project will also test these techniques in real (on-farm) scale in locally produced sealed plastic containers.

In a collaborative agreement with Dr. Bottenberg at IITA (Kano Station, Nigeria), Ocimum basilicum will be grown with their cowpea crop and harvested for use as a postharvest protectant for the cowpeas.

It is recommended to OPROVIA and to the seed storage units in Rwanda, that the essential oil of T. riparia produced in Rwanda be used for protection of seeds. The Lindbergh award has also provided for funding to test hypotheses regarding the use of this essential oil on burlap bags. These bags are the primary storage container for government stored grain and beans in Rwanda and in most developing countries. Laboratory scale tests of the oil and bags were initiated in the summer 1992. Based on the results of these tests, larger scale tests will be designed in collaboration with the OPROVIA staff in Rwanda. It will be recommended to producers that dried, crushed leaves of T. riparia be used as part of a protection plan for beans stored on the farm.

5.2. Impact of project on individual scientists, laboratories and institutions

In Rwanda, the expertise to conduct bioassays, the daily interaction, at times, by fax and phone, when research or equipment questions arise have made, we believe, a major favorable impact on the laboratories that took a role in the project in Rwanda. The research unit of OPROVIA is at this time (August 1992) setting up full scale lab testing of Chrysanthemum cinerarifolium for immediate use in government warehouses. A linkage with the National University of Rwanda has been planned, but not yet funded.

In the US, the MSU laboratory of Dr. Dunkel has now expanded its research from the two Rwandan mints to seven other species of plants with bioactive properties. One of these species, Tagetes patula is now in field trials in Northern Montana and in Nigeria. The HBCU institution, Virginia State University, has a laboratory devoted to the vertebrate toxicity testing of these plant extracts. This was developed in collaboration with this initial PSTC project.

In Morocco studies are being planned to develop their traditionally used mint and sagebrush as an alternative, or additional protection for stored grain. These plans are developing

with the faculty of Institut Agronomique et Veterinaire Hassan II in collaboration with Dr. Dunkel's laboratory.

5.3. Plans for larger scale trials

Larger scale tests are planned, as described in 5.1, will be conducted. On-farm trials are planned for the modifications described for the Q. canum improvements. The studies of this PSTC grant led to other funding to explore the possibilities with neem kernel extract and marigolds, Tagetes spp. Lab scale studies are now nearing completion on these parallel AID-HBCU grants (Dunkel et al. 1990). Since neem trees do not occur in Rwanda at present, this project will be initiated with assistance from an agroforestry unit. Tagetes spp. do occur commonly in Rwanda. Use of Tagetes minuta as a soil fumigant vs. nematodes, as a green manure, and as an extractable product (Clevenger steam distillation) from the roots will be tested in larger scale trials.

The initial Rwanda PSTC Insecticidal Plant study is responsible for the initiation of mountain sagebrush, Artemesia tridentata studies of insecticidal properties. Following this past year of work and preparation of manuscripts, studies of the fumigative properties in larger scale will be undertaken. Soil fumigation and granary fumigation will be the focus of the larger scale testing.

6.0. PROJECT ACTIVITIES/OUTPUTS

6.1. Project planning and evaluation meetings

Three major project planning meetings were held and two evaluation meetings. The third evaluation meeting scheduled for 1991 was postponed to 1992 due to the travel restrictions during the Kuwait war and the civil unrest in Rwanda.

The initial planning workshop was held August 20-25, 1988 in Madison, Wisconsin (University of Wisconsin) 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 Food Science, 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.

Due to the large group of collaborators in five locations, a unified plan of work was developed together during the workplan meetings. Milestones were identified. Areas of work were outlined, details added to the outline, and the estimated time required for their completion were developed. The critical path of work to be accomplished was then determined. Because the required conclusion of the project is publications, a publication policy was developed by consensus with all of the participants of the workshop (Progress Report of this project July 1 to December 31, 1988, p. 10).

In September 1989, a planning and evaluation meeting was held in Rwanda. This visit included collaborators at OPROVIA, CURPHAMETRA, and ISAR. In October 1989, a planning and evaluation meeting was also held at the University of Wisconsin. As a result of these meetings, the objectives of the visit of Dr. W. Burkholder to Rwanda and Dr. I. Butare and Mr. P. Kayinamura to Rwanda were developed. Critically low supplies of the insecticidal plant products were noted.

In October 1990, a series of planning and evaluation meetings were held with the Rwandan and US representatives of the research team. The meetings were held at three locations, Montana State University (MSU), Bozeman MT, University of Wisconsin (UW), Madison WI, Virginia State University (VSU), Petersburg VA and AID - Office of the Science Advisor, Rosslyn VA. Progress in testing hypotheses of the present project was evaluated. One of the most useful products of these meetings was a document, jointly developed by consensus (Annual Report of this project for 1990, Appendix 8). This document outlines recommendations for research in the on-going collaborative effort between MSU, UW, VSU, OPROVIA, and CURPHAMETRA. Funding sources, including this project were identified for each research question. Approximately 40% of this follow-on research plan has already been funded and completed or accomplished under the present project.

In addition to these US-Rwanda planning and evaluation meetings, Dr. Dunkel coordinated US efforts by visiting, on an annual or semi annual basis, the AID Office of the Science Advisor, the University of Wisconsin, and Virginia State University.

6.2. Presentations at professional meetings

- Dunkel, F.V. October 16, 1988. Madison, Wisconsin U.S.A. "Entomological aspects of the postharvest system in Rwanda (East Central Africa)." Entomology Department Colloquium Series University of Wisconsin. 60 min. ca. 60 people attending.
- Dunkel, F.V. February 11, 1989. Selma, Alabama U.S.A. "The Rwandan small farm system." Selma University. Natural Science Division Seminar. 60 min. ca. 40 people attending.
- Dunkel, F.V. May 5, 1989, Bozeman, Montana U.S.A. "Rwandan medicinal plants with insecticidal properties." Entomology Department Seminar Series Montana State University. 50 min. 35 people attending.
- Dunkel, F.V. October 30, 1989. Madison, Wisconsin U.S.A. "The technological basis of traditional Rwandan insecticidal plants." Department of Entomology Seminar. 50 min. 50 people attending.
- Dunkel, F.V., W.E. Burkholder, and J.K. Phillips. December 12, 1989. San Antonio, Texas U.S.A. "Insecticidal properties of Ocimum spp. (Lamiaceae) for the bruchids Zabrotes subfasciatus Bohem and Acanthoscelides obtectus Say." National meetings of the Entomological Society of America. Poster presentation for 1 day. ca. 1000 people attending.
- Dunkel, F.V. May 4, 1990. Bozeman, Montana U.S.A. "Testing hypotheses regarding insecticidal plants." Entomology Department Seminar Series Montana State University. 20 min. 35 people attending.
- Dunkel, F.V., D.K. Weaver, G. Rusuku, N. Kimpe, and L. Van Puyvelde. July 17-22, 1990. Bonn Germany. "Antimicrobial and insecticidal activity of the essential oil from the leaves of Tetradenia riparia." Poster presentation by L. VanPuyvelde. Poster prepared by F. Dunkel and D. Weaver. Internat. Joint Symposium of Besellschaft for Arzneipflanzenforschung, Amer. Soc. Pharmacognosy, Assoc. Francaise pour l'Enseignement et la Recherche en Pharmacognosie, and the Phytochemical Soc. Europe. Poster on display for 5 days. ca. 1000 attending.
- Weaver, D.K., F.V. Dunkel, L.L. Jackson, and W. Burkholder. September 9-14, 1990. Bordeaux, France. "Toxicity of R,S-linalool to four species of storage Coleoptera as influenced by degradation and volatilization." International Workshop on Stored Product Protection. Poster on display for 5 days. ca. 300 people attending.

- Dunkel, F.V., D.K. Weaver, L. VanPuyvelde, A. Serugendo, and J. Cusker. September 14, 1990. Bordeaux, France. "Population suppression effects of Rwandan medicinal plant, Tetradenia riparia (Hochst.) CODD (Lamiaceae) on stored grain and bean insects." International Workshop on Stored Product Protection. 20 min. ca. 100 people attending.
- Dunkel, F.V., I. Butare, and P. Kayinamura. October 17, 1990. Rosslyn, Virginia U.S.A. "Rwandan insecticidal plants: Designing cooperative research to meet country needs." AID-S&T. 60 min. ca. 30 AID officers from the Office of the Science Advisor, the Science and Technology section, and the Africa Bureau.
- Dunkel, F.V. November 1, 1990. Winnipeg, Canada. "The stored grain ecosystem: a global perspective." Keynote address of symposium on "Management of Postharvest ecosystems: Current and future trends." Annual meeting of the Entomological Society of Manitoba. 90 min. ca. 100 attending.
- Weaver, D.K., F.V. Dunkel, L.L. Jackson, and W. Burkholder. December 3, 1990. New Orleans, Louisiana U.S.A. "Toxicity of R,S-linalool to four species of storage Coleoptera as influenced by degradation and volatilization." National meetings of the Entomological Society of America. Poster on display for 1 day. ca. 1000 people attending.
- Dunkel, F.V., D.K. Weaver, L. VanPuyvelde, and J. Cusker. December 3, 1990. New Orleans, Louisiana U.S.A. "Effects of the perennial mint, Tetradenia riparia (Hochst.) CODD (Lamiaceae) on bean bruchid population development." 20 min. ca. 100 people attending. National meetings of the Entomological Society of America. 10 min. ca. 45 people attending.
- Weaver, D.K. January 25, 1991. Bozeman, Montana U.S.A. "Myths and methodologies in the study of plant-derived pesticides." Entomology Department Seminar Series. Montana State University. 50 min. 35 people attending.
- Dunkel, F.V. February 12, 1991. Indianapolis, Indiana U.S.A. "The potential of insecticidal plants for management of stored grain insects." USDA NC-151 Committee on Delivery of Quality Grain and Oilseeds to Foreign and Domestic Markets. 20 min. 55 people attended. Those attending were leaders in stored grain research at US universities or USDA laboratories or represented grower groups and agribusiness firms dealing with postharvest handling or storage of grain.
- Dunkel, F.V. March 14, 1991. Petersburg, Virginia U.S.A. "Insecticidal plants, the postharvest system in Rwanda, possibilities for VSU collaboration." Special Seminar. Virginia State University (VSU). 120 min. 40 people attending. VSU administrators and AES (Agricultural Experiment Station) scientists.

- Dunkel, F.V. July 18, 1991. Bozeman, Montana U.S.A. "Doing Science with insecticidal plants: Pulling us out of the Pesticide Conspiracy." NSF Science Teaching Institute of the Rockies. Montana State University. 50 min. 40 people attending.
- Weaver, D.K., F.V. Dunkel, and L. Van Puyvelde. December 9, 1991. Reno Nevada U.S.A. "Response of storage insects to preparations of the leaves of Tetradenia riparia." National meetings of the Entomological Society of America. 12 min. 40 people.
- Dunkel, F.V. July 15, 1992. Bozeman, Montana. U.S.A. "Doing Science with insecticidal plants: Pulling us out of the Pesticide Conspiracy." NSF Science Teaching Institute of the Rockies. Montana State University. 50 min. 45 people attending.
- Dunkel, F.V. August 14, 1992. Little Falls, Minnesota, U.S.A. "Preserving the harvest and traditional wisdom: Scientific improvement of traditional and natural methods for protection of stored food crops in Rwanda, East Africa." Lindbergh Symposium on Nature versus Technology: Conservation Hanging in the Balance. Lindbergh Foundation. 30 min. 150 people attending.
- Dunkel, F.V. August 14, 1992. Little Falls, Minnesota, U.S.A. "Preserving the harvest and traditional wisdom: Scientific improvement of traditional and natural methods for protection of stored food crops in Rwanda, East Africa." Presentation of Lindbergh Grant Recipients. Lindbergh Foundation. 10 min. 200 people attending.
- Richards, D.C., F.V. Dunkel, and S. Sriharan. December 9, 1992. Baltimore Convention Center, Baltimore MD, USA. "Effect of insecticidal plant extracts on the pirate bug, Xylocoris flavipes (Reuter), a beneficial predator in stored grain." National meetings of the Entomological Society of America. Poster presentation for 10 hours. ca. 1500 people attending.
- Rodriguez, D.C., F.V. Dunkel, D. Richards, and D.K. Weaver. December 9, 1992. Baltimore Convention Center, Baltimore MD, USA. "Fumigative, repellent, and oviposition deterrent properties of mountain sagebrush, Artemisia tridentata, for stored grain insects." National meetings of the Entomological Society of America. Poster presentation for 10 hours. ca. 1500 people attending.
- Raqib, A., F.V. Dunkel, and L. Van Puyvelde. December 7, 1992. Baltimore Convention Center, Baltimore MD, USA. "Efficacy of Tetradenia riparia (Hochst.) CODD (Lamiaceae) essential oil applied to bags for storage insect population." National meetings of the Entomological Society of America. Poster presentation for 10 hours. ca. 1500 people attending.

6.3. Training completed within project

6.3.1. Training in U.S.A.

Rwandans:

Joseph Kayitare - in the USDA-ARS Stored Product Insect Laboratory, Department of Entomology, University of Wisconsin, Madison, Wisconsin. Three months, August-November, 1988. Supervisor was Dr. Wendell Burkholder. Mr. Kayitare conducted preliminary laboratory experiments of behavioral and toxic responses of two species of bruchid to four Rwandan medicinal or insecticidal plants. These plants were: Chenopodium schraderanum Roem. and Schult. (Chenopodiaceae), Tetradenia riparia (Hochst)Codd (Labiatae), Ocimum kilimansharicum Guerke (Labiatae), Ocimum canum Sims (Labiatae), and Capsicum frutescens L. (Solanaceae).

Aussumani Serugendo - in the legume processing/physical properties laboratory, Department of Food Science and Nutrition, University of Minnesota, St. Paul MN. Five months, August-January, 1989. Supervisor was Dr. William Breene. With Dr. Breene and Dr. Zata Vickers, Mr. Serugendo developed a protocol 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.

Marie Nizeyimana - in the stored product insect/insecticidal plant laboratory, Entomology Research Laboratory, Montana State University, Bozeman, Montana U.S.A. Two months, July-August, 1991. Supervisor was Dr. Florence Dunkel. During this period, Ms. Nizeyimana learned the use of Probit, a program for probit analysis of toxicity data; became independent in preparing insects, exposure chambers, and dilutions for acute adult toxicity tests; became independent in monitoring and trouble shooting the proper functioning of the bioassay chamber under rigid test conditions. Ms. Nizeyimana also developed with Dr. Dunkel a plan for reintroduction and improvement of the O. canum and imboho for on-farm storage in Rwanda. Ms. Nizeyimana and Dr. Dunkel also developed a plan for testing a commercially prepared (in Rwanda) extract of Chrysanthemum cinerifolium for use in the national, large scale warehouses in Rwanda.

Throughout their stays, English instruction was provided for each of the Rwandans.

US citizens and other nationals (All supervised by Dr. Dunkel):

James Cusker - With a grant from NSF Science Teaching Institute of the Rockies, Mr. Rodriguez spent one summer session (1990) testing hypotheses of the oviposition behavior of bruchids exposed to the traditional, perennial Rwandan medicinal mint, T. riparia. For the experiments, Mr. Cusker designed an oviposition chamber appropriate to the bruchid species. This work resulted in a refereed publication, a draft of a reviewed publication (Montana Academy of Science), and a presentation at the national meetings of the Entomological Society of America. Mr. Cusker is a master teacher at Sentinel High School in Missoula, Montana.

Brenda Allwardt - As an undergraduate student from Lawrence University (Appleton, Wisconsin) and with her own funding, Ms. Allwardt selected our laboratory and this PSTC project for an independent research experience in the summer of 1990. Ms. Allwardt tested hypotheses regarding the ability of T. riparia to protect grain from oviposition and larval development of the rice weevil, one of the most important stored grain insects in Rwanda (Dunkel et al. 1986). For her research, Ms. Allwardt developed a bioassay process for staining and dissecting the grain kernel to determine the effect of the mint leaves on inhibition of egg and larval development. This technique was used in 1991 for full scale experiments with local Montana plants.

David Rodriguez - With a grant from NSF Science Teaching Institute of the Rockies, Mr. Rodriguez spent two summer sessions (1991 and 1992) testing hypotheses of the insecticidal mode of action of the traditional Rwandan storage structure (the imboho) and the traditional pest protection plant used in its construction. Mr. Rodriguez also designed a two choice chamber for behavioral assays and used it to test the repellency of Rwandan burlap bean and grain storage bags treated with a steam distillate (prepared in Rwanda) of the perennial medicinal mint, T. riparia. Mr. Rodriguez is a master science teacher at Cour de Lene High School, Cour d'Alene, Idaho.

Jeremy Hampton - With a grant from NSF Science Teaching Institute of the Rockies, Mr. Hampton spent one summer session (1991) testing hypotheses of the insecticidal properties of Rocky Mountain plants. Using methods developed in this PSTC project, local US plants were identified with commercial potential for pest management in the U.S.A. This work has resulted in a presentation of the annual meetings of the Entomological Society of America and a manuscript for submission in a refereed journal. Mr. Hampton is a master science teacher at Fernley Junior High School, Fernley (Reno), Nevada.

Libby Nance - With a graduate research assistantship from the Montana Agricultural Experiment Station (MAES), Ms. Nance assisted in the research with additional plant species suggested by the Rwandan local medicinal plant project. Ms. Nance also completed an extensive literature review of the "Optimization of insecticidal components of plants." The results of these findings will be utilized for follow-on studies in 1992-1993 with the Rwandan mints and for the Rocky Mountain plants locally available in Western Montana.

Abdur Raqib - With a graduate assistantship from US-AID Pakistan for a Master of Science degree at the University of Missouri, Mr. Raqib tested the repellency and direct toxicity of storage insects to burlap storage bags treated with the essential oil of T. riparia.

Dr. David Weaver - With a postdoctoral fellowship from MAES and part support from this PSTC project, Dr. Weaver was given training in culture of stored grain and stored bean insects and in understanding the postharvest system in Rwanda. Dr. Weaver, in turn, contributed his expertise in chemistry related to natural products and in conducting behavioral bioassays.

6.3.2. Training in Rwanda

Rwandians:

Evariste Munyarshoka (research scientist) - Mr. Munyarshoka was given training (1989) in preparing serial dilutions for bioassays, the importance of a bioassay system to be used on a regular basis to monitor pesticide resistance, and the importance of alternative pesticides. Mr. Munyarshoka has recently taken a position at OPROVIA as plant protection officer. His new responsibilities include decisions on timing, dosage, and type of pesticide application.

Edouard Nizeyimana (technician) - Mr. Nizeyimana was trained in storage insect mass culturing, maintenance of standard bioassay conditions in test facility, setting up and reading a bioassay, and proper cleanup procedures for toxic chemicals and non-contaminated glassware. Training by Dr. F. Dunkel for two weeks.

Albert Nizeyimana (M.S. student) - Mr. Nizeyimana received training in bioassay procedures and mass culturing of insects. He completed a memoir for his Masters of Science degree at the National University of Rwanda. The topic of his memoir was "Optimization in the growth of the annual Rwandan mint, Q. canum." Dr. Innocent Butare was the major professor, or supervisor of Mr. Nizeyimana.

Patrice Nizeyimana (M.S. student) - Mr. Nizeyimana received training in bioassay procedures and mass culturing of insects. He completed a memoir for his Masters of Science degree at the National University of Rwanda. The topic of his memoir was "Optimization in the growth of the annual Rwandan mint, Chenopodium schraderanum." Dr. Innocent Butare was the major professor, or supervisor of Mr. Nizeyimana.

U.S. Citizens:

Dr. Shobha Sriharan - With parallel funding from the AID-HBCU (Historically Black Colleges and Universities), Dr. Sriharan received training from Dr. Dunkel in integrating toxicity testing and bioassay techniques into research that has meaning in the Rwandan postharvest system.

6.4. Publications

Published or accepted for publication (see appendix for entire article):

Weaver, D.K., F.V. Dunkel, L. Ntezurubanza, L.L. Jackson, and D.T. Stock. 1991. The efficacy of linalool, a major component of freshly-milled Ocimum canum Sims (Lamiaceae), for protection against postharvest damage by certain stored product Coleoptera. *J. Stored Prod. Res.* 27:213-220.

Weaver, D.K., F.V. Dunkel, J.L. Cusker, and L. Van Puyvelde. 1992. Oviposition patterns in two species of bruchids (Coleoptera: Bruchidae) as influenced by the dried leaves of Tetradenia riparia, a perennial mint (Lamiales: Lamiaceae) that suppresses population size. Environ. Entom. in Press (anticipated issue October 1992).

Dunkel, F.V. 1992. The stored grain ecosystem: a global perspective. J. Stored Prod. Res. 28:73-87.

Dunkel, F.V., D.K. Weaver, L. Van Puyvelde, and A. Serugendo. 1991. Growth regulatory effects of a Rwandan medicinal plant Tetradenia riparia (Lamiaceae) on stored grain and bean insects. Proc. Fifth Internat. Conf on Stored Product Protection. Sept. 1990. Vol III, pp. 1609-1617.

Weaver, D.K., F.V. Dunkel, and L.L. Jackson. 1991. Toxicity of R,S-linalool to four species of storage Coleoptera as influenced by volatilization and degradation. Proc. Fifth Internat. Conf on Stored Product Protection. Sept. 1990. Vol III, pp. 1609-1617.

Kayitare, J. and L. Ntezurbanza. 1991. Evaluation de la toxicite et de l'effet repulsif de certaines plantes du Rwanda contre les bruches du haricot: Acanthoscelides obtectus Say et Zabrotes subfasciatus Boheman. Insect Sci. Applic. 12:695-697.

In review (see appendix for entire manuscript):

Weaver, D.K., F.V. Dunkel, L. Van Puyvelde, D.C. Richards, and G.W. Fitzgerald. Toxicity and protectant potential of the essential oil of Tetradenia riparia (Lamiales: Lamiaceae) against Zabrotes subfasciatus (Coleoptera: Bruchidae) infesting dried Pinto beans (Fabales: Leguminosae). to be submitted to J. of Applied Entomology. in USDA-ARS and MSU review.

Weaver, D.K., F.V. Dunkel, R.C. Potter, and L. Ntezurbanza. Contact and fumigant toxicity of milled Ocimum canum Sims to Zabrotes subfasciatus (Boheman) adults. to be submitted to J. Stored Prod. Res. USDA-ARS and MSU review completed.

In preparation:

Dunkel, F.V., A. Serugendo, and W.M. Breene. Sensory evaluation and cookability of dry edible beans (Phaseolus vulgaris L.) stored with insecticidal plant material of local origin. for submission to J. Food Sci. drafted.

Dunkel, F.V., E. Nizeyimana, E. Munyarshoka, T. Wittenberger, and S. Patten. The imboho: a traditional structure for storage of dry pulses in East Africa. for submission to J. Stored Product Res. drafted. (see appendix 8 for entire draft of manuscript)

Note: Many of the articles are senior authored by D.K. Weaver because he was a postdoctoral associate in training in the postharvest / insecticidal plant laboratory and became a collaborator after his hiring December 1989.

All refereed publications pass through an international panel which we set up for this project. The panel consists of:

Dr. B.J.R. Philogene
Department of Biology
l'Universite d'Ottawa
Ottawa, Ontario, Canada

Dr. H. Rembold
Max Planck Institut for Biochemie
8033 Martinsreid
Munich, West Germany

Dr. B. BenJilali
Department of Biochemistry
Institut Agronomique et Veterinaire Hassan II
Rabat Morocco

In addition, each article must pass an MSU departmental review by three entomologists or chemists at MSU. Because one of the authors is now at the USDA (D.K. Weaver), articles which include Dr. Weaver must also pass the USDA review process before submission to the journal.

6.5. Patents pending

Dunkel, F.V., D.K. Weaver, and T. Weaver, III. 1991. "Insecticidal or insect behaviorally active preparations from aromatic plants." U.S. Patent Application Serial No. 07/801,817. Washington D.C.

6.6. Interactions with other projects and the Private Sector

Morocco PSTC and UDLP Storage/Insecticidal Plant projects - Two proposals have been submitted to AID Washington that would link the Institut Agronomique et Veterinaire Hassan II with the private sector in Morocco and Montana. The focus is to be natural products for agricultural and human/veterinary vector systems. A team of 16 faculty, the Ministry of Health, and the Ministry of Agriculture are involved with these proposals. Both proposals passed the preliminary phase and the Principal Investigators (F. Dunkel and K. Bourarach) were invited present full proposals to the University Linkages Development section and to the Office of the Science Advisor.

US - AID Research and University Relations (Historically Black Colleges and Universities) - Concurrent with the present PSTC project, three years of funding for HBCU projects have been received by collaborators. Joint visits in the US with faculty from Selma University and Virginia State University and in Rwanda have been coordinated at least twice each year during the lifetime of the project. Two such meetings are also planned for 1992, one in Rwanda and one in Baltimore, Maryland, U.S.A. A follow-on proposal involving insecticidal plants and Rwanda is currently in the final stages of review with the Office of the Science Advisor. This project proposed is from Virginia State University with F. Dunkel as Co-Principal Investigator.

AID Cape Verde Biocontrol of Locust Projects - The biological control projects coordinated from the same department of Dr. Dunkel has provided efficient interactions. Coordinators of these two projects have kept Dr. Dunkel informed and vice versa regarding project activities and included her in interactions with the private sector.

Rocky Mountain Insecticidal Plant Project - This project is solely funded by the Montana Agricultural Experiment Station. The interdisciplinary team of MSU faculty also collaborate with Dr. Dunkel on the Rwandan mint project to facilitate exchange of techniques, information, etc.

NSF Science Teaching Institute of the Rockies - For three years, mini-grants (four to date) have been provided to master teachers at the secondary school level. These grants are to, in a mentorship relationship with Dr. Dunkel, provide a research experience with insecticidal plants for these teachers.

Grace and Co., Columbia, Maryland U.S.A. - In 1989, pilot studies were completed with their insecticidal product, Margosan-O, produced from the kernel of the neem tree, Azadirachta indica. These studies were conducted collaboratively with the OPROVIA Research Laboratory in Rwanda, Selma University faculty and students in Selma Alabama, and F. Dunkel as consultant at Montana State University. Growth and development studies were completed with the most important stored grain insect species in Rwanda and grain treated with Margosan-O. Sensory evaluations of dry beans (the main source of protein and calories for Rwandans) treated with Margosan-O were completed by a trained sensory panel of Rwandans. Current studies with Margosan-O and Grace and Co. involve testing wheat and barley. Milling, baking, and sensory attributes of products made from treated Montana wheat and barley will be evaluated. Montana strains of destructive and beneficial insects will also be evaluated for their response to Margosan-O.

Basic BioSystems, Missoula, Montana U.S.A. - In 1990, collaborative studies were initiated with Basic BioSystems and their product, Nurture[®], a proteinaceous microparticle. This particle is used for adsorption and absorption of volatile compounds. Our studies investigated the ability of these particles to extend the insecticidal life of one of the most volatile components of the annual Rwandan mint, Ocimum canum. These studies are completed and a refereed publication is in review (see Appendix 5 of this report). Follow-on studies have been agreed

upon to include the use of the Nurture material in enhancing the repellancy of various plant derived compounds. These compounds (from mints, marigolds, and Montana native plants) will also be combined with Nurture to aid in mosquito management in aquatic systems.

Agridyne, Inc., Salt Lake City, Utah U.S.A. - In 1991, discussions were initiated with Agridyne, Inc. regarding their interest in the plant derived products, compounds, and processes we have developed in the Rwandan PSTC project in Rwanda.

University of Missouri, Department of Entomology, Columbia, Missouri U.S.A. - In collaboration with AID funding for graduate students from Pakistan, F. Dunkel contributed to the training of a Master of Science student. General training in management of stored grain was given. Specific research training in testing hypotheses related to bag storage of commodities and use of the Rwandan insecticidal essential oil of T. riparia was also accomplished.

6.7. Parallel and follow-on funding received

US-AID Research and University Relations-HBCU

Selma University 1988-1989

Virginia State University, Year 1 1990-91

Virginia State University, Year 2 1992-93

Dr. S. Sriharan was the P.I. Dr. Dunkel served as consultant for these grants. Total grant funding was \$300,000.

Lindbergh Foundation - The 1992 award in anthropology to Dr. Dunkel for her work in balancing technological advances with traditional wisdom in Rwanda. The award for her work in Rwanda was \$10,580.

NSF Science Teaching Institute of the Rockies - Four mini-grants 1990-1992. These resulted in one refereed publication, one reviewed publication, and two posters presented at the national meetings of the Entomological Society of America. Dr. Dunkel served as mentor to the science teachers receiving the grants. The total awards were ca. \$1600.

Montana Agricultural Experiment Station - A three year approved project (1989-1992) with Dr. Dunkel as P.I. and Dr. L. Jackson, insect biochemist, as co-P.I. The subject of this project is insecticidal plants of Africa and Montana and implications for the agricultural systems of Montana. Laboratory research has led to field applications with the Mint growers association of Montana and the Northern Agricultural Experiment Station in Creston. The total award, including operations, salary for a postdoctoral associate, and capital expenditures was ca. \$86,000. This is in addition to Dr. Dunkel's salary for four years, a portion (ca. 50%) of which was devoted to the insecticidal plant research.

7.0. PROJECT PRODUCTIVITY

The project accomplished all goals with the following exceptions: environmental studies in Rwanda; real scale warehouse study of instrumental hardness and sensory preference; and the intercropping study. The environmental studies and the intercropping study were planned to occur at ISAR, the national Agricultural Research Center of Rwanda. The person originally assigned to this portion of the project, Joseph Kayitare, was promoted to the Ministry immediately after his return from training on the project (1988) at the University of Wisconsin. The person assigned to take his place, Mr. Bizimana was very capable, but had been assigned too many responsibilities. Interaction was complicated by the fact that ISAR, located in Rubona, had no phone or direct fax communication.

The nursery was begun at ISAR, but communication and exchange of plant material and data did not occur with regularity as it did with CURPHAMETRA, Center for Ethnopharmacology in Butare, Rwanda.

Realizing this, we substituted the development of other necessary experiments which gave us information on the natural slow release particles with linalool, component of both of the Rwandan mints. We proceeded with the residual action study and the repellency, oviposition deterrence, and bag study in substitution for the above objectives.

8.0. FUTURE WORK

8.1. Neem studies in Rwanda

Studies conducted with beneficial and destructive storage insects and human sensory preference for beans treated with neem kernel extract (Margosan-O, Grace and Co. Maryland). These studies initiated in Rwanda with the main stored commodities in that country are now being extended to the Montana farming system. Studies with wheat and barley will include insect protection during storage and the milling, baking, rheological and sensory attributes of products made from the treated grain.

8.2. Marigold (Tagetes spp.) studies in Rwanda, USA, and in Nigeria

Current studies underway focus on the response of destructive storage insects and beneficial storage insects (predators and parasitoids) to extracts of flower, foliage and root of Tagetes minuta. Studies have focused on the residual effect of the root extract. Cultures of T. minuta have been initiated in the Plant Growth Center at Montana State University. These cultures will provide a seed increase source of this species which is not commercially available in the USA. These seeds will be used for experimental purposes (primarily for production of the extract) only. Field studies are underway in the Northwestern Agricultural Research Station in Montana. The marigold crop was grown over fields inoculated with Verticillium wilt, the most serious disease in commercial mint fields in the Northeast USA. The second part of the

experiment will conclude in 1993 when mint is grown over the fields where the fallow marigolds were plowed under. The soil in the test plots will also be tested for nematocidal and insecticidal effects with relation to the mint crop.

Field studies are also underway in Kano Station Nigeria using marigolds as an intercrop with cowpeas and using sweet basil, Ocimum basilicum, as a crop to harvest and place into storage with cowpeas. The collaborating scientist, Dr. Bottenberg, is with the International Institute of Agriculture (IITA).

8.3. Exploration of insecticidal properties of Rocky Mountain Plants

Four species, balsamroot, horsemint, mountain sagebrush, and sticky geranium have had initial testing completed and the manuscript is in review. Second stage of research is underway. This involves repellency of storage insects, fumigative properties, and non - storage insects.

8.4. Sagebrush (Artemisia spp.) and mint (Ocimum spp.) studies in Morocco

The P.I. of the present grant has collaborated with Moroccan faculty at the Institut Agronomique et Veterinaire Hassan II for the past six years. The original collaboration was funded by PSTC grant to improve traditional sealed, underground storage structures (matmora). Data from that project indicated there may be resistance to low oxygen atmospheres in the Moroccan storage insect populations (Bartali et al. 1990). We, therefore, proposed that local plants currently in use for insecticidal or medicinal purposes be investigated as possible fumigants that could be added with the grain to the matmora as additional protection. Plant species closely related to those investigated in the present Rwanda PSTC project (Ocimum spp.) and the follow-on Rocky Mountain Plant project in Montana (Artemisia spp.) are traditionally used in Morocco.

The US-AID University Development Linkages Grant will be resubmitted in 1992. Rwanda is not an eligible country for the 1992 competition. The scientific portion of the proposal received a strong rating. A similar focus involving local medicinal/insecticidal plants will be used in Morocco. The primary linkage will be with the Institut Agronomique et Veterinaire Hassan II (IAV). Thirty five faculty at the linked institutions will participate. Insecticidal plants will be evaluated in several systems including preharvest and postharvest cropping systems and insect vectored diseases of human and veterinary importance. Methods for determining efficacy developed in the present PSTC grant will be adapted for the Linkages grant. Methods used to appropriately transmit the results to be used in the present PSTC grant to the Rwandan farming and marketing system will be adapted for the Moroccan farming, marketing and private enterprise system.

The University Linkages proposal will promote the development of environmentally sustainable pest management systems and their use in strengthening the private sector. Because women in the workforce is an issue of importance in Morocco, the team members and objectives

have been selected to address this issue both in academia and the private sector. (Postscript: In April 1992, the preproposal, or Letter of Intent, was accepted. On June 2, 1992, the full proposal was submitted to US-AID.)

The AID-PSTC full proposal invited from IAV faculty and Dr. Dunkel will be submitted August 1992. It will involve the use North African plants and inovative encapsulation, adsorption and absorption methods for extending efficacy.

8.5. Development of Tetradenia oil as a warehouse bag treatment

This PSTC project established that the oil is a strong oviposition deterrent and an insecticide for stored bean and grain insects. Our research in this project also established that the oil does not effect germination of beans and maintains its insecticidal properties for at least 210 days. Based on these results, we have initiated studies with treatment of the burlap and plastic bags used for all large scale government warehouse storage in Rwanda. These bags are also used throughout the developing countries for grain and bean storage. Initial studies indicate that the oil has repellent properties when applied to the bag material. Studies both in Rwanda and at Montana State University will continue with this essential oil. The importance for further research in this area for Rwanda is that the plant is readily available throughout Rwanda. The essential oil is already being produced for commercialization in the private sector there. The portion of the essential oil that we worked with is a byproduct of the production of a medicinal oil. This medicinal oil is from the most popular traditional medicines of Rwanda. The Lindbergh Award was given to F. Dunkel to continue these studies with Rwanda. Additional details have been described in greater detail in section 5.1 and 5.3 of this report.

8.6. Improvement of Ocimum canum as an on-farm stored bean protectant.

This PSTC project established that the annual mint, Ocimum canum had about 27 distinct compounds in extracts of its leaf. One of these, linalool, is a strong insecticide with fumigant action. The leaf was found to be more insecticidal if it was finely processed. This process has a patent pending and will be developed in collaboration with the private sector which produces biorational pesticides in the USA. This process will also be reintroduced into Rwanda for use on the subsistence farms that made the original suggestion to me. It will also be introduced into other areas of Rwanda for on-farm protection of stored beans and sorghum in sealed traditional containers (e.g. clay pots and gourds). The funding for this has been provided by the Lindbergh award to F. Dunkel.

Our studies also indicated that the insecticidal action of the leaf is not completely explained by the action of linalool. Several other compounds of the leaf will be studied in pure form for their insecticidal properties.

These follow-on projects have been described in greater detail in section 5.1 and 5.3 of this report.

8.7. Efficacy studies with crude pyrethrin extracts (Chrysanthemum cinerarifolium) produced within Rwanda.

Full scale studies are now underway in the laboratory in Rwanda (OPROVIA/GRENARWA II - Recherches) to test the efficacy of this product available locally. Storage insects, the four primary destructive insects used for the present PSTC test will be used. The goal of the experiments is to use the product in OPROVIA warehouses as an alternative to Actellic. Strains of three of these major Rwandan storage insects have been collected from OPROVIA warehouses and identified as resistant to Actellic (Srinaran et al. 1990).

8.8. Collaboration with private sector in development of natural products which allow slow release of volatile plant products

A continuation is planned of the initial studies described the manuscript Appendix 5. Both Rwandan plants and the Artemesia spp. Montana and Morocco are targeted for development. These studies are in collaboration with Basic BioSystems, Missoula, Montana USA.

In conclusion, there is not doubt that both the Rwandan pest management system and their private sector producing pest management material has benefited from this project. In addition, an area of research is expanding at Montana State University and at Virginia State University in use of local plants for pest management. This is a direct result of this PSTC project. The USA studies which follow are already involved with the private sector, both with small, new companies and with large scale, older companies. The benefit has also been experienced or will be by collaborating scientists in Morocco, Nigeria, and Pakistan. It has been and will be a useful contribution to both the developing and the developed world.

9.0. LITERATURE CITED

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10.0. APPENDICES .

Appendix 1. Published refereed journal article. "The efficacy of linalool, a major component of freshly-milled Ocimum canum Sims (Lamiaceae), for protection against postharvest damage by certain stored product Coleoptera." Journal of Stored Product Research. 27:213-220.

THE EFFICACY OF LINALOOL, A MAJOR COMPONENT OF FRESHLY-MILLED *OCIMUM CANUM* SIMS (LAMIACEAE), FOR PROTECTION AGAINST POSTHARVEST DAMAGE BY CERTAIN STORED PRODUCT COLEOPTERA

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Abstract—Linalool was present at 8.6 ± 0.9 mg/g in the dried leaves of *Ocimum canum* Sims, an annual mint used in Rwanda to protect against postharvest insect damage. Direct exposure of adults of *Zabrotes subfasciatus* (Bohem.) to milled, dried *O. canum* leaves resulted in 100% mortality of males and 50% mortality of females after 48 hr. Dose-response curves for linalool were completed with adult *Z. subfasciatus*, *Acanthoscelides obiectus* (Say), *Rhyzopertha dominica* (F.), and *Sitophilus oryzae* (L.) using a filter paper bioassay. The LC₅₀ values were: 428 µg/cm² for *Z. subfasciatus*; 405 µg/cm² for *A. obiectus*; 428 µg/cm² for *R. dominica*; 427 µg/cm² for *S. oryzae*. Knockdown was occasionally followed by recovery at doses less than the LC₅₀ for all species. There are significant differences in the LC₅₀ and LT₅₀ values for male and female *Z. subfasciatus*. At the lower dosages hyperactivity rarely preceded moribundity and mortality where these occurred, while at higher dosages hyperactivity occurred soon after initial exposure and preceded imminent death. A concentration increase from 250 to 750 µg/cm², representing a tripling of dosage, spanned the 10–100% response mortality for all species at 24 hr. Air-exposure of linalool-treated papers (500 µg/cm²) for up to 24 hr significantly decreased toxicity to both sexes of *Z. subfasciatus*. Quantitative analysis showed the only significant decrease in the amount of linalool to occur after 0.25 hr, and this did not fully correlate with the resulting decrease in efficacy against both sexes of *Z. subfasciatus*. The results are discussed in terms of the efficacy of using *O. canum* for the protection against loss due to insects in the traditional food storage systems of Rwanda.

INTRODUCTION

In Rwanda, some farmers store dry edible beans, *Phaseolus vulgaris* (L.), in traditional closed structures (imboho). Whole leaves of *Ocimum canum* Sims are usually added to the stored foodstuff to prevent insect damage within these structures (Dunkel *et al.*, 1986). Linalool is a major component of the essential oil of this annual mint, representing 60–90% of the total volatiles collected (Ntezurubanza, 1987).

Linalool (3,7-dimethyl-1,6-octadien-3-ol) is a common component of floral scents and is an olfactory cue in the seeking of host plants by numerous phytophagous invertebrates. It is an oxygenated monoterpene which acts as a reversible competitive inhibitor of acetylcholinesterase (Ryan and Byrne, 1988) and has been suggested as an alternative to conventional insecticides in controlling all life stages of the cat flea, *Ctenocephalides felis* (Bouche) (Hink *et al.*, 1988). The acute oral LD₅₀ for rats is 2.8 g/kg and the acute dermal LD₅₀ for rabbits is 5.6 g/kg (Opdyke, 1979). The LC₅₀ value of adult *C. felis* is 39 µg/cm² (Hink *et al.*, 1988). The LC₅₀ value for adult red flour beetles, *Tribolium castaneum* (Herbst) is 2.5×10^4 ppm (Ryan and Byrne, 1988).

At the national government warehouses of Rwanda (OPROVIA: Office National pour le Développement et la Commercialisation des Produits Vivriers et des Productions Animales) a search is underway to identify preparations (Dunkel *et al.*, 1992a, b) or procedures that will replace pirimiphos-methyl, the sole insecticide used in grain and bean storage. Pirimiphos-methyl has been used prophylactically since 1983 (Dunkel *et al.*, 1986) and populations of *Acanthoscelides obiectus*

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Say and *Sitophilus oryzae* (L.) with increased resistance to pirimiphos-methyl have been identified in OPROVIA Warehouses (Sriharen *et al.*, 1992).

This article describes the quantification of linalool from dried leaves of *O. canum* and experiments using milled dried leaves against adults of *Zabrotes subfasciatus* (Bohem.). The basic information is followed by an evaluation of the toxicity of linalool to adults of stored-product insects. The degradation and volatilization of linalool from treated filter papers is subjected to insect bioassay and quantitative chemical analysis. The use of this material in postharvest systems, on farm and in national (OPROVIA) warehouses, of Rwanda is discussed.

MATERIALS AND METHODS

Insect rearing. All four species were reared at $27 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ r.h. and a 12:12 light:dark photoperiod. *Z. subfasciatus* and *A. obtectus* were reared on a diet of dried Pinto beans (13.7% equilibrium moisture content oven dry $110 \pm 1^\circ\text{C}$). *Rhyzopertha dominica* (F.) and *S. oryzae* were reared on a diet of 96:2:2 (w/w) soft white wheat:whole wheat flour:brewers yeast (17.1% equilibrium moisture content). All insect cultures have been laboratory reared for many years except for *R. dominica*, which was collected in a Montana storage bin in 1989.

Quantification of linalool from *Ocimum canum*. Leaves of *O. canum* were harvested at the plant nursery of the Center for Ethnopharmacology (CURPHAMETRA) in Butare Prefecture, Rwanda. Leaves were oven dried (40°C) and express-shipped intact to Montana State University, U.S.A., where they were stored in a -20°C freezer prior to bioassay and chemical analysis. The leaves (1.0 g in each of four replicates) were milled in a Waring blender for 2 min and extracted immediately in 60 ml of 1:4 isopropanol:hexane (containing $40 \text{ ng}/\mu\text{l}$ decane as an internal standard) in a 125 ml Erlenmeyer flask for 24 hr. The flasks were occasionally agitated and covered to prevent photodegradation. The resulting supernatant solution was directly injected into a gas chromatograph for quantitative analysis. All solvents were purchased from the Fisher Chemical Co., Fair Lawn, N.J., U.S.A. Gas chromatography (GC) was performed on a Varian Model 3700 equipped with a flame-ionization detector containing a $50 \text{ m} \times 0.25 \text{ mm}$ i.d. HP-1 column with 0.11μ film thickness; helium (He) carrier gas velocity was $31 \text{ cm}^3/\text{s}$ (220°C). The temperature program consisted of the following: initial temperature 60°C , initial hold 8 min, temperature increase $4^\circ\text{C}/\text{min}$, final temperature 260°C .

The linalool peak was identified using narrow-bore capillary GC-Mass Spectrometer (MS). The GC was a Varian Model 3700 equipped with a flame ionization detector and a $30.0 \text{ m} \times 0.25 \text{ mm}$ i.d. DB5 column with 0.25μ film thickness: column conditions used were: He carrier gas velocity $-30 \text{ cm}^3/\text{s}$ (220°C); temperature programming—initial temperature 50°C , initial hold 4.0 min, temperature increase 5.0°C , final temperature 280°C , final hold 10 min; injector temperature 260°C ; detector temperature 290°C . The electron impact mass spectra were obtained on a VG Analytical model VG 70 EHF MS operating at 70 eV with a source temperature of 200°C .

Leaf exposure bioassay. Ten adult *Z. subfasciatus* (5 male and 5 female; 0–3 days post-adult eclosion) were added to 5.5 cm dia glass Petri dishes in which 1.0 g of freshly milled, dried leaves of *O. canum* had been distributed evenly throughout the dish. Ten replicates of the treatment and ten replicates of the control consisting of an empty Petri dish were conducted. The bioassay conditions were $28 \pm 1^\circ\text{C}$ at $65 \pm 5\%$ r.h. with a 12:12 light:dark photoperiod. Bioassays were evaluated by viewing mortality at 24 and 48 hr. Mortality was evaluated if the insect was immobile and did not react to three probings with a blunt dissecting probe.

Linalool dose-response bioassays. A Whatman No. 1 filter paper (9 cm dia) was placed in a glass Corning Petri dish (10 cm dia). An aliquot of the appropriate dilution of R,S linalool (Sigma Chemical Co., St Louis, Mo, U.S.A.) was delivered to the filter paper in 1.0 ml absolute ethanol (Quantum Chemical Co., Tuscola, Ill.). The ethanol was allowed to evaporate for 20 min prior to the addition of the insects. Mortality was evaluated as above. Moribundity was assessed by selecting those insects that were on their backs and ambulating very weakly. These insects were subsequently righted and viewed carefully. Those that immediately fell onto their backs again as a result of intoxication were classified as moribund. At higher dosages all moribund insects subsequently died. Recovery occurred occasionally at lower dosages. The bioassay conditions for this and the following bioassay were $27 \pm 2^\circ\text{C}$ at $65 \pm 8\%$ r.h. with a 12:12 light:dark photoperiod.

Z. subfasciatus were sexed with 5 males and 5 females per replicate; 10 adults of unknown sex were used for the other species. In a separate experiment, dose-response curves were also prepared for male and female *Z. subfasciatus* using 5 replicates of 10 individuals for each dosage. To avoid disturbance of volatile chemical equilibria and disturbance of the insects in the covered dishes counts of mortality and moribundity were conducted at 24 hr, but knockdown was recorded at 0.25, 6, and 18 hr. Dosages of 0, 10, 100, 1000 and 10,000 $\mu\text{g}/\text{cm}^2$ were used in preliminary experiments and dosages of 0, 250, 300, 350, 400, 450, 500 and 750 $\mu\text{g}/\text{cm}^2$ were used in later tests.

Bioassay of linalool with increasing duration of air exposure. The protocol was similar to that for the dose-response bioassays. The ethanol in an aliquot delivering 500 $\mu\text{g}/\text{cm}^2$ on the filter paper was allowed to evaporate for 20 min and ten replicates of *Z. subfasciatus* (5 male, 5 female; 0-1 days post-adult eclosion) were added immediately. Other replicates were covered though they contained no insects. Ten *Z. subfasciatus* (as above) were added at 0.25, 6, 18 and 24 hr post-ethanol evaporation. Mortality/moribundity were determined as above and evaluated 24 hr after introduction of the insects into each trial. Ten replicates of an ethanol control were conducted simultaneously for each trial. The bioassay conditions were $28 \pm 1^\circ\text{C}$ at $65 \pm 5\%$ r.h. with a 12:12 light:dark photoperiod.

Quantitative chemical analysis of linalool-treated substrates with increasing duration of air exposure. Four additional replicates in the air exposure procedure (above) were used. At the time of insect introduction each filter paper in these additional replicates was handled with forceps, cut into ca 0.5 cm^2 pieces and transferred into 125 ml of 1:1 isopropanol:hexane (containing 40 $\text{ng}/\mu\text{l}$ decane as an internal standard) in a 250 ml Erlenmeyer flask. The flasks were covered to prevent photodegradation and occasionally agitated during a 24 hr interval. The resulting solution was directly injected into a gas chromatograph for quantitative analysis and GC-MS for identification (as above).

Statistical analysis. The linalool dose-response bioassay data were subject to probit analysis for determination of LC_{50} and LT_{50} values (Finney, 1971). Abbott's formula (Abbott, 1925) was used to adjust for control mortality when necessary. Since the dominant response of moribund insects was to die after prolonged exposure for more than 24 hr, moribundity and mortality were pooled for analysis in LC_{50} determinations. LT_{50} values were calculated for 500 $\mu\text{g}/\text{cm}^2$ for all species using knockdown data for 0.25, 6, and 18 hr and pooled moribundity and mortality data for 24 hr. Significant recovery never occurred at 500 $\mu\text{g}/\text{cm}^2$ during these trials. The percent mortality data were normalized by arcsine transformation and were subjected to analysis of variance (Sokal and Rohlf, 1981). If significant interaction occurred between species (or sex) and dosage, then LSD multiple comparisons were conducted with $\alpha = 0.05$ (Sokal and Rohlf, 1981).

RESULTS

Extraction and quantitative gas chromatographic analysis indicated that linalool was present in milled, air-dried leaves of *O. canum* at 8.6 ± 0.9 mg/g. This represented $64 \pm 4\%$ of the total extract from the leaves. The leaf exposure bioassay indicated 100% mortality of male *Z. subfasciatus* at 24 hr and only 50% mortality of the females at 48 hr (Table 1).

Synthetic R,S-linalool caused two distinct response patterns in the test insects (Table 2). For higher doses; i.e. greater than the LC_{50} value, the insects would first become very hyperactive, with concomitant withering of antennae. Elytra and wings would become distorted and twisted due to rapid "fanning" and due to the inability of the insects to remain upright during these bursts of activity. This pattern would continue to increase in intensity until the insect could not successfully

Table 1. Acute mean mortality (\pm SD) of females of adult *Z. subfasciatus* (Bohem.) exposed to 1.0 g of milled, dried leaves of *O. canum* Sims

	% Mortality (hr)*			
	24		48	
Preparation	♂	♀	♂	♀
<i>O. canum</i>	100	36 ± 12	100	50 ± 14
Control	0	0	0	0

*Ten replicates of 5♂ or 5♀ at $28 \pm 1^\circ\text{C}$, $65 \pm 5\%$ r.h. and 12:12 L:D.

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Table 2. Percentage pooled mortality and moribundity (\pm SD) induced by linalool at different concentrations for four species of stored products Coleoptera after 24 hr

Insect species*	Linalool ($\mu\text{g}/\text{cm}^2$)						
	250	300	350	400	450	500	750
	Mean % moribundity and mortality†						
<i>Z. subfasciatus</i> (0-1)	0 ^a	2 \pm 4 ^a	4 \pm 8 ^a	24 \pm 22 ^a	62 \pm 31 ^a	98 \pm 6 ^a	100 ^a
<i>A. obtectus</i> (0-1)	1 \pm 3 ^{ab}	6 \pm 10 ^{ab}	25 \pm 12 ^b	40 \pm 15 ^a	66 \pm 28 ^a	99 \pm 3 ^a	100 ^a
<i>R. dominica</i> (0-6)	5 \pm 7 ^{ab}	14 \pm 13 ^b	23 \pm 15 ^b	34 \pm 20 ^{ab}	53 \pm 31 ^a	84 \pm 31 ^a	100 ^a
<i>S. oryzae</i> (0-)	7 \pm 13 ^b	8 \pm 13 ^{ab}	18 \pm 16 ^b	26 \pm 24 ^{ab}	53 \pm 42 ^a	95 \pm 13 ^a	100 ^a

*Values in parentheses denote the number of days post adult emergence (range). 10 insects per replicate; 10 replicates per dose. Adults were not sexed except for *Z. subfasciatus* 5♂ and 5♀. Bioassays were conducted at 27 \pm 2°C; 65 \pm 8% r.h. and 12:12 L:D.

†Means associated with the same letter in a column are not significantly different (LSD test ($P < 0.05$) following F test ($P < 0.05$)).

right itself. It would continue to attempt to weakly ambulate upon its back. This activity would gradually decrease and eventually cease entirely. Inactivity would be an indicator of profound moribundity and, presumably, subsequent death, all occurring in < 6 hr. For lower doses; i.e. less than the LC_{50} value, there was seldom any observable hyperactivity. Susceptible individuals seemed to be unable to right themselves after a longer duration of exposure. These individuals would continue to ambulate weakly for a longer period of time than the more acute response of individuals exposed to higher dosages. Profound moribundity and, presumably, death did occur, but rarely within 24 hr. Certain individuals that were characteristically moribund, particularly at lower dosages, would recover and appear normal during later observations.

Initial experiments using exponential increases in dosage from 10 to 10,000 $\mu\text{g}/\text{cm}^2$ resulted in a complete span of concentrations resulting in any partial mortality in the test population. This occurred between 100–1000 $\mu\text{g}/\text{cm}^2$, so subsequent experiments encompassed this range.

All χ^2 tests showed a reasonable linear relationship between probit-transformed mortalities and \log_{10} -transformed concentrations and times, except for *S. oryzae*, with increasing concentration (Table 3) and *R. dominica* with increasing duration of exposure to linalool at 500 $\mu\text{g}/\text{cm}^2$ (Table 4). The heterogeneity in the responses of *S. oryzae* are due to a linear additivity (rather than an exponential response) occurring at doses less than 400 $\mu\text{g}/\text{cm}^2$ (Table 2). Probit analysis of the mortality data for this *S. oryzae* trial using only the data for doses ≥ 400 $\mu\text{g}/\text{cm}^2$ (i.e. deleting the data which displayed additivity) gives a probit line with a χ^2 value of 6.52 with an LC_{50} value of 436 (428, 444) $\mu\text{g}/\text{cm}^2$ which was not significantly heterogeneous at $\alpha = 0.10$ (d.f. = 3). This is comparable to the originally determined LC_{50} of 427 $\mu\text{g}/\text{cm}^2$ given in Table 3. The heterogeneity of *R. dominica* with increasing duration of exposure is due to mortality/moribundity increasing from 3 to 93% between 0.25 and 6 hr with 3% recovery occurring in the next 12 hr.

Mortality/moribundity data for each species with increasing dosage of linalool at 24 hr is summarized in Table 2. *R. dominica* is more susceptible to linalool at 300 $\mu\text{g}/\text{cm}^2$ than *Z. subfasciatus*, and *A. obtectus* is more susceptible to linalool at 350 $\mu\text{g}/\text{cm}^2$ than *Z. subfasciatus*.

The linalool dose-response bioassays indicated that the LC_{50} values for all four species were similar (Table 3). However, the slopes for the species indicate that the dosage range for mortality in the bruchid populations is more narrow than that of the populations of *R. dominica* and *S. oryzae* (Table 3). The LT_{50} values for these species at 500 $\mu\text{g}/\text{cm}^2$ are quite similar, as well (Table 4). *R. dominica* is probably more susceptible than *A. obtectus*, but the regression for probit line for *R. dominica* is not homogeneous enough to be certain of the validity of the LT_{50} value (Table 4).

Table 3. Probit analyses of pooled mortality and moribundity data obtained from bioassays using increasing concentrations of linalool against four species of stored product Coleoptera after 24 hr

Insect species*	Intercept	Slope	χ^2 test†	LC_{50} ($\mu\text{g}/\text{cm}^2$) with 95% fiducial limits (range)
<i>Z. subfasciatus</i> (0-1)	-39	17	10.28	428 (419, 436)
<i>A. obtectus</i> (0-1)	-26	12	11.52	405 (395, 415)
<i>R. dominica</i> (0-6)	-20	10	11.11	428 (416, 441)
<i>S. oryzae</i> (0,6)	-17	8	40.26	427 (413, 441)

*Values in parentheses denote the number of days post adult emergence (range). 10 insects per replicate; 10 replicates per dose. Adults were not sexed except for *Z. subfasciatus* 5♂ and 5♀. Bioassays were conducted at 27 \pm 2°C; 65 \pm 8% r.h. and 12:12 L:D.

†Goodness of fit test for probit line: values showed no significant heterogeneity at the level of $\alpha = 0.05$ (d.f. = 6), except for *S. oryzae* which was significantly heterogeneous at $\alpha = 0.001$ (d.f. = 6).

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Table 4. Probit analyses of knockdown and mortality + moribundity data obtained from bioassays using linalool at 500 $\mu\text{g}/\text{cm}^2$ against four species of stored products Coleoptera at 0.25, 6, 18 and 24 hr \ddagger

Insect species†	Intercept	Slope	χ^2 test‡	LT ₅₀ (hr) with 95% fiducial limits (range)§
<i>Z. subfasciatus</i> (0-1)	1.1	1.9	3.60**	2.8 (2.2, 3.5)
<i>A. obiectus</i> (0-1)	0.6	2.0	0.72*	3.9 (3.2, 4.8)
<i>R. dominica</i> (0-6)	2.0	1.5	17.14	2.0 (1.7, 2.9)
<i>S. oryzae</i> (0.6)	1.8	1.5	6.27***	3.3 (2.6, 4.2)

†Values in parentheses denote the number of days post adult emergence (range). 10 insects per replicate; 10 replicates per dose. Adults were not sexed except for *Z. subfasciatus* 5♂ and 5♀. Bioassays were conducted at 27 ± 2°C; 65 ± 8% r.h. and 12:12 L:D.

‡Goodness of fit test for probit line—values showed no significant heterogeneity at the level of: * $\alpha = 0.50$ (d.f. = 3); ** $\alpha = 0.10$ (d.f. = 3); *** $\alpha = 0.05$; except for *R. dominica* which was significantly heterogeneous at $\alpha = 0.001$ (d.f. = 3).

§Knockdown data for 0.25, 6, and 18 hr. Mortality + moribundity data for 24 hr.

Table 5. Relationship between knockdown (±SD) and mortality (±SD) from bioassays using linalool at 400 $\mu\text{g}/\text{cm}^2$ against four species of stored products Coleoptera at 0.25, 6, 18 and 24 hr

Insect species*	Mean % knockdown (% mortality) at time (hr)†				
	0.25	6.0	18.0	24.0	(24.0)
<i>Z. subfasciatus</i> (0-1)	9 ± 16 ^b	5 ± 7 ^a	61 ± 22 ^b	24 ± 22 ^a	4 ± 7 ^a
<i>A. obiectus</i> (0-1)	0 ^a	12 ± 9 ^{ab}	28 ± 14 ^a	40 ± 15 ^b	6 ± 10 ^{ab}
<i>R. dominica</i> (0-6)	1 ± 3 ^a	53 ± 22 ^c	33 ± 20 ^a	34 ± 20 ^{ab}	31 ± 20 ^c
<i>S. oryzae</i> (0.6)	0 ^a	17 ± 14 ^b	33 ± 37 ^a	26 ± 24 ^{bb}	20 ± 26 ^{bc}

*Values in parentheses denote the number of days post adult emergence (range). 10 insects per replicate; 10 replicates per dose. Adults were not sexed except for *Z. subfasciatus* 5♂ and 5♀. Bioassays were conducted at 27 ± 2°C; 65 ± 8% r.h. and 12:12 L:D.

†Means associated with the same letter in a column are not significantly different [LSD test ($P < 0.05$) following F test ($P < 0.0001$)].

The relationship between knockdown and mortality for the four species at 400 $\mu\text{g}/\text{cm}^2$ shows that *R. dominica* is more susceptible than either bruchid species at 6 hr and that 20% recovery occurs by 18 hr (Table 5). *S. oryzae* is more susceptible than *Z. subfasciatus* at 6 hr and shows a 7% recovery between 18 and 24 hr. *Z. subfasciatus* is more susceptible than any other species at 18 hr but shows a 37% recovery at 24 hr. These knockdown data are quite distinct from the actual mortality data, which indicate that the bruchid populations are less susceptible than the populations of *R. dominica* and *S. oryzae*, although *S. oryzae* is not significantly more susceptible than *A. obiectus*.

The sexual dimorphism of *Z. subfasciatus* indicated a differential response of male and female *Z. subfasciatus* in the initial dose response trial (Table 6). Dose-responses of male and female *Z. subfasciatus* indicated different LC₅₀ values. The LC₅₀ value for females was 453 and 405 $\mu\text{g}/\text{cm}^2$ for males (Table 6). This difference in susceptibility was also evident in the LT₅₀ values at 500 $\mu\text{g}/\text{cm}^2$ with the value for females being twice that for the males (Table 6). At 400 $\mu\text{g}/\text{cm}^2$ male *Z. subfasciatus* also displayed 18% recovery between 6 and 24 hr whereas females showed no

Table 6. Probit analyses of pooled mortality + moribundity data obtained using increasing dosages of linalool against male and female *Z. subfasciatus* (Bohem.) after 24 hr and of knockdown and mortality + moribundity data obtained using linalool at 500 $\mu\text{g}/\text{cm}^2$ at 0.25, 6, 18 and 24 hr

	Sex†	Intercept	Slope	χ^2 test‡	LC ₅₀ ($\mu\text{g}/\text{cm}^2$) with 95% fiducial limits (range)§
					LT ₅₀ (hr) with 95% fiducial limits (range)¶
Dose response	♂ (0-2)	-22	10	16.04****	405 (389, 421)
	♀ (0-2)	-19	9	13.37***	453 (433, 475)
Temporal response for 500 $\mu\text{g}/\text{cm}^2$	♂ (0-2)	1.9	1.6	5.22**	2.3 (1.6, 3.3)
	♀ (0-2)	1.8	1.4	0.84*	4.7 (3.3, 6.6)

†Values in parentheses denote the number of days post adult emergence (range). 10 insects per replicate; 5 replicates per dose. Bioassays were conducted at 27 ± 1°C; 65 ± 5% r.h. and 12:12 L:D.

‡Goodness of fit test for probit line—values showed no significant heterogeneity at the level of: **** $\alpha = 0.025$ (d.f. = 6); *** $\alpha = 0.01$; (d.f. = 6); ** $\alpha = 0.50$ (d.f. = 3); * $\alpha = 0.10$ (d.f. = 3).

§Pooled mortality + moribundity data for 24 hr.

¶Knockdown data for 0.25, 6 and 18 hr. Mortality + moribundity data for 24 hr.

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Table 7. Relationship between knockdown (\pm SD) and mortality (\pm SD) from bioassays using linalool at $400 \mu\text{g}/\text{cm}^2$ against male and female *Z. subfasciatus* (Bohem.) at 0.25, 6, 18 and 24 hr

Sex*	Mean % knockdown (% mortality) at time (hr)†				
	0.25	6.0	18.0	24.0	(24.0)
♂ (0-2)	8 \pm 8	58 \pm 22	48 \pm 15	40 \pm 10	12 \pm 8
♀ (0-2)	2 \pm 5	16 \pm 11	22 \pm 8	22 \pm 13	4 \pm 6

*Values in parentheses denote the number of days post adult emergence (range). 10 insects per replicate; 5 replicates per dose. Bioassays were conducted at $27 \pm 1^\circ\text{C}$; $65 \pm 5\%$ r.h. and 12:12 L:D.

†Comparisons between sex were inappropriate ($F = 2.09$; $P = 0.1212$).

recovery at this dosage (Table 7). The knockdown of the male population was 58% at 6 hr and 48% at 18 hr, while for the female population the knockdown was 16 and 22% at 18 and 24 hr, respectively (Table 7).

The difference in susceptibility of male and female *Z. subfasciatus* is also evident in trials where treated substrates were aerated in covered Petri dishes for increasing periods of time prior to the assay (Table 8). Males drop from 96% susceptibility at 6 hr to 12-24% susceptibility subsequently, whereas females were 76% susceptible initially, but were only 34% susceptible 0.25 hr later (Table 8).

Quantitative chemical analysis of the treated substrates in additional replicates of the above experiment indicated that the decrease in susceptibility of female *Z. subfasciatus* may be correlated to a $15 \mu\text{g}/\text{cm}^2$ drop in concentration (Table 9), but no other variability in the concentration of linalool or other detectable chemical components in the system is evident (Table 9).

DISCUSSION

The large amount of linalool in the dried leaves of *O. canum* confirms the earlier reported percentage found in the essential oil of this species (Ntezurubanza, 1987). The milled leaves of this species are quite toxic to *Z. subfasciatus* males, however, the amount of linalool present does not correlate with the amount required for activity in filter paper dose-response trials (Table 2); the amount of linalool present in a Petri dish at the $450 \mu\text{g}/\text{cm}^2$ dosage is 28.6 mg as compared to 8.6 mg in the milled leaf trials. The Petri dishes used in the milled leaf trials are smaller than those in the synthetic linalool trials, but calculations of the two-dimensional surface area of the dish gives a concentration of $362 \mu\text{g}/\text{cm}^2$. This is still significantly lower than required, particularly since the

Table 8. Acute mean mortality (\pm SD) of *Z. subfasciatus* (Bohem.) exposed to linalool-treated ($500 \mu\text{g}/\text{cm}^2$) papers with increasing duration of air exposure prior to bioassay

Treatment†	Sex	Pre-exposure interval (hr)*				
		0	0.25	6	18	24
Linalool	♂	100	98 \pm 4	96 \pm 8	12 \pm 14	24 \pm 24
	♀	76 \pm 38	34 \pm 34	40 \pm 22	6 \pm 10	2 \pm 6
Control	♂	0	0	0	0	0
	♀	0	0	0	0	0

* $28 \pm 1^\circ\text{C}$; $65 \pm 5\%$ r.h.; 12:12 light:dark photoperiod.

†10 replicates; 5♂ and 5♀ each; mortality and moribundity data after 24 hr of exposure; ethanol allowed to evaporate 20 min in all trials including controls.

Table 9. Amount ($\mu\text{g}/\text{cm}^2$) (\pm SD) of R, S-linalool* and degradation products extracted from filter paper with increasing duration of air exposure†

Compound	Amount* (μg compound/ cm^2) at time following linalool application (hr)†					
	0‡	0§	0.25¶	6¶	18¶	24¶
β -Myrcene	0.6 \pm 0.1	1.1 \pm 0.2	1.2 \pm 0.2	1.3 \pm 0.1	1.3 \pm 0.1	1.2 \pm 0.4
d-Limonene	1.1 \pm 0.1	1.6 \pm 0.4	1.7 \pm 0.2	1.4 \pm 0.3	1.5 \pm 0.3	1.7 \pm 0.4
R, S-Linalool	483.0 \pm 11.7	402.5 \pm 19.5	387.4 \pm 25.4	385.3 \pm 18.1	385.1 \pm 22.6	388.5 \pm 20.1
3,7-Dimethyl-1-octen-3-ol	3.0 \pm 1.4	2.5 \pm 0.2	2.4 \pm 1.5	2.8 \pm 2.1	2.7 \pm 1.3	1.3 \pm 0.6

*Linalool applied at ca $500 \mu\text{g}/\text{cm}^2$; 4 replicates. Extractions concomitant with introduction of insects for bioassay of same protocol (Table 9).

†Air exposure at $28 \pm 1^\circ\text{C}$; $55 \pm 5\%$ r.h.; 12:12 light:dark.

‡Aliquot delivered directly into extraction flask.

§Aliquot applied to filter paper and ethanol evaporated (20 min).

¶After ethanol evaporation.

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depth of the milled leaf material was approx. 3 mm in this experiment giving a three-dimensional surface area that is much greater than the LC_{50} value surface area. These differences may be due to two methodological differences. The first is that the insects in the milled leaf trials are rapidly coated with sticky leaf particles (due to their own movement) that adhere to them. The insects, therefore, receive a topical chemical and physical treatment whereas the filter paper trials provide little direct topical treatment initially. After the insects become intoxicated and fall upon their dorsal surfaces frequently they may also become coated with residual linalool from the surface of the filter paper. The second problem is that the active surface area of the filter paper ($1.09 \text{ m}^2/\text{g} = \text{ca } 0.6 \text{ m}^2$ per 9.0 cm dia Whatman No. 1) is much greater than its two dimensional area ($\text{ca } 0.0063 \text{ m}^2$), so the actual amount per cm^2 is approx. 100 times less than that reported.

The narrow dosage range required to conduct probit analyses and the resultant high numerical values for the slopes suggest linalool is primarily fumigative in its activity in these trials. This is further supported by the rapid withering of antennae after exposure. The loss of activity in less than 24 hr seems to confirm this as well. The fumigative activity may also explain why *R. dominica* and *S. oryzae*, smaller insects, are more susceptible.

The LC_{50} values reported here differ greatly from the $39 \mu\text{g}/\text{cm}^2$ reported for *Ctenocephalides felis* adults (Hink *et al.*, 1988). However, this cat flea bioassay involved saturated papers treated with linalool in a water/Tween 80 solution, which was not evaporated, thus allowing greater potential contact (and subsequent topical coating) than the bioassay method used in the present study. The LC_{50} values do compare very favorably with the $2.5 \times 10^4 \text{ ppm}$ ($526 \mu\text{g}/\text{cm}^2$) reported for an insecticide-susceptible strain of *Tribolium castaneum* (Ryan and Byrne, 1988). This bioassay was very similar to the one used in the present study, except that the solvent was acetone and was evaporated in 1 min.

The greater susceptibility of male as compared to female *Z. subfasciatus* is also probably a function of the larger size and lower activity of the females of this species. This lower susceptibility is likely the source of differences in the responses of the sexes to the milled *O. canum* leaves (Table 1) and in the trial with increasing duration of air exposure of linalool-treated filter papers (Table 8).

The data indicate a surprising degree of similarity in LC_{50} values for the four selected insect species. However, the slopes of the probit lines vary, the lesser slopes indicating that the responses of *R. dominica* and *S. oryzae* are more heterogeneous. This may be due to greater genetic variability in the susceptibility of the populations of these species or merely to the greater age range of individuals used for these two species (0–6 days). The *Z. subfasciatus* and *A. obtectus* were 0–1 day old.

The recovery of the insects at lower dosages is probably a function of sub-lethal fumigation by linalool, which was found to be a reversible inhibitor of acetylcholinesterase for an insecticide-susceptible strain of *T. castaneum* (Ryan and Byrne, 1988).

The quantitative chemical analysis indicates that linalool appears to be "bound" to the filter papers in these trials. This could be a function of interactions between the hydroxyl group and polar sites on the filter paper. Such interactions only have to be of greater energy than the forces of volatilization and gaseous dissolution to be maintained. This is a particularly compelling possibility because it would mean that linalool could have a much lower LC_{50} than the apparatus used here suggests. Theoretically, the mortality we report would therefore be a function of the initial volatile component and the topical component contacted on the surface of the filter paper only. Most unprocessed foodstuffs (i.e. grain or beans) do not have polar surfaces so the dosage required for protection against postharvest damage may be considerably less. We are currently investigating this possibility, with full awareness that the hydrophobic component of the linalool molecule may interact significantly with surface waxes on the foodstuffs.

The efficacy of *O. canum* in providing protection against insect damage in the Rwandan postharvest system may be partly due to the high concentration of linalool in its leaves. However, the efficacy of linalool as a component of *O. canum*, or when purchased as a synthetic preparation appears limited to a relatively short time frame. This suggests that the freshly milled leaves of *O. canum* provide the greatest protection against insect damage when initially added to the stored foodstuff and longer term control may be less reliable. However, control may be achieved by behavioral effects such as repellency or oviposition deterrence which require lower dosages of chemicals. The plant preparation may also contain other compounds that provide a surrounding

chemical matrix which may decrease volatility and therefore enhance the potential mortality induced by topical encounter with linalool. We are currently investigating these phenomena.

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Appendix 2. Published refereed journal article. "The stored grain ecosystem: a global perspective." Journal of Stored Product Research. 28:73-87.

THE STORED GRAIN ECOSYSTEM: A GLOBAL PERSPECTIVE*

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Abstract—Ecosystem principles and processes, such as biological succession, population dynamics, niche concept, limits to growth, and food webs apply to grain storage systems, as well as to forests, oceans, lakes, prairies, and preharvest agroecosystems. In 1989, Odum classified the food-storage system as a human-subsidized, solar-powered ecosystem, one of the four main ecosystems of the world. A grain storage ecosystem is a complex system that can be described in several scales. One may consider the storage structure and its contents as an entire system, or one may consider a group of storage structures as an archipelago of ecological islands linked by transportation and commerce. Government policies and consumer demands are part of the environment that must be considered in effective economic management of the stored grain ecosystem. The grain storage system is part of the postharvest ecosystem, a larger system which extends from harvest to consumption. An understanding of the interrelationship of biological and physical factors, and selection pressures in the system is essential to making sound decisions about the long term directions of postharvest research, as well as the daily management of grain and other food commodities in the postharvest system. In this paper, ecosystem principles are used to draw together progress which has been made in understanding: (1) the physical elements, defined in part by the structure, (2) the biological elements, including human society, and (3) the interrelationships of physical and biological elements in postharvest systems throughout the world. Suggestions given for future directions in ecosystem-based research on the postharvest grain system are drawn from systems in several areas of the world and include examples of reverse technology.

INTRODUCTION

In 163 B.C., in China, Emperor Wen issued this edict: "Why is the food of people so scarce? . . . Where does the blame lie? . . . I have been unable to attain a proper balance between important and unimportant affairs. Let this matter be debated. . . . Let all exhaust their efforts and ponder deeply whether there is some way to aid the people" (World Bank, 1986). Now, at the close of the 20th century, we are less concerned than Emperor Wen about actual food scarcity as we are about the conservation of energy resources used to produce and protect food and the timely distribution of the food that has already been produced. We are debating the most efficient ways to make available, at the appropriate time and place, more of what has already been produced for the people of the world. I propose that better long-term management of the stored grain ecosystem can be achieved by applying the same principles that have been used to study and manage other ecosystems.

In this paper, ecosystem principles are used to draw together progress which has been made in understanding: (1) the physical elements, defined in part by the structure, (2) the biological elements, including human society, and (3) the interrelationships of physical and biological elements in postharvest systems throughout the world. Suggestions given for future directions in ecosystem-based research on the postharvest grain system are drawn from systems in several areas of the world and include examples of reverse technology.

An ecosystem is an arbitrarily defined combination of interacting biotic communities which in turn interact with their abiotic environment. Odum (1989) divided all the earth's ecosystems into four classes based on their source and level of energy use. These basic classes of ecosystems that differ in approximate average annual energy flows in Joules per m² consumed annually are: unsubsidized natural solar-powered (8400 kJ/m²); naturally subsidized solar-powered (84,000 kJ/m²); human-subsidized solar-powered (84,000 kJ/m²); fuel-powered urban-industrial (8,400,000 kJ/m²). Stored grain ecosystems are human-subsidized solar-powered ecosystems that are composed of dormant autotrophs (i.e. seeds) that serve both as an energy source and as a habitat for many heterotrophic species of fungi, bacteria, insects and mites.

*Based on a paper presented at a symposium on "Management of Postharvest Ecosystems: Current and Future Trends" held at Winnipeg, Canada, in November 1990, at the annual meeting of the Entomological Society of Manitoba.

There are a diversity of stored grain/stored product ecosystems. Some postharvest protection systems address the issue of energy conservation and conservation of petroleum resources and others do not. Some systems are simply grain and beans stored in the home in baskets, boxes, gourds, and other small containers such as one finds in Rwanda (Dunkel *et al.*, 1986), subsaharan Africa, Poland, India (Hyde *et al.*, 1973), and in Central and South America. Other systems are bags, either on pallets under plastic sheeting to create a controlled atmosphere, such as in warehouses in China (Dunkel, 1982), or bags on pallets without plastic, such as those in warehouses in India and subsaharan Africa (Dunkel *et al.*, 1986). Many storage systems consist of many kinds of bins, located in the house or outside, owned by the farmer, a cooperative group of farmers, by the government, or by a grain handling company. These systems generally require energy or petroleum-based input, such as residual or fumigant pesticides, and drying and aeration equipment.

Underground storage systems are presently used in most countries except those of Europe, the U.S.A. and Canada (Dunkel, 1985). Many of these systems circumvent the use of petroleum-based chemicals and fans requiring electrical energy input. There are many traditional, on-farm types of underground food storage such as the North African "matmora" (Bartali, 1986), the patra in India (Gilman and Boxall, 1974), and pits in Madagascar and other countries in Southern Africa (Dunkel, 1985). There are also several types of ancient and modern large scale underground warehouses such as the ancient Chinese national warehouse, the modern Chinese spherical bins (Sterling *et al.*, 1983), the plastic underground bins in Brazil (Sartori, 1987), the 7500 metric ton concrete structures of Argentina (Munro, 1966; Dunkel, 1985). The limestone cavern storage warehouses under Kansas City, Mo. in the U.S.A. (Sterling *et al.*, 1983; Dunkel, 1985; Stauffer, 1977), the plastic tubes in a pit design for North American farms (Dunkel *et al.*, 1988), and the box-shaped design with sand and plastic which is successful in wet, termite infested soil in East Africa (Hanegreefs *et al.*, 1986) were recently developed by Europeans and North Americans of European derivation.

Earth-bermed structures use the earth differently than do the completely underground structures. Bermed structures use the earth primarily as a support, whereas underground structures use the earth for support, as well as for the insulative effect. Although earth-bermed structures are designed to be airtight, condensation of water inside the structure is a more important consideration than in the completely underground structure. Examples of earth bermed storage systems are the 1450 metric ton capacity conical silos in Kenya (Hyde *et al.*, 1973), the very large Australian earth bermed structures (Champ and McCabe, 1984; Woolcock and Amos, 1977; Dunkel, 1985), and the ferro-cement silos designed for Africa (Smith and Boon-Long, 1970; Dunkel, 1985).

Above ground elevators on large grain farms, country elevators, and terminal elevators collectively hold billions of metric tons, but for relatively short periods of time. Refrigerated storages, those in a residence (refrigerators) and large-scale, commercial units are also storage systems. Temporary facilities such as river barges, ocean transport vessels, railroad cars, or piles of grain on the ground are other grain storage systems.

INTERACTION OF STORED GRAIN ECOSYSTEMS WITH OTHER ECOSYSTEMS

Problems in a system are usually attacked by using a model of that system. If the model in one's mind is restricted, or incomplete, the solutions one derives for the problem are also often restricted. Solutions that are developed to problems in a stored grain ecosystem may be different if the interaction of stored grain ecosystems with the preharvest agroecosystem is considered, rather than just the stored grain ecosystem in isolation. The following are examples of how necessary it is to understand this interaction between the stored grain ecosystem and other ecosystems.

In South China, in early March, farmers experience problems with their triple crop system. A crop of wheat is planted between two crops of rice each year. In early March rice seedlings need to be transplanted, so farmers need to harvest the wheat crop because it grows in the same fields that need to be flooded to receive the rice seedlings (Dunkel *et al.*, 1982b, 1985). Ideally, the flooded field is left to rest for at least 1 week before the rice seedlings are transplanted into the field. This flooding insures that the Pyralid, *Chilo plejadellos* Zincken, a serious pest, that may have survived

in the wheat, is killed before the new rice crop is planted. There are two temptations facing the farmer who has rice ready to transplant. One is to omit the resting period of the flooded field after the wheat harvest. The other is to harvest the wheat before the grain is sufficiently field dried. If the farmer succumbs to the temptation of early harvest, there is the risk of not having enough sun for drying the grain after the wheat harvest. This may increase loss due to fungal development in the postharvest grain ecosystem. Good preparation of the wheat for storage (that is sufficient field maturation and drying), on the other hand, may greatly influence loss due to *Chilo plejadellus* in the next rice crop.

Another example of the interaction of the preharvest agroecosystem and the storage ecosystem occurs in Midwestern (Ill.) and Southeastern (Ga) U.S.A. In these regions, *Aspergillus flavus* Link sometimes becomes a storage problem and reduces kernel weight in corn and peanuts. Sometimes, because of mycotoxin (aflatoxin) production by *Aspergillus flavus*, an entire harvested corn crop is condemned and has to be disposed of as a toxic waste. The inoculation of a new crop with this fungus is accomplished by the Nitidulid *Carpophilus hemipterus* (L.), in the field the following year (Lillihøj *et al.*, 1978; Wicklow *et al.*, 1988).

In Rwanda, and most bean producing areas, the bruchid, *Acanthoscelides obtectus* (Say) moves from its sylvatic reservoir, a natural prairie or savannah ecosystem, to the bean field, a preharvest agroecosystem, and then to the stored product system. In the bean field, the insect lays its eggs on the bean pod and the first instar larva penetrates the pod and bean coat to take up residence in a bean (Lamb and Dunkel, 1987). The bruchid is transferred to the storage ecosystem in beans where its progeny will infest other beans. A similar story can be told for most other insects which are able to survive in the storage ecosystem; suggesting that most "storage" insects also occur in other ecosystems.

In the U.S.A., the natural habitat of *Rhyzopertha dominica* (F.) is probably the forest ecosystem. In the Southern U.S.A., *Rhyzopertha dominica* can be obtained in traps baited with its aggregation pheromone (Cogburn *et al.*, 1984) miles from storages or other agroecosystems (R. Cogburn, 1991, pers. commun.). This species has been found in pastures, in soybean fields, as well as in standing grain. The borer enters the preharvest grain system, then is transported within the kernel of the newly harvested grain to the storage system. This movement from the natural habitat through two ecosystems may also occur in the northern U.S.A., and in Canada.

Another example of the interaction of non-storage ecosystems with stored grain ecosystems is the movement of *Cynaesus angustus* LeConte. This beetle has recently (in 1900s) entered the stored grain ecosystem and is moving quickly north and east in North America (Dunkel *et al.*, 1982a). The movement of this insect from desert to storage to urban ecosystems has been documented within the past 75 yr. With a center of origin in the Southwest U.S.A., *Cynaesus angustus* is associated primarily with the agave, *Agave parryi* Engelm., in desert ecosystems. *Cynaesus angustus* is a strong flyer and probably orients by olfactory cues (Kao *et al.*, 1984) to grain bins, flour mills, or to field stored residue from the cotton ginning process (Morrison and Dunkel, 1983). When encountering stored products such as upholstered furniture in transit, this beetle has also been able to cause significant feeding damage (Dunkel, F., unpublished data).

ECOLOGICAL SUCCESSION AND DEVELOPMENT OF THE COMMUNITY

A community is an assemblage of populations of several species of living organisms sharing the same living areas or habitat. Krebs (1972) characterizes a community as having five measurable attributes: (1) species diversity, or what kinds of plants and animals live in the community; (2) growth form and structure or the way each species grows and the type of structure each species will have, such as mycelia or broad leaves from a tree; (3) dominance, or which species exerts the most influence on the others because of its numbers, size, toxin production, or other attributes; (4) relative abundance or the relative proportion of different kinds in a community; and (5) trophic structure, or what consumes what and how the energy flows. In the stored grain ecosystem, there are usually three well-represented communities: (1) the fungal community including those that can compete at atmospheric oxygen tension and those that can only compete in a reduced oxygen tension atmosphere, (2) the insect community including predators, parasitoids and scavengers, as well as those insects which actually cause loss, and (3) the mammal and avian communities.

The fungal community in the stored grain ecosystem is often much more complex than that of the mammal community. Which species are present is regulated by a combination of oxygen tension, equilibrium moisture content (water activity) and grain type. For example, in starchy cereal seeds (wheat, barley, oats, rye, rice, millet, maize, sorghum and triticale) at an equilibrium relative humidity (er.h.) of 65–70% (=M.C. of 13–14%) and oxygen tensions near atmospheric, only *Aspergillus halophilicus* Christiansen, Papavizas, and Benjamin will be found (Christiansen and Sauer, 1982). With a small increase in er.h. to 71–75% (=M.C. of 14.5–15%), three other fungal species will germinate, *Aspergillus restrictus* G. Smith, *Aspergillus glaucus* Link: Fr., and *Wallemia sebi* (Fries) van Arx (Christiansen and Sauer, 1982). At er.h. of 75–80% (=M.C. of 15.5–16.0%), *Aspergillus flavus* and *Penicillium* spp will be added to the fungal community. If, however, the oxygen tension decreases below atmospheric, these filamentous storage fungi will not develop, but instead, yeast species will predominate.

Succession in the insect communities of stored grain ecosystems is not as strictly regulated by M.C. % and oxygen tension as it is in fungal communities. Time of harvest and life style of the insect, that is, whether an insect is a parasitoid, predator, primary feeder, or scavenger in the storage insect community, is more relevant to the prediction of which insect species will be present. Composition and percentage of the foreign material is also a good predictor of insect species composition. For example, if the grain is freshly harvested with little or no fines or broken kernels, the species in abundance will be those that attack the whole grain, obligate internal feeders and germ feeders. As the grain mass develops an abundance of insect cadavers from these primary feeders, there will be a rise in the populations of scavengers such as the Dermestidae. If the grain mass develops a large percentage of broken kernels by the activity of internal infesting insects or by rough handling during transportation, it is more likely to have non-internal feeders such as *Cryptolestes* spp as part of the insect community. If moisture begins to rise due to high insect populations, the fungal community will develop, and if mycotoxins harmful to insects have not been secreted by the fungi, populations of fungivorous insect species (e.g. Mycetophagidae) are likely to be present.

Relatively few species comprise most mammal communities associated with stored grain or stored product ecosystems. Rodents, voles and both new world and old world mice can be associated with this system. Squirrels and shrews are other pest species that may appear in a storage system. Domestic mammals and humans are the intended end-users of the system. The needs and preferences of this portion of the mammal community often change due to their nutritional or sensory requirements for the end product. For example, one segment of the human population is searching for a reduced or altered fat content of steak and other meat products, or flour which contains a certain quantity of fiber, or other flour characteristics which provide a more nutritious pasta. Another segment of the human population is searching for special taste of cooked dry beans with a certain texture (Edmister *et al.*, 1986). Another part of the human population is searching for food products low or absent in pesticides and synthetic hormones (Ames *et al.*, 1987).

POPULATION DYNAMICS

A population consists of groups of interbreeding, or potentially interbreeding, individuals of one species living together at one locality. The ideal growth of a population can be expressed by a differential equation that describes a sigmoidal or logistic curve (Verhulst, 1838). The curve has 4 phases, the lag or slow acceleration period, the exponential increase period (=logarithmic phase), the deceleration period, and the stationary (equilibrium with the environment) period. The stationary phase is achieved when the population increases to the carrying capacity of the environment and the ecosystem. This carrying capacity, however, may change. An example is the case of storage fungi and adults of *Tribolium castaneum* Herbst and *Tribolium confusum* Du Val, where toxic substances are produced by the fungus or the insects, or both insects and fungi. As the number of these beetles in the storage system increases, the concentration of antifungal compounds (quinones) secreted by the beetles will increase and the size of the fungal populations will decrease. The carrying capacity of the grain mass for fungal populations is lower if it is infested with *Tribolium castaneum* or *Tribolium confusum* than if there are no quinone producing insects in the ecosystem.

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Many species of fungi and some species of insects, when observed in the natural preharvest and storage conditions, yield another type of curve, the J-shaped growth curve. This curve was obtained in Manitoba by Sinha and Wallace (1965) with a population of *Aspergillus flavus* in a damp grain pocket in a 13.6 t wheat bulk. The population increased at an accelerating rate. Then, instead of leveling off, the population precipitously decreased its rate of increase to zero. The large population of fungi died because of the fungus-mediated temperature of 64°C.

In the studies of Cahagnier and Poisson (1973) in Nantes, France, this J-curve phenomenon was observed in freshly harvested wet maize with several species of field fungi in the genera *Cephalosporium*, *Cladosporium* and *Verticillium*, as well as, storage fungi in the genera *Mucor* and *Penicillium*. Rapid, unrestricted, exponential growth, without a lag period, up to the carrying capacity of the environment was recorded. The experiment was terminated at this point, before any major environmental shift caused a precipitous fall in the population.

NICHE CONCEPT AND INTERACTIONS AT THE SPECIES LEVEL

In 1959 G. E. Hutchinson redefined the niche, and this definition has become that most accepted to date. Hutchinson described a niche as a habitat supplying the environmental conditions, within the range of the species, that the species needs for living and reproducing. He distinguished between the fundamental and the realized niche. The fundamental niche of the species has a potential unlimited number of environmental variables. The realized niche is the actual, limited spectrum of conditions and resources that allows a species to maintain a viable population despite the presence of competitors and predators. Faced with severe competition, such as that in stored grain fungal communities, the individual species may be completely displaced from several parts of its fundamental niche and may thrive only in areas where its competitors cannot. Therefore these areas comprise its realized niche.

The niche of fungal species found in the storage ecosystem is narrowly defined by: water activity of the substrate (the grain, beans, etc.), oxygen tension, and sensitivity to mycotoxin production of other fungal species. Examples of how fungal species change when there are minute changes in their microhabitat were presented in the succession discussion earlier in this paper.

THE CELLULAR LEVEL

To understand the successful adaptations that have been made by insects, mites and fungi in the stored grain ecosystem, and how these adaptations impact on management techniques, the cellular level must be understood. Morphological, physiological, and biochemical characteristics of insects, mites, and fungi are directed by activities of individual cells or groups of cells. Creative management of pest and beneficial insects, mites, and fungi in the storage ecosystem can be improved by a more thorough understanding of the function and products of certain cells.

For example, many stored grain Coleoptera have cryptonephridia, a special aggregation of their malpighian tubules attached to their hind gut and enveloped in a perinephric membrane. This concentrated, highly convoluted mass of malpighian tubules provides one last chance to reabsorb scarce molecules of water in the dry stored product environment. Data indicate this powerful water absorbing organ is present in stored grain beetle species of the families Tenebrionidae (Saini, 1964) and Dermestidae (Dunkel and Boush, 1968). In *Tenebrio molitor* L., these cellular arrangements of malpighian tubules create osmotic gradients (O'Donnell and Machin, 1991) which absorb water from fecal pellets in the rectum (Ramsay, 1964), and gain water vapor from the atmosphere through the open anal canal (Machin, 1976). The arrangement of these cells dictates the difference between an insect species that will survive the rigors of the stored grain ecosystem and a species that will not adapt to this xeric environment.

Insects associated with storage also have many interesting cells for reception and production of behavior altering compounds which, within the same species, may alter growth and reproduction. Cellular products such as aromatic ketones are produced by adult *Tribolium castaneum* and are potent inhibitors of insect prostaglandin synthetase (Howard and Mueller, 1987; Jurenka *et al.*, 1986). Based on analogies from other species (Destephano *et al.*, 1982; Loher *et al.*, 1981; Yamaja *et al.*, 1980; Dadd and Kleinjan, 1984), these local cell hormones may have a role in regulating

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reproductive behavior in *Tribolium castaneum*. Egg spacing chemicals produced by cells associated with the ovipositor of some bruchid females are also used for population regulation within the species (Messina *et al.*, 1987). Mandibular glands of pyralid larvae associated with stored grain, produce an epideictic pheromone. This pheromone regulates development of late instar larvae and oviposition behavior of adult females of the same species. Last instar larvae of *Anagasta kuehniella* (Zeller), upon meeting other individuals, deposit drops of secretion from their mandibular glands on the substrate beside their silk thread (Corbet, 1971). These cellular secretions were found to regulate total numbers of *Anagasta kuehniella* in a closed system and dispersion of individuals within the system. Larvae responded to these droplets by varying length of larval development, dispersion and size (Cotter, 1974). Adult females responded to these mandibular gland droplets by laying more eggs at low secretion levels and significantly lower numbers of eggs at higher levels (Corbet, 1973). These products of mandibular gland cells may have potential for population management of phycitid destroyers of stored grain and cereal products. Certain cells in male *Cryptolestes ferrugineus* (Stephens), *Cryptolestes pusillus* (Schoenherr), *Cryptolestes turcicus* (Grouvelle), *Oryzaephilus mercator* (Fauvel), and *Oryzaephilus surinamensis* (L.), produce aggregating pheromones that attract both sexes of the same species (Vanderwel *et al.*, 1989). These pheromone producing cells use fatty acids and terpenoids in the insect diet to produce seven structurally related macrolides. Species specific combinations of these macrocyclic lactones function as aggregation pheromones. Other specialized cells located in the seventh abdominal segment of female dermestid beetles (Hammack *et al.*, 1973) produce pheromones which activate reception cells in male antennae.

Many stored grain insects also have cells which produce secretions that alter the behavior of other species. Many of these secretions are for defense. The best known are quinones in the tenebrionid beetles such as *Tribolium* spp (Tschinkel, 1975). Other cellular secretions which signal between species are those involved in host finding by parasitoids. Semiochemicals are produced by many storage insects and some are used by their parasitoids to find them, and these compounds may initiate oviposition activities by the parasitoids. Cells of the mandibular glands which produce the epideictic pheromone in *Anagasta kuehniella* also produce a kairomone. This kairomone attracts the ichneumonid parasitoid, *Venturia canescens* (Grav.) to initiate oviposition movements (Corbet, 1971). The major component of this cellular secretion from mandibular glands is similar in the related species, *Plodia interpunctella* (Huebner), *Ephestia elutella* (Huebner) and *Ephestia cautella* (Walker) (Mudd and Corbet, 1973).

Unlike insects and mites, which are distinct functional units, fungi lack comparable discrete units that define an "individual". Clones are a common and important feature of fungal population structure (Sinha, 1990). Part of the activity of these cells, if they are from filamentous fungi, contributes to loss of grain mass. Part of the activity of fungi causes the production of cellular wastes from primary metabolism and the accumulation of secondary metabolites such as mycotoxins. These cellular products actually turn the dominant plant of the ecosystem into a toxic substance. The list of toxic metabolites produced by stored grain fungi is expanding (CAST, 1989). Increasingly, more scientists are involved in this area of research. When environmental conditions are right, some strains of certain fungal species produce mycotoxins (Sinha, 1990). For instance if shelled maize is damp patulin is produced by *Penicillium* spp. If cob maize is damp, zearalenone and trichothecenes may be produced. Aflatoxins are produced by *Aspergillus flavus* and *Aspergillus parasiticus* Speare in the field or in the storage structure if the moisture content is above 16% and the oxygen tension is atmospheric. Ochratoxins are produced by other *Aspergillus* species if the moisture content is above 14.5% and the oxygen tension is atmospheric. In low temperatures, penicillic acid is produced by *Aspergillus ochraceus* K. Wilh. and *Penicillium aurantiogriseum* Dierckx (= *Penicillium cyclopium* Westing).

THE POLITICAL LEVEL

Politics play an important role in this human subsidized ecosystem. In the U.S.A., many changes are exerted on the system due to changes in regulations established by the Federal Grain Inspection Service and price supports provided for in the Farm Bill. Government rules such as, price subsidies, federal grain grades and standards, and mycotoxin tolerances, have an impact on how the system

is managed. For example, the apparently simple change of separating broken corn from foreign material in the federal grades for shelled maize could have major economic consequences. Consumer requirements, such as pesticide-free wheat or barley, have an influence on how the system is managed. The use of penalties and incentives at the first point of sale also has a profound effect (Bylenga *et al.*, 1987). For example, in order to obtain the high premium for malting-quality barley, producers in the Western U.S.A. are adding aeration systems with electronic, computer driven controls to their storages. A strict control of moisture is necessary because the new barley varieties designed for malting are extremely sensitive to increase in moisture content. With small increases in water content of the grain, "chitting" or the physiological beginning of germination occurs. Chitting causes the barley to lose its malting designation, and the producer loses the premium price (Northwestern Montana malt barley farmers pers. commun., 1991). The impact of introducing penalties at the point of sale in Rwanda, where no formal grading system existed, was analyzed and found to have a potential negative impact on the amount of beans that would be purchased by the national warehouses (Bylenga *et al.*, 1987).

OUTLOOK FOR FUTURE RESEARCH

As environmental protection agencies around the world increase the restrictions placed on pesticides used in many ecosystems, including in stored grain, we will be searching at the cellular level and at the community and population level for new ways to manage without the presently registered chemicals. Because of the increasing proportion of resistant individuals in insect and mite populations, we must intensify our search for alternative management methods. The practice of prophylactic application of the same chemical year after year for stored grain "insurance" will soon disappear. Integrated pest management is established as a viable alternative to the "pesticide treadmill" for many agroecosystems. The "pesticide conspiracy" described by Van den Bosch (1978) is less and less influencing research areas chosen by scientists and management practices developed by industry.

The future is promising for entomologists who have been investing their research career in alternatives to the dependence on prophylactically-used, petroleum-based chemicals. Instead of being told at regional, national and international professional meetings that they should "get practical" with their research, and that they should switch to a commonly accepted chemical group to investigate, they are being sought out and pressed for answers to the present pesticide crisis. The alternatives may not be sprayable, or may not attack every pest species in the system. The decision to use a particular alternative will be based on monitoring information, risk/benefit analysis, and a specific prescription for the most economically important pest. This prescription-based pest management will require an information organizing tool, such as a computer-based expert system, for its most efficient use. Computer-based expert systems are presently being developed for stored grain ecosystems (Flinn and Hagstrum, 1990). This type of artificial intelligence will need specific answers from research. Research scientists and the extension services will need to cooperate more closely than ever to make a success of management advised by expert systems.

How much energy is lost by mismanagement of the stored grain ecosystem?

Solar energy is put into the agroecosystem and then transferred to the stored grain ecosystem. In addition, fossil fuel, and human energy are put into the stored grain ecosystem directly. Many estimates have been made of actual postharvest losses, and of storage loss, a subset of postharvest loss (Adams, 1976; Adams, 1977a-c; Adams and Harman, 1977; Dunkel *et al.*, 1985, 1986; Durnez and Dejaegher, 1980; Rowley, 1984). Many times these loss figures are quoted to suit the purpose of the government providing the data. In Morocco, the government's general estimate of annual stored grain (wheat) loss in the country was 25%. Subsequent studies in Morocco based on weight loss, fungal and insect damage (Bartali *et al.*, 1990) indicated that this was an accurate figure. Figures for postharvest loss in Rwanda were published by FAO (1977) as 25%. Actual surveys of losses during storage in Rwanda (1984-1986) showed that there was a 1-2% weight loss at the farm level and a 12% loss due to end-user rejection (Dunkel *et al.*, 1986). Clearly, even comparing the FAO figure with the sum of all postharvest loss, there is a wide discrepancy between the published figure and the actual measurable loss.

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How does an ecosystem approach lead to better management, more sustainable approaches and more economical techniques?

Solutions currently in use may not be the more sustainable approach or the more economical technique. Research and extension entomologists, agricultural engineers, plant pathologists, and academicians in these areas in the classroom can internalize the principles described herein for ecosystem analysis. How can monitoring the impact of changes in structures and farmer practices be important in managing insects, mites and fungi in storage? How can knowing the actual preference of consumers who will use the grain, beans or other product impact on storage management methods? How will understanding the special niche requirements of fungi associated with stored products make management of them more efficient? How will actually knowing the amount of loss experienced at different levels in a nation's stored grain system help achieve self-sufficiency? How will understanding the insect and mite community, and the sylvatic reservoirs of each species, help scientists devise non-pesticidal stored grain management schemes?

Sustainable approaches are those which use renewable resources, and which do not pollute the environment. Sustainable approaches are those which do not promote the development of resistant pest populations. Integrated management (IM) of an ecosystem, such as stored grain, requires knowing the components of the specific system in question, the individual species in each community, their niche requirements, their development cycle, and their behavioral patterns. Integrated management requires that successional changes in communities are monitored. Integrated management of a stored grain ecosystem also requires knowing how the populations of individual species in the system are changing at appropriate intervals and what immigration there may be into and emigration from the stored grain system. It is also important to know what passive dispersal of individual species there is in the grain itself or in transportation vehicles.

Frequently, an integrated management plan which focuses on sustainable techniques is also more economical. In IM, high cost pesticides are used only as an emergency solution. Monitoring abiotic and biotic properties of the system can often alert the grain manager of the best time to use or move the grain. An ecosystem approach also places emphasis on the intended primary consumer (humans or livestock that eat the grain as grain) and on the secondary intended consumer (humans that eat the grain as meat from the livestock which ate the grain). Understanding the quality that is particularly important for each country's market and understanding the specialty markets within each country can allow the producer and grain manager to manage the system to meet these criteria. Meeting these special criteria may mean a sale or a premium price.

How can these ecosystem principles be applied on a global scale?

Storage ecosystems are connected by transportation and commerce. Grain and other stored products move around our planet on a global scale. The insects and mites that are pests of these commodities can move at an even faster rate, by simply accompanying travelers on jet airplanes.

In our research, extension, and academic presentations, we can internationalize our efforts. By example, we can encourage our colleagues and students to think globally, and sustainably, and ask the ecosystem questions. We can ask: what are the components of the system with which we are working and, in a practical sense, how can we solve the postharvest problems of this group? We can also ask: how can the postharvest problems of this group be solved without, or at least with a minimum, use of pesticides? We can inquire: what is already available in local materials and in indigenous practices that can be used to solve the pest problems within the country?

The following five case studies were chosen to provide some ideas of how ecosystem principles can be applied on a global scale. Each region has its own combinations of environmental conditions, uniquely evolved farmer practices, economic milieu, governmental regulations, and end-user requirements. In applying ecosystem principles to these regions, the unique aspects of the region also need to be considered. The following are also examples of reverse technology, how we can assist other countries while learning from them in managing our own stored grain ecosystems.

Case Study: People's Republic of China (P.R.C.)

During my work in stored grain ecosystems of the P.R.C., I encountered many ideas suitable for reverse technology, that is, ideas adaptable to other countries, including the U.S.A. Safe

storage in the P.R.C. was primarily based on physical manipulation of the environment and on non-synthesized chemicals. Management of stored grain systems in the P.R.C. was also based on an intense early monitoring of the whole ecosystem beginning with the place at which the grain was sold to the State (Dunkel, 1982). The most common type of large scale storage of grain was in bags on pallets. The stacks of bags were always covered with locally produced plastic sheeting. Carbon dioxide was injected under the plastic. The carbon dioxide was produced naturally from a slurry of yeast cultures and rice bran, etc. (Dunkel, 1982). Underground grain storage of rice and millet "saved nations" in Ancient China (e.g. during the Sui and Tang Dynasties). Large scale underground storage of wheat and other commodities was now being practiced efficiently with modern methods of remote temperature monitoring. At least one of these underground units also included a mill to prepare the wheat flour (Sterling *et al.*, 1983).

The Chinese have successfully implemented a national monitoring plan for the main communities (insects, fungi and rodents) in their stored grain for over 15 yr (Dunkel, 1982). Most grain is held in long term storage (over 3 months) by the government, in local, prefectural, or city warehouses. At each of these storages, there is a monitoring plan which includes remotely sensed temperature, analysis of samples for insects, mites, and fungi, moisture content analysis, and several other measures specific to rice. Once or twice a month, depending on the time of year and the previous history of the grain, these data are collected and the report sent to the next higher level of organization. For example, reports from the warehouse at the first point of sale are sent to the province where they are reviewed and then sent to the federal level. If there is grain at risk, a team of people with extension responsibilities is sent from the unit receiving the report to those with the problem. If the reports are consistently good, that is if the grain has the "4 Nos" (No insects or mites, no high moisture, no rodents or birds, no dangerous residues) then the storage unit receives an award. When these observations were made, these "4 No" awards were highly respected (Dunkel, 1982). Therefore, as national policy, the Chinese used the physical limits to growth (equilibrium moisture content, temperature, and oxygen tension) of the fungi, insects and other arthropods. The monitoring system developed in the P.R.C. particularly took advantage of the connectedness of the entire system of storage structures linked in different locations by transportation.

In South China, however, there were special problems at the interface of their agroecosystem and the stored grain system. The problem was the "triple crop squeeze" (Dunkel *et al.*, 1982b). In the period immediately after harvest, most of the wheat was lost because it did not have sufficient time in the field to dry, and often there were not enough sunny days for the sun drying to get the grain out of a high risk condition. At this point, the grain was on the farm. It was too early in the storage system for the intricate monitoring system and the carbon dioxide supplemented controlled atmosphere storage to be implemented to save the grain. One solution tested was the use of a locally produced food preservative (2,4 hexadienoic acid, sorbic acid) to stop germination of field and storage fungi. This same compound, later in the storage period, prevented the F1 generation of the main storage insect pests in South China, *Sitophilus oryzae* (L.), *Rhizopertha dominica*, and *Sitotroga cerealella* (Olivier) (Dunkel *et al.*, 1982b).

Case Study: Morocco

The stored grain system in Morocco consists of matmora, sela, the local markets, transportation of many different types and terminal elevators. The matmora and sela are used on-farm, although large farms may also have larger bins and flat storages. The matmora is a flask-shaped underground bin carved from the earth in areas of Morocco which have a particularly favorable soil type, and the sela is an aboveground bin woven from local reeds. Transportation consists of grain trucks, wagons, baskets on donkeys, and rail cars. The present problems in the storage ecosystem are as follows: (1) The government of Morocco wants to achieve self-sufficiency in wheat and barley, (2) the percent of wheat needed to be imported each year was roughly equal to the amount estimated lost each year due to conditions in the storage system; and (3) the Moroccan government knows that storage conditions have to be improved, but they did not want to invest in expensive terminal structures which are often difficult to maintain.

After 5 yr of collaborative research and surveys, the following was a program to address these problems. A new matmora was designed, with a sealed plastic liner. With the liner, fungal

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population development, particularly *Aspergillus flavus* and *Aspergillus parasiticus*, did not increase significantly during storage. On the other hand, the possibility of insect resistance to low oxygen atmospheres was detected in areas where underground storage (matmora) had been used for centuries, perhaps millennia. A sound infrastructure of stored grain scientists spanning eight disciplines was developed at the Institute Agronomique et Veterinaire Hassan II. These professors are now committed to posing and testing hypotheses that will lead to the application of ecosystem principles for better management of the postharvest system and to convey their research results directly to producers and to the government extension service. The team is particularly sensitive to the niche requirements of insects and fungi, and to end user quality requirements.

Case Study: Rwanda

In Rwanda, 95% of the population are subsistence farmers. Mean farm size is one hectare and it is decreasing. The primary commodities stored are dry edible beans, sorghum and fermented cassava. Some peas and dry maize are also stored. Storage structures are located on-farm (primarily in the house), in cooperative silo/hangars, in merchant storages, in large scale government storages in bags on pallets, and in long term strategic storage (similar to the government storages).

The Government of Rwanda decided to improve long term, large scale strategic storage for Rwanda, and develop monitoring and other management techniques. As a result, a long term, multidisciplinary collaborative research and survey plan was developed to study the entire stored grain and stored bean ecosystem. The following collaborative research and surveys were completed: survey of local bean varieties (over 300 land races or varieties) which produced the bean catalogue (Lamb and Hardman, 1986), a survey of storage management procedures and the resulting quality of beans and sorghum (Dunkel *et al.*, 1986), genetic resistance of the bean varieties to bruchids which invade the bean seeds after senescence of the plant (Lamb and Dunkel, 1987), the effects of storage on bean cookability and consumer sensory preference (Edmister *et al.*, 1986), development of alternative storage methods (Hanegreefs *et al.*, 1986), and development of grades and standards (Bylenga *et al.*, 1987). A summary of conclusions and recommendations was prepared for the agricultural marketing board and for the Government of Rwanda (Dunkel *et al.*, 1988). This document formed the basis of another 5 yr period of implementation and research.

We found that actual levels of loss were not as high as predicted (Dunkel *et al.*, 1988). Producers had developed over 300 land races (varieties) of dry edible beans, *Phaseolus vulgaris* (L.) (Lamb and Hardman, 1986). Some of these varieties have genetic resistance as dry beans to the two main insect (bruchid) causes of loss in Rwanda (Lamb and Dunkel, 1987). Underground, sealed storage could be successfully adapted to wet, termite infested soil in Rwanda (Hanegreefs *et al.*, 1986). Resistance of storage insects to pirimiphos methyl was predicted to be developing in older government warehouses (Dunkel *et al.*, 1988) and has been confirmed with subsequent research (Sriharan *et al.*, 1991).

We learned that Rwandan farmers evolved many practices using locally available plant, animal, and soil materials for storage structures and for pesticidal properties (Dunkel *et al.*, 1986). Some of these practices have been examined in depth and found to have a scientific basis for their efficacy (Dunkel *et al.*, 1991; Weaver *et al.*, 1991a,b). These practices, through reverse technology, may provide some possibilities for novel insecticides that are effective with postharvest and preharvest insects in other areas of the world, as well as, in Rwanda on farms and, with minor adaptations, in long term government storages.

Case Study: Minnesota, U.S.A.

In Minnesota, grain (maize, wheat and barley) is primarily stored on the farm. Field drying is used for most wheat, but maize is generally low or high temperature dried after harvest. Aeration is used each fall and spring to decrease temperature gradients in the bins. Farmers generally store for multiple years to take advantage of the market fluctuations. There is a small profit margin which is decreasing due to cutbacks in government subsidies. There is easy access to terminal elevators (there are more terminal elevators in Minnesota than in any other state in the U.S.A.). Transportation possibilities consist of river barge to New Orleans, rail or truck to Chicago, and ocean vessel from Duluth.

The problem with the present storage system in Minnesota is that insect contamination of the grain which Minnesota sells can be a threat to marketing of the grain. Research results have shown that insect species thought to be important in storage were not (e.g. *Sitophilus*) and other insects not considered important in Minnesota were important [e.g. *Plodia interpunctella*, *Ahasverus advena* Waltl., *Typhea stercorea* (L.), *Cryptolestes* spp, *Cybaeus angustus*] (Barak and Harein, 1981a). Insects not previously thought important were all associated with high moisture conditions and the fungal community. Some resistance to malathion and other insecticides has been detected. There is no incentive from elevators for the farmer to decrease insect infestation (Barak and Harein, 1981b). One of the "new" storage insects is the larger black flour beetle, *Cybaeus angustus* which has moved within the past 80 yr from a desert ecosystem and is now able to thrive in diverse storage ecosystems (Dunkel *et al.*, 1982a; Barak *et al.*, 1981; Morrison and Dunkel, 1983; Kao *et al.*, 1984).

Therefore, the situation in Minnesota is such that alternatives to malathion need to be developed and tested. The "new" storage insects and their special niche requirements need to be considered in developing a management plan for the storage ecosystem in Minnesota.

Case Study: Montana, U.S.A.

In Montana, most grain stored is wheat and barley and it is primarily stored on the farm. Grain is primarily dried in the field, before or after swathing. There are few farms that use either dryers or aeration equipment. Grain is often stored for more than 1 yr. Some producers use malathion or chlorpyrifos methyl prophylactically. Monitoring of grain quality is done visually and generally without the aid of insect traps or remote temperature systems. Profit margins are low for these crops and they are decreasing. Terminal elevator access is often difficult. The nearest port is Seattle or Portland. Most grain is sold to buyers outside North America, primarily Japan. Some grain is sold within the U.S.A. to California for feed and some to breweries in Missouri or Colorado. Rail or truck is the primary transportation route out of the state. Recently, grain sold to California from three Montana counties has been quarantined [for *Oulema melanopus* (L.)] and entry is refused unless it is fumigated. Cost of fumigating, in many cases, eliminates the profit margin in the sale of this grain. *Oulema melanopus* is part of the preharvest grain agroecosystem and without effective predators or parasitoids it may be a problem. In stored grain ecosystems this species may survive a short period as an adult which is the overwintering stage.

Oulema melanopus is apparently a short term problem awaiting the completion of convincing storage research on its survival before the California quarantine is lifted. Longer term problems with the present storage ecosystem in Montana are *Rhyzopertha dominica* which is increasing in frequency (A. Williams, pers. commun., 1991) and registered insecticides that do not seem to work well in its management. The decline of already low profit margins for wheat and barley is another problem in this system. The solution being investigated to solve these problems is the initiation of a statewide grower awareness program on *Rhyzopertha dominica* and laboratory studies on sustainable alternatives to current insecticides for this insect. A statewide survey of this borer in forest, prairie, agro- and storage ecosystems is being planned. Marketing of pesticide free wheat and barley is being investigated and research on underground storage systems is in progress. Some producers that store over 100,000 bushels are considering investing in computer controlled aeration systems. Overdrying of the grain results in loss at point of sale. Therefore, in Montana the ecosystem approach is leading to a careful watch over the population fluctuations and distribution of the insect community within the state. Careful attention to end user requirements may be an economically important consideration.

DOES THE MOVEMENT OF GRAIN THROUGHOUT THE WORLD FOLLOW THE SAME PATH AS THE MOVEMENT OF RESISTANCE GENES AND INSECTS MINUS THEIR REGULATING PARASITES AND PREDATORS?

Although grain follows the worldwide trade routes, small amounts of grain, dry edible beans, and other products are often carried by travelers when they move from country to country and continent to continent. Even with customs inspection, insects move in these small amounts of stored products. The "founder effect" occurs when one, or very few reproductive females arrive in a mated condition in new territory and establish a colony.

With this grain in transit, insects move, the genetic material which confers on the individual susceptibility (or resistance) to insecticides moves, and fungi move. The fungi, *Aspergillus flavus* and *Aspergillus parasiticus*, primarily found on nuts and maize are now located around the world. Wherever they occur, there is the possibility of their producing mycotoxins which are difficult to degrade. Stored grain insects resistant to malathion are now found in most countries. The genetic material that confers resistance may have independently evolved in many places. Some populations may have become resistant by the founder effect. In 1983, resistance in *Tribolium castaneum* in Manitoba, Canada was rare and no evidence was found for malathion resistance in populations of *Cryptolestes ferrugineus* (White and Watters, 1984). However, all populations sampled at the Canadian coast on board freight liners which transported grain were resistant to malathion in 1983. By 1985, three of twenty three strains of *Cryptolestes ferrugineus* collected from Canadian farms were resistant (White and Loschiavo, 1985). In 1991, two-thirds of the *Tribolium castaneum* populations were moderately resistant to malathion (N. D. G. White, 1991 pers. commun.). In Rwanda, populations of the main storage insects are showing increased resistance to pirimiphos-methyl (Sriharan *et al.*, 1991). In 1982, the government storage pest management system switched from prophylactic use of malathion to prophylactic use of pirimiphos-methyl. Reports of resistance to phosphine and high carbon dioxide/low oxygen atmospheres are appearing in the literature. There is now increasing concern about the discovery in a number of developing countries of strains of stored product insects, mainly *Rhyzopertha dominica* and *Tribolium castaneum* that are showing resistance to phosphine (Prevett, 1990; Sartori *et al.*, 1991; Rajendran, 1991). As international trade routes increase in diversity and international travel by the ordinary citizen increases, resistance to insecticides will spread more and more rapidly. Insects not normally associated with storage ecosystems may by chance enter the system and will be rapidly transported throughout the storage systems of the world (Calderon, 1981).

Insect pests often move without their population regulating natural enemies. One example is *Prostephanus truncatus* Horn. This insect probably evolved with maize in Central America. Food aid shipments of maize to Tanzania resulted in a serious problem years later. The founding population apparently migrated without the parasites and predators which regulated its populations. Stored maize and cassava are now seriously threatened by the expanding populations of *Prostephanus truncatus* which are not easily managed by malathion and pirimiphos-methyl. This insect was subsequently brought to Togo and Burma by a similar type of emergency food shipment.

Through history, movements of storage pests often resulted in encounters with new commodities, some of which were more favorable for development than the substrate where the pest evolved. *Cynaeus angustus* may be one of these insects. This species is able to reach much higher population levels in cotton gin residue or medium moisture stored maize than in its probable original food namely agave stem pulp 1 yr after flowering. *C. angustus* represents one of the newest examples of improved success with a commodity switch (Dunkel *et al.*, 1982a). An interesting historical question is the coevolution of *Sitophilus* spp and their domesticated food sources, rice, wheat and maize. Did each species, *Sitophilus oryzae*, *Sitophilus granarius* (L.), and *Sitophilus zeamais* Motschulsky evolve in these commodities? The centers of origin of maize, rice and wheat were in three separate continents. At some point, maize eating humans perhaps met wheat eating humans, etc. Their main grains were grown and stored together and their *Sitophilus* spp were exchanged.

On our planet Earth, there are some ecosystems upon which we depend to support our lives. It is extremely urgent that we, as the Chinese Emperor Wen requested of his agricultural advisors in 163 B.C. "exhaust all our efforts and ponder deeply" how we might manage the energy input and pest management in the stored grain ecosystems of the world in a sustainable, economical and safe manner.

SUMMARY AND CONCLUSIONS

The stored grain ecosystem is a set of island systems, connected by the transportation systems, the systems of commerce and international food aid distribution systems. The concepts of modern ecology should be applied to the management of insects, mites and fungi in stored grain ecosystems throughout the world. In several areas involving fungi in stored grain ecosystems, the physical,

chemical and biological limits of these organisms have been determined. Additional efforts should be undertaken to develop energy budgets for grain bulks infested with fungi and/or arthropods. Prophylactic use of pesticides throughout the world increases insect genetic resistance to these materials. Safe, sustainable alternatives to currently used insecticides need to be developed.

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Appendix 3. In press refereed journal article. "Oviposition patterns in two species of bruchids (Coleoptera: Bruchidae) as influenced by the dried leaves of Tetradenia riparia, a perennial mint (Lamiales: Lamiaceae) that suppresses population size." Environ. Entom. in Press (anticipated issue October 1992).

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Oviposition patterns in two species of bruchids (Coleoptera: Bruchidae)
as influenced by the dried leaves of *Tetradenia riparia*,
a perennial mint (Lamiales: Lamiaceae) that suppresses population size.

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1 **ABSTRACT-** *Tetradenia riparia* (Hochst.) Codd. (Lamiales:
2 Lamiaceae) is a Rwandan traditional medicinal plant reported to
3 be used to prevent postharvest insect damage in traditional
4 storage situations. Addition of milled or crushed leaves of *T.*
5 *riparia* at $\geq 4\%$ (w/w) significantly decreased fecundity and
6 fertility of *Zabrotes subfasciatus* (Boheman) and *Acanthoscelides*
7 *obtectus* (Say), which resulted in smaller F_1 populations. A
8 concentration of 10% leaves (w/w) also increased cumulative
9 larval and pupal mortality for *Z. subfasciatus*. The increased
10 mortality may be caused by increased competition between *Z.*
11 *subfasciatus* larvae within the bean. Competition could be
12 influenced by the great decrease in percentage of beans
13 oviposited upon by *Z. subfasciatus*. The control achieved against
14 the F_1 populations did not persist. Hormoligosis, in the form of
15 increased fecundity, was also found for leaves from one supply at
16 the 1% concentration. In general, the initial inhibitory
17 effects were found to be reproducible using different supplies of
18 leaves. Results were also quite consistent for the two species
19 of Bruchidae, despite innate behavioral differences between them
20 at oviposition and hatch.

21

22 **Key words-** Insecta, *Tetradenia riparia*, oviposition suppression,
23 Bruchidae.

1 AN AGE-OLD PRACTICE of subsistence farmers is to mix leaves from
2 a local plant with stored foodstuffs to reduce pest damage. For
3 example, leaves of neem, *Azadirachta indica* A. Juss., were
4 reported to be added to foodstuffs to repel storage insect pests
5 (Pruthi 1937). In Rwanda, *Tetradenia riparia* (Hochst.) Codd. is
6 cultivated near homes and is claimed to be effective against a
7 wide range of diseases and ailments including malaria, angina,
8 gastroenteritis, gonorrhoea, diarrhoea and fevers, among others.
9 The leaves have also been reported to be used to protect
10 foodstuffs in traditional silos (Van Puyvelde et al. 1975, Van
11 Puyvelde 1976). A survey of 50 farmers throughout Rwanda
12 conducted over 1.5 yr, however, indicated that only 4% of farmers
13 interviewed still used traditional protectants, and that the
14 plant species used were primarily *Capsicum frutescens* L., and
15 *Ocimum canum* Sims. No evidence of usage of *T. riparia* in
16 traditional storage was obtained (Dunkel et al. 1986). However,
17 screening of methanol extracts of dried leaves of *T. riparia*
18 indicated significant antimicrobial activity (Boily & Van
19 Puyvelde 1986). Antimicrobial activity was found for 8(14),15-
20 sandaracopimaradiene-7 α ,18-diol (Van Puyvelde et al. 1986) which
21 was isolated and identified from the dried leaves of this plant
22 (De Kimpe et al. 1982). Numerous novel large terpenoids have
23 been identified from *T. riparia* (Zelnik et al. 1978, Van
24 Puyvelde et al. 1979, Van Puyvelde et al. 1981, and Van Puyvelde
25 et al. 1987).

26 Stored, dried beans (*Phaseolus vulgaris* L.) represent the

1 main source of proteins and calories in the Rwandan traditional
2 diet. Bruchids, *Acanthoscelides obtectus* (Say), the bean weevil,
3 and *Zabrotes subfasciatus* (Boheman), the Mexican bean weevil,
4 cause significant losses of stored beans in Rwanda. Damage is
5 particularly severe from *A. obtectus* (Dunkel et al. 1986). These
6 two species have different oviposition behaviors. *A. obtectus*
7 deposits its eggs in interstices among the beans, whereas *Z.*
8 *subfasciatus*, like most of the economically important bruchids,
9 adheres its eggs directly to the testa of the bean. Therefore, a
10 newly-hatched *A. obtectus* larva must actively seek a suitable
11 bean to penetrate (and might be quite vulnerable to insecticidal
12 or behaviorally-active preparations while foraging), while a
13 newly-hatched *Z. subfasciatus* larva penetrates the bean directly,
14 from beneath the protective outer layer of the chorion.

15 A preliminary study (Munyemana 1986) indicated that *T.*
16 *riparia* leaves may inhibit the population growth of *A. obtectus*.
17 As such we assessed the efficacy of the leaves against bruchid
18 oviposition and the subsequent developing populations at discrete
19 intervals by using both unprocessed leaves and leaves subjected
20 to a simple mechanical procedure. We tested the hypotheses that:
21 1. Milled *T. riparia* leaves have greater activity than the
22 crushed leaves against *Z. subfasciatus*. 2. The bruchid
23 suppression by *T. riparia* leaves does not significantly vary
24 between leaf harvests. 3. *T. riparia* significantly reduces
25 populations of more than one insect species. 4. Adequate insect
26 control can be achieved without additional concentrative

1 processing.

2 **Materials and Methods**

3 Leaves of *T. riparia* were collected near Butare, Rwanda
4 (2°35'S, 29°44'E) and dried at 40°C for 24 hr. The dried leaves
5 were then crushed and shipped via courier to Montana State
6 University, Bozeman, Montana. Plant material was stored at -20°C
7 until used. Two lots of plant material were used and were
8 collected from the same sites. The first was collected and
9 shipped in February, 1990 (Lot A) and the second in June, 1990
10 (Lot B). All experiments were begun within 14 days of the
11 receipt of the plant material. Voucher specimens were deposited
12 in the Montana State University Herbarium- " Voucher F. V. Dunkel
13 1 (MONT; MSU Herbarium)". The collection consists of the
14 florescence plus an apical whorl of leaves collected by F.V.D. in
15 the Arboretum of the National University of Rwanda, Butare,
16 Rwanda on November 14, 1986. It also consisted of basal leaves
17 collected March 10, 1991 by L.V.P. at the same location. The
18 habitat of this area is relatively level, well-drained and is
19 located at approximately 1,330 meters above sea level. *Z.*
20 *subfasciatus* and *A. obtectus* were reared on dried Pinto beans
21 (*Phaseolus vulgaris* L.) at 27±1°C, 65±5% RH, with a 12:12 (L:D)
22 photoperiod. The stock cultures had been maintained in the
23 laboratory for many years. Experiments were conducted under
24 conditions similar to those for culture maintenance.

25 **Experiment 1.** The efficacy of milled and crushed plant
26 material as a suppressant of *Z. subfasciatus* populations was

1 compared. Milled leaves (Lot A) were prepared by grinding for 40
2 s in an electric coffee grinder (SHG 75, SHG, Italy) giving a
3 mode particle size range of 0.3 - 0.6mm with a range of 0.15 -
4 1.1mm. Crushed leaves were used as shipped, with a mode particle
5 size range of 3 - 5mm and a particle size range of 0.75 - 15mm.
6 Leaf preparations of the dried leaves of *T. riparia* were added to
7 ten replicates of 20g of dried Pinto beans (approximately 50
8 beans; 16.07% moisture content as determined by 24 hr oven drying
9 at 110°C) at concentrations of 0.1, 0.5, 1 and 10% weight/wet
10 weight. Ten control replicates, without *T. riparia*, were also
11 prepared for both the milled and the crushed preparations. Plant
12 materials were mixed by shaking and placed in a 10 dram plastic
13 shell vial. Five male and five female *Z. subfasciatus* (0-3 d
14 old) were added to each vial and all vials were sealed with a
15 perforated lid. Adults were allowed to oviposit until death,
16 which occurred within 17 d for all individuals. At 25 days,
17 individual beans were examined to count the number of hatched and
18 unhatched eggs. At 55 d both live and dead emerged adults were
19 counted. Live insects were returned to the vials and the
20 experiment was continued another 30 d at which time all vials
21 were frozen and the emerged adults were again counted. An
22 earlier study (Howe & Currie 1964) and our previous experience
23 (D.K.W. & F.V.D., unpublished data) suggested that counts should
24 be conducted at these intervals to maximize the accuracy of
25 counts for both eggs and adults. These count dates are critical
26 for accurate comparison between treatment and control numbers,

1 particularly if developmental delay occurs as a function of sub-
2 lethal treatment as was reported for low concentrations of ground
3 black pepper (Su 1977). Assessing efficacy earlier than the
4 final possible day that conserves experimental accuracy might
5 inflate treatment effects by giving lower hatch and emergence
6 numbers for the treatment than actually would occur.

7 **Experiment 2.** Concentrations of 1, 2, 3, 4 and 10%
8 weight/wet weight of crushed, dried leaves (Lot B) were prepared,
9 as above, again with *Z. subfasciatus* (0-2 d old) as the test
10 insect. Ten replicates of each concentration and ten control
11 replicates were used. Counts were conducted as above.

12 **Experiment 3.** The dosages and plant material supply used in
13 experiment 2 were evaluated with *A. obtectus* as the test insect.
14 The scale of this experiment was reduced because of the
15 difficulty posed in locating and separating loose, delicate eggs
16 from the crushed plant material and beans. Concentrations were
17 thus prepared as percentage by weight of three beans only
18 (approximately 1.3 g), to which four male and four female *A.*
19 *obtectus* (1-2 d old) were added. Insects were sexed according to
20 Halstead (1963). Preliminary experiments with our stock culture
21 indicated that this number of beans had no negative effect upon
22 oviposition (J.L.C., F.V.D. & D.K.W., unpublished data). The *A.*
23 *obtectus* were removed from the plant material after 6 d and
24 hatched and unhatched eggs were counted at 15 d. The plant
25 material and insects were placed in 3 ml glass vials which were
26 sealed with perforated filter paper. Ten replicates of each

1 dosage and of a control were initially prepared, but scrutiny of
2 the dead parental cohorts eliminated some of the replicates due
3 to errors made during live sexing.

4 **Experiment 4.** The dosages, replicate numbers, plant
5 material and shell vial size used in experiment 2 were evaluated
6 with *A. obtectus* as the test animal. This experiment used 5 male
7 and 5 female adults (0-2 d old). Replicate numbers were again
8 reduced following post-mortem evaluation of live-sexing accuracy.
9 This experiment was conducted because potential damage caused by
10 handling of the loose and fragile eggs of this species prohibited
11 counting the eggs and relating this number to the number of F_1
12 progeny for the fecundity study in Experiment 3 (as noted by
13 Lambert et al. 1985). Therefore, in this additional experiment,
14 the vials were left undisturbed for fifty-five days and then
15 frozen. F_1 adults were sexed and counted subsequently. Fifty-
16 five d was chosen to allow maximal emergence of the F_1 adults
17 prior to the emergence of the first F_2 adult (Howe & Currie 1964,
18 Szentesi 1972, D.K.W. & F.V.D., unpublished data).

19 **Statistical analyses.** Controls for both plant preparations
20 in Experiment 1 were not significantly different and were thus
21 pooled. Means and standard errors were calculated for all
22 variables and were plotted against concentration. The data in
23 which the response variables were clearly dependant on the
24 structured concentration of leaves were subjected to regression
25 analysis (Sokal & Rohlf 1981). Many response variables were
26 significantly influenced by concentration, but the regressions

1 were dominated by the response to the maximal concentration only.
2 In such cases regression analyses were inappropriate despite the
3 structured experimental design. Therefore, such data from
4 Experiment 1 were subjected to two way analysis of variance.
5 Similar data from all other experiments were subjected to one way
6 analysis of variance. Comparisons of means were conducted using
7 t-tests after a significant effect of concentration was indicated
8 in the ANOVA. The only comparisons made were between a specific
9 treatment and the control and only if the effect of the treatment
10 appeared biologically significant. If the two-way analysis of
11 variance indicated a significant interaction between process and
12 concentration in Experiment 1 (for data for which regression
13 analysis was inappropriate), an additional pairwise comparison
14 was made between preparations at the same concentration if the
15 difference was biologically relevant. If the two-way ANOVA
16 showed no significant interaction between preparation and
17 concentration for a variable in Experiment 1, a one way analysis
18 of variance was conducted and individual data of biological
19 interest for particular concentrations were compared to the
20 control when concentration was significant in the ANOVA. Count
21 data were normalized by square root transformation and
22 proportion/percent data were normalized by arcsine transformation
23 prior to analysis (Sokal & Rohlf 1981). All statistical analyses
24 described above were conducted using MSUSTAT Version 4.12 (Lund
25 1989). Regression analysis was used to estimate EC_{50} or EC_{25}
26 values for probit-transformed percentage suppressions (0 percent

- 1 suppression in controls) regressed against log concentration,
- 2 where possible (Finney 1971).

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Results

Higher concentrations of milled or crushed leaves of *Tetradenia riparia* influence oviposition by both *A. obtectus* and *Z. subfasciatus*. Count numbers for selected variables per *Z. subfasciatus* parental cohort are reported in Tables 1-4. The milling of these dried leaves increased efficacy by decreasing total eggs laid at the 10% concentration for *Z. subfasciatus*. The 10% concentration of crushed leaves reduced *Z. subfasciatus* oviposition approximately 20% for both Lot A (Table 1) and Lot B (Table 2), as opposed to a reduction of 40% for the 10% concentration of the milled leaves of Lot A (Table 1). The percentage of hatched eggs was also significantly (approximately 15%) lower for both processes at the 10% concentration (Table 1) which resulted in 50% fewer larvae (hatched eggs) for the 10% milled concentration and 30% fewer larvae for the 10% crushed concentration, relative to the control. These effects were replicated using leaves from Lot B at the 10% concentration (Table 2) and a concentration-dependant effect on the proportion of eggs hatched was evident (Figure 1). The EC_{25} for these data is 29% (95% fiducial limits- 21%, 40%; $\chi^2 = 17.22$, $df = 4$; data not significantly heterogenous at $\alpha = 0.001$). There was an additional 10% mortality during development for the 10% milled concentration and an additional 20% mortality for the 10% crushed concentration, relative to the control (Table 1) and the data from Lot B are almost identical (Table 2).

1 The 1% concentration of leaves from Lot A stimulated
2 oviposition, though not significantly for the crushed leaves.
3 This hormoligotic effect (Luckey 1968) was not found for leaves
4 from Lot B at the same concentration. The percentage of beans
5 oviposited on by *Z. subfasciatus* was strongly concentration-
6 dependant (Figure 2) resulting in a 90% decrease in beans
7 penetrated at the 10% concentration of crushed leaves from Lot B.
8 The EC_{50} for these data is 4.0% (95% fiducial limits- 3.5%, 4.5%;
9 $\chi^2 = 7.82$, $df = 4$; data not significantly heterogenous at $\alpha =$
10 0.05).

11 There was a clear concentration-dependant effect of crushed
12 leaves from Lot B on total eggs per parental cohort of *A.*
13 *obtectus* (Figure 3). The number of eggs laid decreases to 40% of
14 the control for the 10% concentration. The EC_{50} for the total
15 number of eggs is 5.7% (95% fiducial limits- 4.4%, 7.5%; $\chi^2 =$
16 3.47, $df = 4$; data not significantly heterogenous at $\alpha = 0.1$),
17 while the EC_{50} for the number of hatched eggs is 4.8% (95%
18 fiducial limits- 3.8%, 6.1%; $\chi^2 = 4.17$, $df = 4$; data not
19 significantly heterogenous at $\alpha = 0.1$). The proportion of eggs
20 hatched for this species varied from 0.84 to 0.72 with no evident
21 concentration-dependant effect.

22 The percentage of live adult *Z. subfasciatus* at 55 days is
23 39% greater at the 1.0 milled concentration and 33% greater at
24 the 0.5% crushed concentration for leaves from Lot A relative to
25 the control (Table 3). For the leaves from Lot B there is a 30%
26 increase in the percentage of live adults at 55 days for the 1%

1 and 2% concentrations and a 40% increase in the percentage of
2 live adults at 55 days at concentrations $\geq 3\%$ w/w (Table 4).
3 There seemed to be minimal difference between the sexes for
4 percentage composition of the live adults for either leaf supply
5 (Table 3 and Table 4). The leaves from Lot A caused some
6 fluctuations in the F_1 sex ratio as a function of concentration
7 but the variance among cohorts at the 10% dosage was too great
8 for this to be significant (Table 3). This was not found for
9 leaves from Lot B (Table 4). The productivity of F_1 females
10 appeared to be hampered by effects of density (for the lower
11 concentrations and the control) as the carrying capacity of the
12 food supply was approached (Table 3 and 4). The density of F_1
13 adults was relatively low for the 10% concentrations from both
14 leaf supplies (Table 3 and 4). This resulted in little
15 difference between the initial parental female and F_1 female
16 productivity at these concentrations (Table 3 and Table 4). The
17 overall number of adult progeny at 85 days was similar to the
18 control for most concentrations (Table 3 and Table 4). The
19 milled leaves from Lot A reduced the number of adult progeny by
20 25% relative to the control at 10% w/w (Table 3). The level of
21 control that was caused by the 1% crushed concentration from Lot
22 A (Table 3) was anomalous relative to the 10% crushed
23 concentration from the same leaf supply, and was not reproduced
24 using leaves from Lot B (Table 4).

25 There was a moderate concentration-dependance for A.

26 *obtectus* adult numbers at 55 days using crushed leaves from Lot B

1 (Figure 4). The number for the 10% concentration is 30% of the
2 control number (Figure 4), which suggests that approximately 10%
3 more mortality occurs from egg deposition to adult emergence
4 relative to the control (based on the oviposition data from
5 Experiment 3, Figure 3). The EC_{50} for the males is 5.2% (95%
6 fiducial limits- 3.2%, 8.4%; $\chi^2 = 10.66$, $df = 4$; data not
7 significantly heterogenous at $\alpha = 0.025$), while the EC_{50} for the
8 females is 7.5% (95% fiducial limits- 4.5%, 12.5%; $\chi^2 = 10.03$, df
9 = 4; data not significantly heterogenous at $\alpha = 0.025$).

10 Discussion

11 Some traditional insect control practices using unprocessed
12 plant material have been reported to have little acute toxic
13 effect upon insects (Lambert et al. 1985 and this study) but can
14 influence the size of resulting populations. Research has
15 repeatedly demonstrated the efficacy of extracted or steam-
16 distilled components from traditionally used anti-insectan
17 plants. Such procedures, common in the search for novel natural
18 source insecticides, use relatively limited technology. However,
19 these types of processing are generally not used by subsistence
20 farmers. Isolation and distillation practices frequently
21 concentrate phytochemicals beyond levels at which they occur
22 naturally, giving results that may be greatly exaggerated from
23 those achieved via the simple cultural practices from which they
24 were inspired. Such research does not provide an accurate
25 representation of the usefulness of the traditional procedure in

1 the farming system where they evolved. However, Throne (1990)
2 and Baker et al. (1991) have demonstrated that non-lethal
3 inhibition of population may result in prolonged significant
4 reductions in stored product insect populations. The possibility
5 that simple addition of anti-insectan plant material to stored
6 foodstuffs might have similar effects is certainly compelling.

7 Here we have demonstrated that the dried leaves of a
8 commonly cultivated medicinal plant reduce F_1 populations of two
9 economically important bruchids. There may be a strong
10 temptation to state that the effect of the preparations evaluated
11 here are "weak", because of the amount of material required.
12 This is a matter of the economic perspective of potential end-
13 users. Ecologically, at the higher doses tested, the effects on
14 F_1 populations are dramatic. The decrease of potential loss by
15 70% (*A. obtectus*, Experiment 4) is certainly better than nothing
16 and is without cost to the subsistence farmer in the Rwandan
17 agroecosystem. The technologically favored approach of adding
18 minute amounts of very toxic material to food is not accessible
19 or appropriate in the context of subsistence farming. An average
20 dried leaf of *T. riparia* weighs 1.25g and the plant is a large
21 (2-4m) perennial, so it is certainly practical to use, despite
22 the quantity required. This is particularly true in the Rwandan
23 farming system, since *T. riparia* is grown beside the homestead
24 for medicinal usage.

25 Milling the leaves increased the release of volatile
26 components contained in the trichomes of this mint, as evidenced

1 by the increase in intensity of the characteristic odor of this
2 plant species, but it is unlikely that the efficacy of these
3 leaves, milled or crushed, is a function of their phytochemical
4 nature only. The specific anti-insectan activity we observed may
5 have been due to the innate physical disturbance caused by
6 foreign material in the foodstuff in combination with either
7 behavioral (general repellency or specific oviposition
8 deterrence) or physiological (decreased ovariole
9 size/productivity) effects. At all the higher concentrations for
10 both supplies of leaves, the percentage of unhatched *Z.*
11 *subfasciatus* eggs was similar indicating that the embryonic
12 insect may have been susceptible to volatiles above a specific
13 threshold or that maternal productivity was directly affected.
14 The decrease in the percentage of beans that were oviposited on
15 by *Z. subfasciatus* clearly had a behavioral component since the
16 decrease in beans exploited was much greater than the
17 corresponding decrease in fecundity.

18 Our data show a different pattern than that found for *A.*
19 *obtectus* by Lambert et al. (1985). They found that the
20 traditional protectants *Hyptis spicigera* Vahl and *Cassia*
21 *nigricans* Lam. in powdered form at $\leq 3\%$ concentrations (w/w)
22 caused variable effects (i.e., enhanced, decreased and unaltered
23 oviposition over 3 replicates of *C. nigricans*) or insignificant
24 changes in oviposition patterns (*H. spicigera*). However, these
25 authors found a significant concentration-dependant effect for an
26 ethanol extract equivalent to these concentrations for both

1 species (Lambert et al. 1985). We found that the proportion of
2 unhatched eggs remained relatively constant for *A. obtectus* with
3 increasing concentration although the total fecundity was
4 reduced. Su (1977) found that ground black pepper at 625 ppm,
5 which caused only an ca. 8% increase in cumulative mortality of
6 *S. oryzae* at 5 weeks, decreased F₁ progeny 66% relative to the
7 controls. This indicates that low toxicity preparations of other
8 types of plant material may also significantly decrease
9 fecundity.

10 Similarly, a portion of our data parallels the hormoligosis
11 (Luckey 1968) observed in *A. obtectus* emergence by Lambert et al.
12 (1985) in response to low dosages of *H. spicigera*. We found that
13 the 1% doses of *T. riparia* increased both oviposition and
14 resultant emergence (adults per eggs laid) of *Z. subfasciatus* in
15 Experiment 1. This did not occur in Experiment 2 or with *A.*
16 *obtectus*, so this effect may depend upon seasonal changes in the
17 chemical composition of this perennial mint. It would be
18 desirable to avoid such effects when using these simple
19 preparations of plant material simply by using high dosages that
20 are reliable and using consistent harvest dates and locations,
21 although our data are insufficient to offer any immediate
22 suggestions. The implications of hormoligosis induced by plant
23 material may be significant for those screening for novel
24 insecticides in a low-technology environment. Actually, low to
25 moderate dosages of plant material used in combination with a
26 local storage insect and foodstuff may be an indicator of control

1 potential when either inhibitory or stimulatory effects are
2 noted. The stimulatory effects would suggest that with further
3 concentration or isolation there may be an inhibitory effect.

4 The results of the F₁ adult emergence patterns suggests that
5 the plant material may have had an effect on developmental period
6 of *Z. subfasciatus* since there are increasing numbers of live F₁
7 insects with increasing concentration (i.e., delayed emergence
8 from the bean). This effect, although obvious, can not be
9 measured because of our experimental design. If developmental
10 delay were great enough this could be of considerable benefit as
11 was implicit in the recent models of Throne (1990) and Baker et
12 al. (1991). Su (1977) also demonstrated this effect for *S.*
13 *oryzae* with 625 ppm ground black peppercorns, which had low
14 toxicity but increased the median emergence date and the range of
15 emergence for F₁ adults. It is also possible, but unlikely, that
16 the increased number of live *Z. subfasciatus* at moderate
17 concentrations is due to enhanced longevity of emerged adults
18 (Lambert et al. 1985). There was also significant larval
19 mortality (adults per eggs hatched) for *Z. subfasciatus* for the
20 10% crushed concentration from Lot A and Lot B. This may have
21 been largely due to oviposition on a greatly reduced number of
22 beans, which would increase larval competition within the bean.
23 This does not coincide with sex ratio alteration which occurred
24 only in Experiment 1 for *Z. subfasciatus*, although it might be
25 logical to assume that the larger females (Howe and Currie 1964)
26 were more competitive inside the bean.

1 In summary, the efficacy of the dried leaves of *T. riparia*
2 is governed primarily by decreases in the fecundity and, to a
3 lesser extent, the fertility of the parental females with high
4 concentrations of the plant material. F₁ larval mortality was
5 also increased at higher concentrations. Bean protection was
6 more likely to be due to behavioral effects induced by physical
7 and/or chemical properties of the odorous leaves of this
8 perennial mint, because in replicates that contained greater
9 quantities of leaves, oviposition by *Z. subfasciatus* was observed
10 upon fewer beans. Any physiological effects upon the fecundity
11 or the fertility of individual females is less likely, since the
12 parental adults were removed directly from the stock culture and
13 added to the experiment without any prior exposure to *T. riparia*
14 volatiles.

15 In an earlier study (Dunkel et al. 1986) it was found that
16 beans with bruchid emergence holes were unacceptable for either
17 planting or food and were discarded by Rwandan consumers. At the
18 4% crushed concentration in our experiments there was nearly a
19 50% reduction in the number of beans oviposited upon by *Z.*
20 *subfasciatus* and at the 10% crushed concentration there was a 70%
21 reduction in F₁ emergence by *A. obtectus*. This indicates that
22 there will be fewer emergence holes and that the net losses in
23 stored beans to Rwandan consumers may be considerably reduced by
24 using the crushed dried leaves of *T. riparia* at these levels.

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Figure Legends

Figure 1. Mean proportion of eggs hatched (\pm S.E.) per *Z. subfasciatus* parental cohort (5♀, 5♂) exposed to crushed leaves of *T. riparia* (Lot B).

Means \pm standard errors are for illustrative purposes. Regression based on raw data for each cohort, $F = 59.64$; $df = 1, 58$; $P < 0.0001$.

Figure 2. Mean percentage beans oviposited on (\pm S.E.) per *Z. subfasciatus* parental cohort (5♀, 5♂) exposed to crushed leaves of *T. riparia* (Lot B).

Means \pm standard errors are for illustrative purposes. Regression based on raw data for each cohort, $F = 166.51$; $df = 1, 58$; $P < 0.0001$.

Figure 3. Mean total number of eggs (\pm S.E.) per *A. obtectus* parental cohort (4♀, 4♂) exposed to crushed leaves of *T. riparia* (Lot B).

Means \pm standard errors are for illustrative purposes. Regression based on raw data for each cohort, Total eggs- $F = 84.84$; $df = 1, 44$; $P < 0.0001$, Hatched eggs- $F = 78.04$; $df = 1, 44$; $P < 0.0001$.

Figure 4. Mean total number of adults at 55 days (\pm S.E.) per *A. obtectus* parental cohort (5♀, 5♂) exposed to crushed leaves of *T. riparia* (Lot B).

Means \pm standard errors are for illustrative purposes.

Regression based on raw data for each cohort, Females- $F = 18.02$;
df = 2, 44; $P < 0.0001$, Males- $F = 28.90$; df = 2, 44; $P < 0.0001$.

Running Head: Weaver et al. Medicinal mint inhibition of
bruchid populations.

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Journal Section: **Physiology and Chemical Ecology**

13

Table 1. Mean oviposition and developmental parameters (\pm S.E.) per *Z. subfasciatus* parental cohort (5♀, 5♂) exposed to milled or crushed leaves of *T. riparia* (Lot A).

Process	Conc. (% w/w)	*Total Eggs At 25 Days	^b Hatched Eggs At 25 days	^c Percent Hatched At 25 Days	^d Percent Adults Per Eggs Laid	^e Percent Adults Per Eggs Hatched
Control	0	143 \pm 6	136 \pm 6	94.53 \pm 0.01	80.31 \pm 0.01	84.88 \pm 0.01
Milled	0.1	167 \pm 9	160 \pm 9	96.04 \pm 0.00	76.25 \pm 0.02	79.66 \pm 0.02
	0.5	156 \pm 8	146 \pm 9	93.20 \pm 0.01	73.83 \pm 0.02 [*]	79.38 \pm 0.02
	1.0	168 \pm 4 [*]	157 \pm 4 [*]	93.15 \pm 0.02	81.63 \pm 0.02	86.75 \pm 0.01
	10.0	84 \pm 14 [*]	67 \pm 12 ^{1*}	78.97 \pm 0.04 [*]	61.92 \pm 0.05 [*]	76.59 \pm 0.04 [*]
Crushed	0.1	156 \pm 13	147 \pm 12	94.26 \pm 0.01	76.00 \pm 0.01	80.66 \pm 0.01
	0.5	155 \pm 7	147 \pm 12	94.78 \pm 0.01	75.59 \pm 0.03	79.72 \pm 0.03
	1.0	172 \pm 6 [*]	156 \pm 6	90.87 \pm 0.01	75.16 \pm 0.02	82.59 \pm 0.02
	10.0	116 \pm 10 [*]	94 \pm 7 ^{1*}	82.00 \pm 0.03 [*]	53.61 \pm 0.04 [*]	65.94 \pm 0.06 [*]

*Total Eggs At 25 Days. Process * Concentration- $F = 2.63$; $df = 3, 72$; $p = 0.0563$.

Milled Concentration- $F = 15.48$, $df = 4, 55$; $p < 0.0001$. *Concentration vs Control, 1% - $t = 2.02$; $df = 28$; $p = 0.0484$, 10% - $t = 5.74$; $df = 28$; $p < 0.0001$.

Crushed Concentration- $F = 5.21$, $df = 4, 55$; $p = 0.0006$. *Concentration vs Control, 1% - $t = 2.45$; $df = 28$; $p = 0.0177$, 10% - $t = 4.33$; $df = 28$; $p < 0.0001$.

^bHatched Eggs At 25 Days. Process * Concentration- $F = 2.97$; $df = 3, 72$; $p = 0.0373$. *Concentration vs Concentration- $t = 2.99$; $df = 18$; $p = 0.0036$. *Concentration vs Control, 1% Milled - $t = 2.06$; $df = 28$; $p = 0.0425$, 10% Milled - $t = 7.41$; $df = 28$; $p < 0.0001$, 10% Crushed - $t = 3.95$; $df = 28$; $p < 0.0001$.

^cPercent Hatched At 25 Days. Process * Concentration- $F = 0.90$; $df = 3, 72$; $p = 0.4442$.

Milled Concentration- $F = 13.18$; $df = 4, 55$; $p < 0.0001$. *Concentration vs Control - $t = 6.54$; $df = 28$; $p < 0.0001$.

Crushed Concentration- $F = 13.38$; $df = 4, 55$; $p < 0.0001$. *Concentration vs Control, 1% - $t = 2.61$; $df = 28$; $p = 0.0115$, 10% - $t = 6.73$; $df = 28$; $p < 0.0001$.

^dPercent Adults Per Eggs Laid. Process * Concentration- $F = 1.48$; $df = 3, 72$; $p = 0.2282$.

Milled Concentration- $F = 9.05$; $df = 4, 55$; $p < 0.0001$. *Concentration vs Control, 0.5% - $t = 2.10$; $df = 28$; $p = 0.0387$, 10% - $t = 5.33$; $df = 28$; $p < 0.0001$.

Crushed Concentration- $F = 14.61$; $df = 4, 55$; $p < 0.0001$. *Concentration vs Control - $t = 7.57$; $df = 28$; $p < 0.0001$.

^ePercent Adults Per Eggs Hatched. Process * Concentration- $F = 1.50$; $df = 3, 72$; $p = 0.2207$.

Milled Concentration- $F = 4.59$; $df = 4, 55$; $p = 0.0014$. *Concentration vs Control - $t = 2.49$; $df = 28$; $p = 0.0146$.

Crushed Concentration- $F = 6.15$; $df = 4, 55$; $p = 0.0001$. *Concentration vs Control - $t = 5.30$; $df = 28$; $p < 0.0001$.

Table 2. Mean oviposition and developmental parameters (\pm S.E.) per *Z. subfasciatus* parental cohort (5♀, 5♂) exposed to crushed leaves of *T. riparia* (Lot B).

Conc. (% w/w)	^a Total Eggs At 25 Days	^b Hatched Eggs At 25 Days	^c Percent Adults Per Eggs Laid	^d Percent Adults Per Eggs Hatched
0	122 \pm 6	118 \pm 6	82.83 \pm 0.01	85.39 \pm 0.02
1	109 \pm 8	105 \pm 8	77.37 \pm 0.03	80.91 \pm 0.03
2	120 \pm 4	114 \pm 4	77.92 \pm 0.03	81.79 \pm 0.03
3	125 \pm 6	120 \pm 6	78.77 \pm 0.04	82.28 \pm 0.04
4	120 \pm 9	110 \pm 10	71.55 \pm 0.05 ^e	79.22 \pm 0.06
10	90 \pm 5 ^e	78 \pm 5 ^e	53.57 \pm 0.04 ^e	62.29 \pm 0.05 ^e

^aTotal Eggs At 25 Days. Concentration- $F = 4.44$; $df = 5, 54$; $P = 0.0010$. Concentration vs Control- $t = 3.63$; $df = 18$; $P < 0.0001$.

^bHatched Eggs At 25 Days. Concentration- $F = 6.01$; $df = 5, 54$; $P = 0.0001$. Concentration vs Control- $t = 4.52$; $df = 18$; $P < 0.0001$.

^cPercent Adults Per Eggs Laid. Concentration- $F = 7.67$; $df = 5, 54$; $P < 0.0001$. Concentration vs Control, 4% - $t = 2.19$; $df = 18$; $P = 0.0330$, 10% - $t = 5.47$ $df = 18$; $P < 0.0001$.

^dPercent Adults Per Eggs Hatched. Concentration- $F = 3.80$; $df = 5, 54$; $P = 0.0031$. Concentration vs Control- $t = 5.38$; $df = 18$; $P < 0.0001$.

Table 3. Mean F₁ population and productivity parameters (\pm S.E.) per *Z. subfasciatus* parental cohort (5♀, 5♂) exposed to milled or crushed leaves of *T. riparia* (Lot A).

Process	Conc. (% w/w)	^a Total Adults At 55 Days	^b Live Adults At 55 Days	^c Live ♀ At 55 Days	^d Sex Ratio (♀/♂) At 55 Days	Adults/ F ₁ ♀ At 85 Days	^e Adult Progeny At 85 Days
Control	0	116 \pm 6	22 \pm 2	11 \pm 1	1.14 \pm 0.04	3.3 \pm 0.4	301 \pm 9
Milled	0.1	128 \pm 9	16 \pm 3	10 \pm 2	1.11 \pm 0.04	2.2 \pm 0.4	280 \pm 15
	0.5	115 \pm 6	25 \pm 4	15 \pm 2	1.12 \pm 0.06	3.7 \pm 0.5	330 \pm 21
	1.0	137 \pm 4 ^e	43 \pm 5 ^h	30 \pm 3 ^h	1.07 \pm 0.04	2.7 \pm 0.2	326 \pm 15 ⁱ
	10.0	54 \pm 11 ^e	7 \pm 2 ^h	4 \pm 1 ^e	1.39 \pm 0.26	7.9 \pm 1.3 ^e	231 \pm 20 ^h
Crushed	0.1	119 \pm 11	11 \pm 2 ^e	8 \pm 1	1.27 \pm 0.13	2.8 \pm 0.5	278 \pm 18
	0.5	117 \pm 7	33 \pm 5 ^e	17 \pm 2 ^e	1.08 \pm 0.08	3.9 \pm 0.5	338 \pm 22
	1.0	129 \pm 5	14 \pm 2 ^h	9 \pm 1 ⁱ	1.23 \pm 0.07	1.4 \pm 0.1 ^e	228 \pm 8 ^h
	10.0	62 \pm 7 ^e	13 \pm 2 ^h	8 \pm 1	1.44 \pm 0.16	7.6 \pm 1.0 ^e	307 \pm 36 ⁱ

^aTotal Adults At 55 Days. Process * Concentration- $F = 0.79$; $df = 3, 72$; $P = 0.5059$.

Milled Concentration- $F = 19.08$; $df = 4, 55$; $P < 0.0001$. Concentration vs Control, 1% - $t = 2.013$; $df = 28$; $P = 0.0470$, 10% - $t = 7.15$; $df = 28$; $P < 0.0001$.

Crushed Concentration- $F = 13.21$; $df = 4, 55$; $P < 0.0001$. Concentration vs Control- $t = 5.79$; $df = 28$; $P < 0.0001$.

^bLive Adults At 55 Days. Process * Concentration- $F = 12.53$; $df = 3, 72$; $P < 0.0001$. Concentration vs Concentration¹, 1% - $t = 5.60$; $df = 18$; $P < 0.0001$, 10% - $t = 2.28$; $df = 18$; $P = 0.0252$. Concentration vs. Control, 0.1% Crushed- $t = 3.24$; $df = 28$; $P = 0.0017$, 0.5% Crushed- $t = 2.45$; $df = 28$; $P = 0.0161$, 1% Crushed- $t = 2.22$; $df = 28$; $P = 0.0287$, 10% Crushed- $t = 2.52$; $df = 28$; $P = 0.0134$, 1% Milled- $t = 4.25$; $df = 28$; $P < 0.0001$, 10% Milled- $t = 5.15$; $df = 28$; $P < 0.0001$.

^cLive Females At 55 Days. Process * Concentration- $F = 14.72$; $df = 3, 72$; $P < 0.0001$. Concentration vs Concentration¹, 1% - $t = 6.66$; $df = 18$; $P < 0.0001$, 10% - $t = 2.35$; $df = 18$; $P = 0.0208$. Concentration vs Control, 0.5% Crushed- $t = 2.11$; $df = 28$; $P = 0.0373$, 1% Milled- $t = 6.45$; $df = 28$; $P < 0.0001$, 10% Milled- $t = 4.37$; $df = 28$; $P < 0.0001$.

^dSex Ratio At 55 Days. Process * Concentration- $F = 0.32$; $df = 3, 72$; $P = 0.8087$.

Milled Concentration- $F = 0.88$; $df = 4, 55$; $P = 0.4986$.

Crushed Concentration- $F = 1.83$; $df = 4, 55$; $P = 0.1213$.

^eAdults Per F₁ Female At 85 Days. Process * Concentration- $F = 2.33$; $df = 3, 72$; $P = 0.0817$.

Milled Concentration- $F = 12.43$; $df = 4, 55$; $P < 0.0001$. Concentration vs Control- $t = 5.65$; $df = 28$; $P < 0.0001$.

Crushed Concentration- $F = 17.97$; $df = 4, 55$; $P < 0.0001$. Concentration vs Control, 1% - $t = 3.77$; $df = 28$; $P < 0.0001$, 10% - $t = 5.69$; $df = 28$; $P < 0.0001$.

^fAdult Progeny At 85 Days. Process * Concentration- $F = 6.15$, $df = 3, 72$; $P < 0.0001$. Concentration vs Concentration¹, 1% - $t = 3.70$; $df = 18$; $P < 0.0001$, 10% - $t = 2.77$; $df = 18$; $P = 0.0068$. Concentration vs Control, 1% Crushed- $t = 3.28$; $df = 28$; $P = 0.0015$, 10% Milled- $t = 3.29$; $df = 28$; $P = 0.0014$.

Table 4. Mean F₁ population and productivity parameters (\pm S.E.) per *Z. subfasciatus* parental cohort (5♀, 5♂) exposed to crushed leaves of *T. riparia* (Lot B).

Conc. (% w/w)	^a Total Adults At 55 Days	^b Live Adults At 55 Days	^c Live ♀ At 55 Days	^d Sex Ratio (♀/♂) At 55 Days	^e Adults/ F ₁ ♀ At 85 Days	^f Adult Progeny At 85 Days
0	101 \pm 5	49 \pm 7	23 \pm 3	1.00 \pm 0.07	5.1 \pm 0.5	348 \pm 22
1	84 \pm 5	61 \pm 6	33 \pm 5	1.18 \pm 0.08	4.5 \pm 0.5	278 \pm 18 [*]
2	93 \pm 4	63 \pm 6 [*]	30 \pm 3	1.07 \pm 0.07	4.8 \pm 0.4	314 \pm 17
3	100 \pm 7	93 \pm 8 [*]	47 \pm 4 [*]	1.05 \pm 0.07	4.2 \pm 0.7	291 \pm 13
4	86 \pm 9	81 \pm 9 [*]	41 \pm 5 [*]	1.16 \pm 0.06	4.6 \pm 0.5	279 \pm 17 [*]
10	48 \pm 5 [*]	45 \pm 6	22 \pm 3	1.07 \pm 0.10	11.1 \pm 2.4 [*]	286 \pm 27 [*]

^aTotal Adults At 55 Days. Concentration- $F = 11.85$; $df = 5, 54$; $P < 0.0001$. ^{*}Concentration vs Control- $t = 6.61$; $df = 18$; $P < 0.0001$.

^bLive Adults At 55 Days. Concentration- $F = 6.66$; $df = 5, 54$; $P < 0.0001$. ^{*}Concentration vs Control, 2%- $t = 2.02$; $df = 18$; $P = 0.0483$, 3%- $t = 4.74$; $df = 18$; $P < 0.0001$, 4%- $t = 3.63$; $df = 18$; $P < 0.0001$.

^cLive Females At 55 Days. Concentration- $F = 6.28$; $df = 5, 54$; $P < 0.0001$. ^{*}Concentration vs Control, 3%- $t = 4.25$; $df = 18$; $P < 0.0001$, 4%- $t = 3.45$; $df = 18$; $P = 0.0015$.

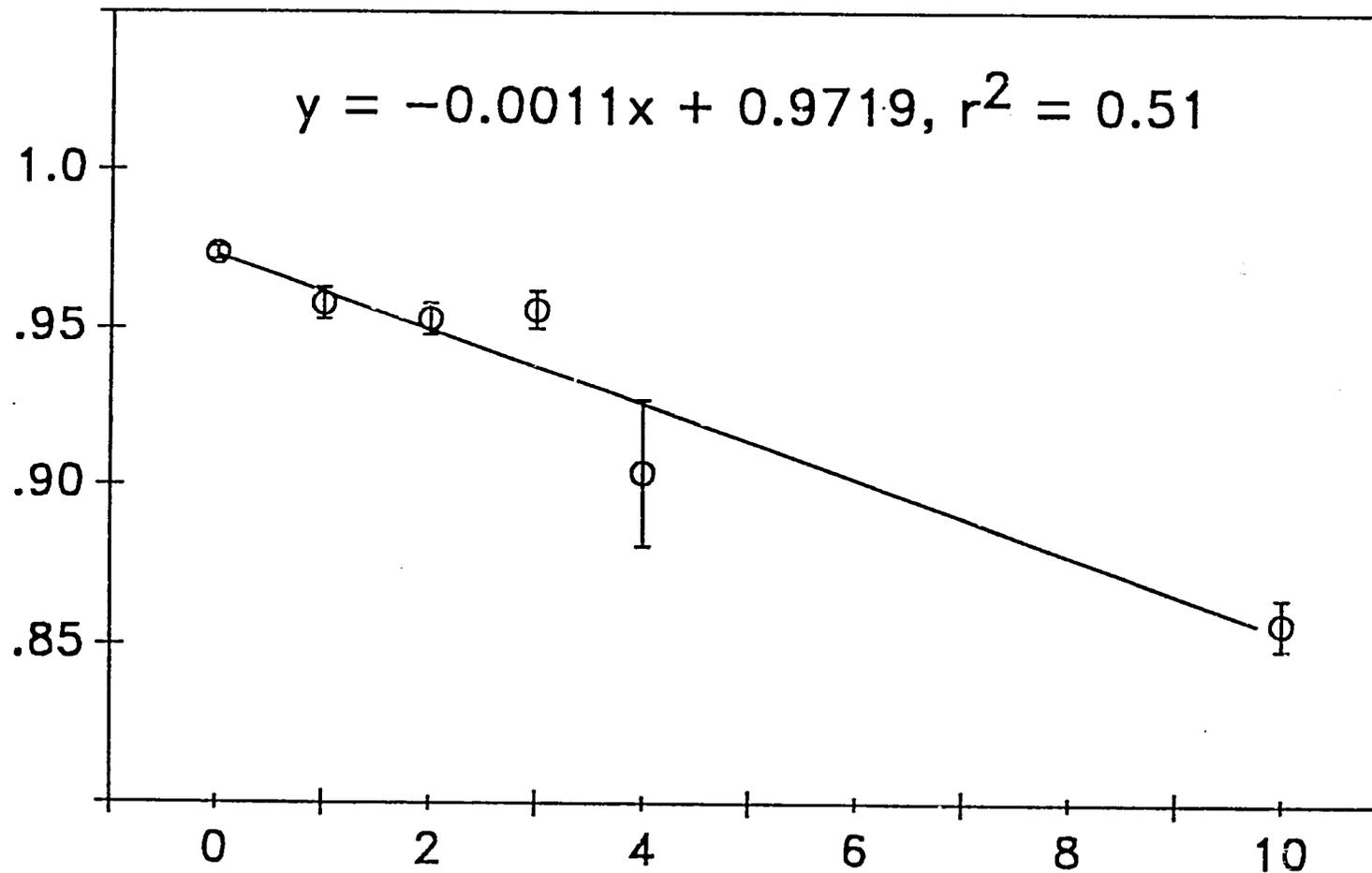
^dSex Ratio At 55 Days. Concentration- $F = 0.93$; $df = 5, 54$; $P = 0.4807$.

^eAdults Per F₁ Female At 85 Days. Concentration- $F = 10.69$; $df = 5, 54$; $P < 0.0001$. ^{*}Concentration vs Control- $t = 5.49$; $df = 18$; $P < 0.0001$.

^fAdult Progeny At 85 Days. Concentration- $F = 1.92$; $df = 5, 54$; $P = 0.0938$. ^{*}Concentration vs Control, 1%- $t = 2.48$; $df = 18$; $P = 0.0162$, 4%- $t = 2.46$; $df = 18$; $P = 0.0173$, 10%- $t = 2.30$; $df = 18$; $P = 0.0252$.

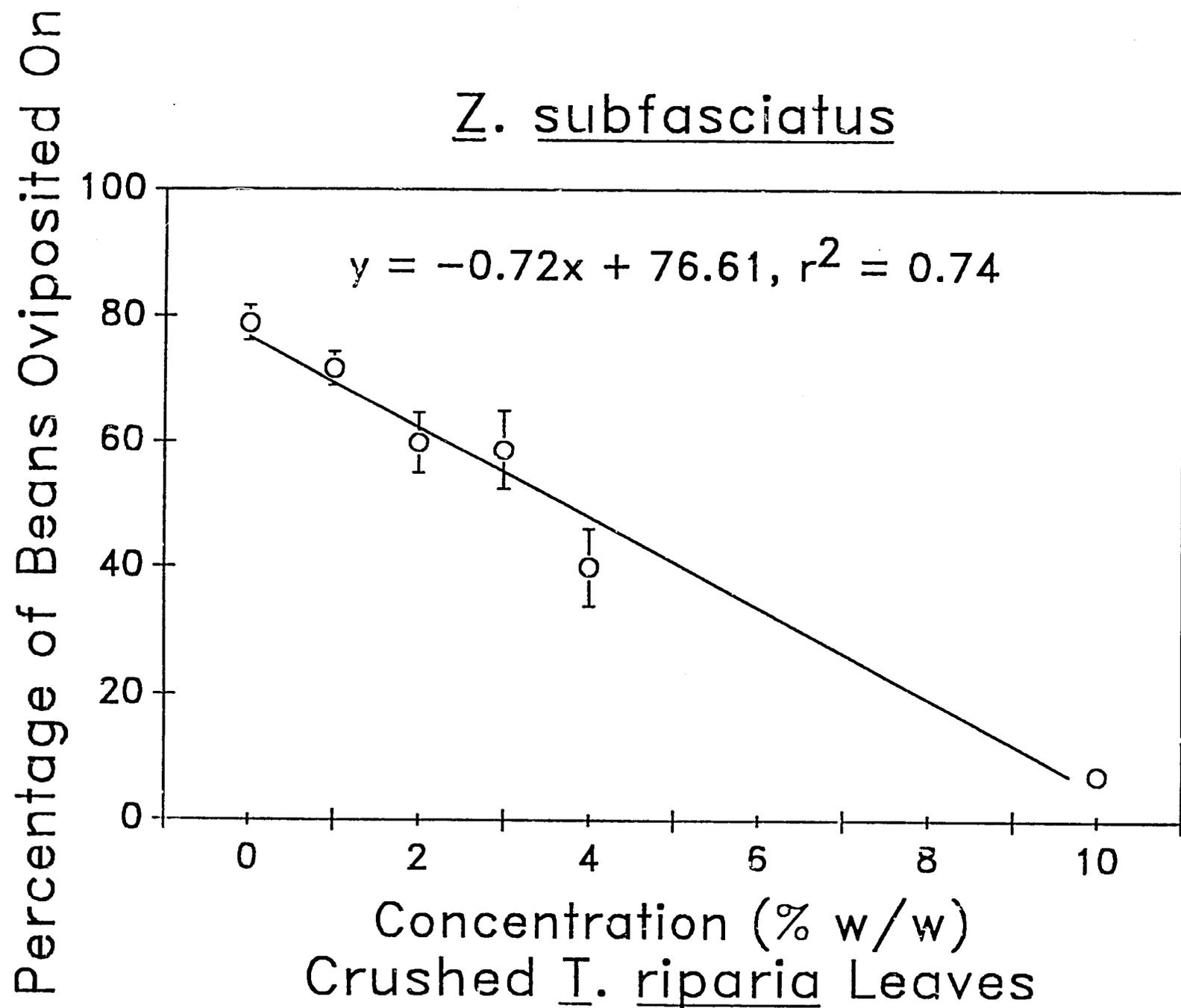
Z. subfasciatus

Proportion of Eggs Hatched

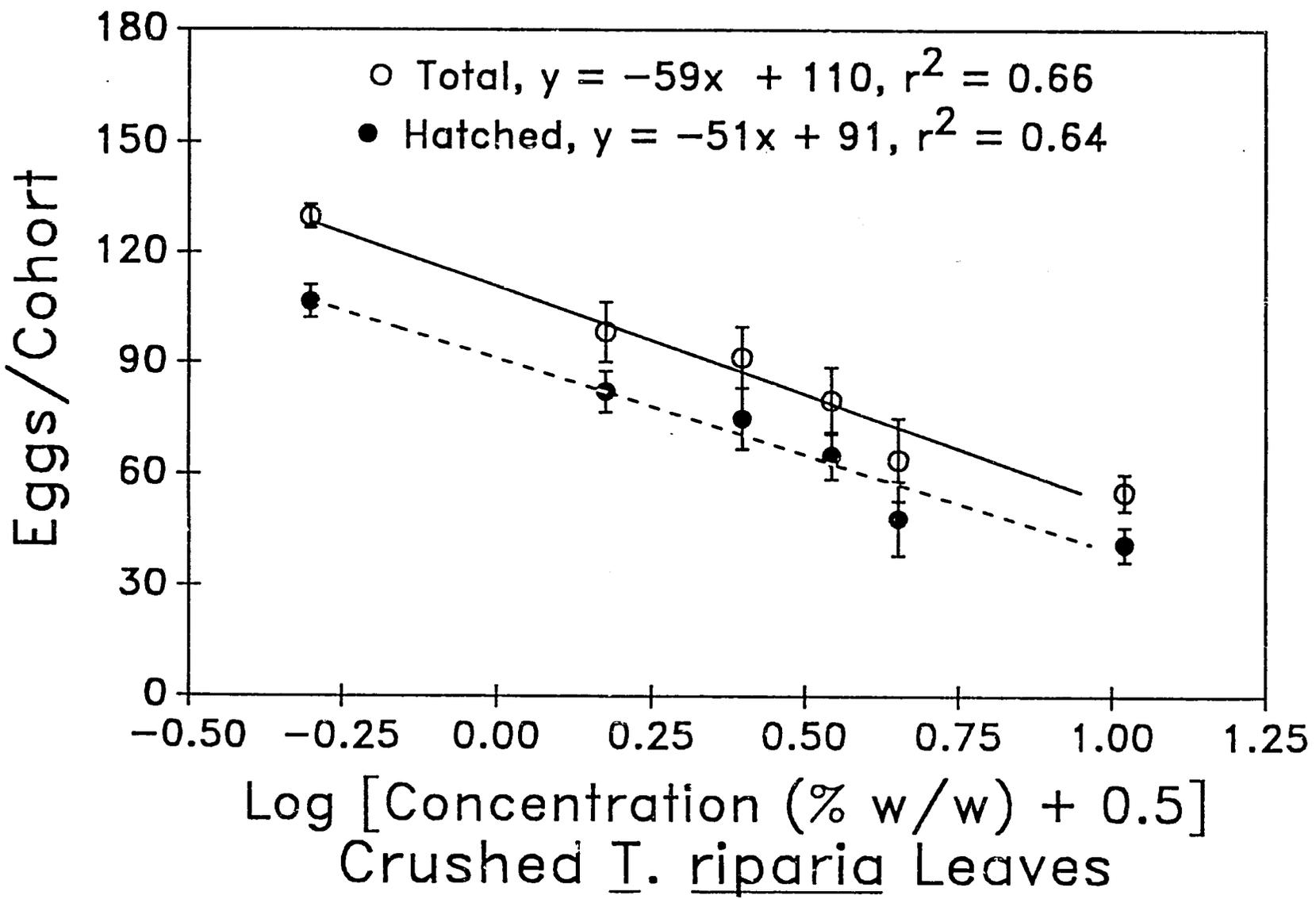


Concentration (% w/w)
Crushed T. riparia Leaves

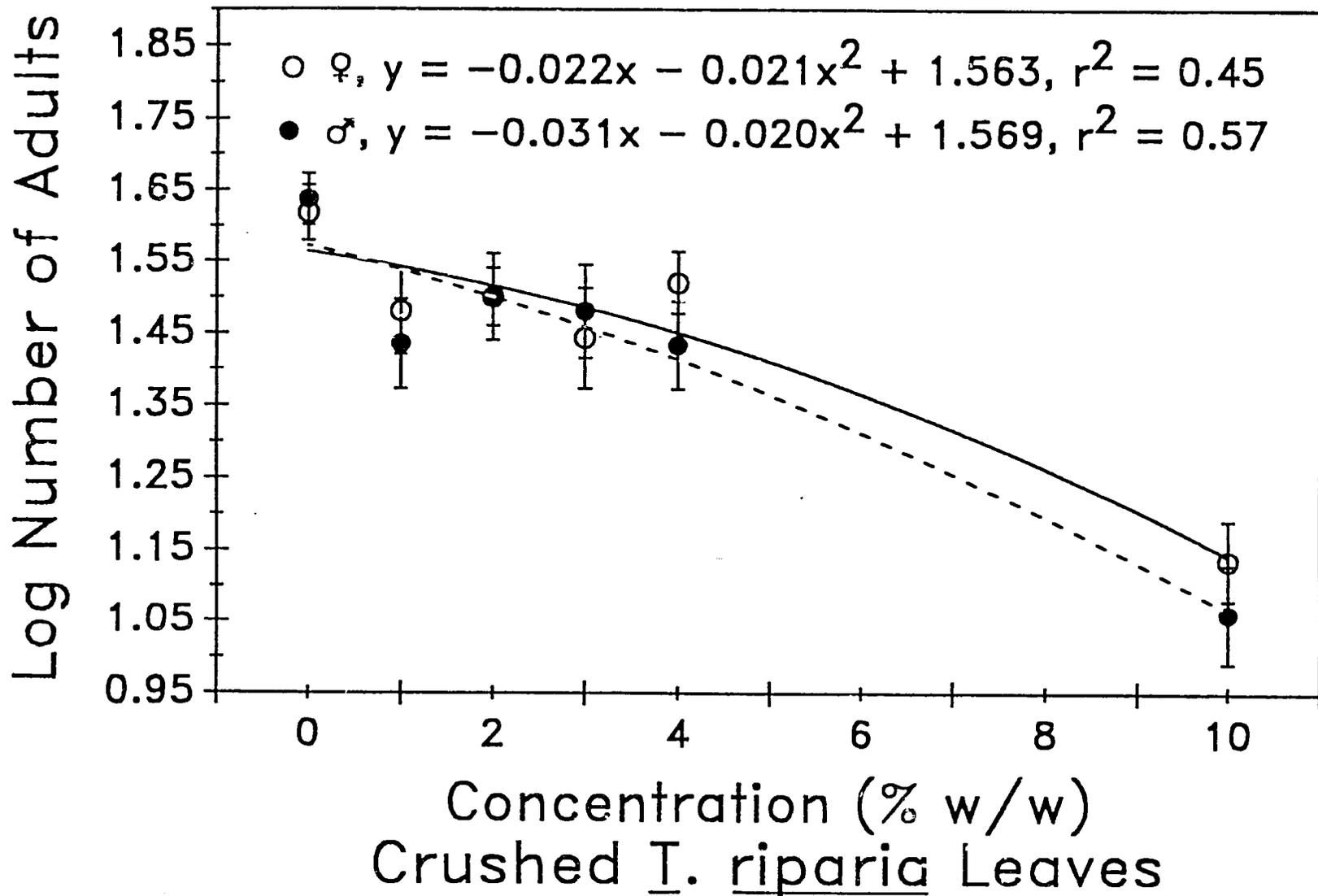
Z. subfasciatus



A. obtectus



A. obtectus



Appendix 4. Refereed journal article in review. "Toxicity and protectant potential of the essential oil of Tetradenia riparia (Lamiales: Lamiaceae) against Zabrotes subfasciatus (Coleoptera: Bruchidae) infesting dried Pinto beans (Fabales: Leguminosae)." to be submitted to the Journal of Applied Entomology. in USDA-ARS and MSU review.

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Toxicity and protectant potential of the essential oil
of *Tetradenia riparia* (Lamiales: Lamiaceae) against
Zabrotes subfasciatus (Coleoptera: Bruchidae) infesting
dried Pinto beans (Fabales: Leguminosae).

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1 We conclude that the essential oil of *T. riparia* may make an effective seed
2 treatment against *Z. subfasciatus* at 1.7 liters/tonne. Additional research must
3 evaluate mammalian toxicity and subsequently, if practical, palatability of these
4 treated beans.

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1 SINGH and RAO 1985, SU 1991a, SU 1991b). In addition, the efficacy of powders
2 and extracts of powders of tissues of various plant species have been determined
3 for adults of several species of bruchids (SU 1984a; 1984b, DON-PEDRO 1985,
4 LAMBERT et al. 1985, DELOBEL and MALONGA 1987, SU and HORVAT 1987, SU 1990,
5 WEAVER et al. 1991, Weaver et al. In press). However, no research has directly
6 determined the ovicidal and larvicidal properties of an essential oil against a
7 bruchid species on a commodity, although many have evaluated protectant activity
8 against adults in both choice and no choice tests. Here we report the ovicidal,
9 larvicidal, and acute adult toxicity of the essential oil of *T. riparia* to *Z.*
10 *subfasciatus*. In addition, long term protectant experiments were conducted in
11 both choice and no-choice tests. The effect of the essential oil on seed
12 germination was also evaluated at 6 months.

13 2 Materials and methods

14 2.1 Insect culture

15 *Z. subfasciatus* were maintained on dried Pinto beans (*Phaseolus vulgaris* L.)
16 at $27 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH, with a 12:12 (L:D) photoperiod. The beans (0.5 liter,
17 equilibrium moisture content = 13.8%) were placed in 0.95 liter jars and
18 inoculated with ca. 300 adult bruchids. Culture cohorts were discarded after the
19 emergence of two subsequent generations. The stock culture had been laboratory
20 maintained for many years.

21 2.2 Preparation and characterization of the essential oil

1 Fresh leaves of *T. riparia* were collected from the area surrounding Butaré,
2 Rwanda and the essential oil was prepared on a pilot-scale Clevenger-type
3 hydrodistillation apparatus (yield- 0.07% w/w). The oil was express-shipped to
4 Montana State University and stored at -20°C until usage. A large composite
5 sample (40 ml) was prepared to eliminate potential variability among experiments.
6 The density of this essential oil was 0.92 g/ml.

7 Oil from this composite sample was subjected to gas chromatography- mass
8 spectroscopy (GC-MS) analysis at the initiation and termination of these
9 experiments. GC was conducted on a Varian Model 3700 equipped with a 30m X
10 0.25mm (i.d.) DB-5 column with a 0.25 μ m film thickness. GC conditions: He
11 carrier gas velocity- 30 cm₃/s (220°C); temperature programming- initial
12 temperature- 50°C, initial hold- 4.0 min, temperature increase- 5°C/min, final
13 temperature- 280°C, final hold- 10 min, injection port temperature- 260°C,
14 detector temperature- 290°C. Electron impact MS were obtained on a VG Analytical
15 VG 70EHF operating at 70 eV with a source temperature of 200°C.

16 2.3 Adult toxicity bioassay

17 A 5.5 cm diam Whatman #1 filter paper was placed in the inverted lid of a
18 5.0 cm (internal diameter) glass Corning petri dish. A 0.5 ml aliquot of the
19 appropriate dilution of *T. riparia* essential oil in absolute ethanol was applied
20 to the filter paper. The control was absolute ethanol only. Ethanol was
21 evaporated for 20 min prior to the addition of the insects (0-2 days post-adult
22 eclosion, 5♂ and 5♀). Concentrations of oil used were 396, 791, 1583 and 3165
23 μ g/cm² of filter paper. Knockdown was assessed at 0.25, 6, and 18 hr after the
24 addition of the insects. Mortality and moribundity were assessed at 24 hr.

1 Moribundity was evaluated by righting individuals ambulating weakly while resting
2 on their dorsal surfaces. Those that were unable to remain upright were
3 classified as moribund. The criteria for mortality was the failure to elicit any
4 motion after three probings with a blunt dissecting probe. Insects which were
5 moribund after exposure to higher concentrations of oil died within 24hr, so
6 mortality and moribundity were pooled for probit analysis. These bioassays were
7 conducted at $23 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ RH with a photoperiod of 14L:10D. There were
8 ten replicates of each treatment and ten of the control.

9 2.4 Determination of Pinto bean surface area

10 Pinto bean masses were determined on a Mettler AT-250 Digital Balance
11 (sensitivity- 0.1 mg). Subsequently, beans were soaked in reagent grade acetone
12 for 24 hours. The resultant endosperm mass was reduced so that the testa was
13 loose and readily removed using a sharp scalpel. The removed fragments of the
14 testa were pressed flat between two microscope slides that were taped tightly
15 together using transparent tape. The surface area of the testa for individual
16 beans was determined three times using a Li-Cor Model LI-3100 Area Meter (Li-Cor,
17 Inc., Lincoln, Nebraska, U.S.A.) set at 0.001 cm² sensitivity. The means of the
18 triplicate-determined surface areas were then regressed against initial mass to
19 derive a predictive equation, surface area (cm²) = 4.51 X mass (g) + 0.70, r² =
20 0.92; $t = 14.5$, $P < 0.0001$; $F_{1,18} = 211.18$, $P < 0.0001$. Thus, each 20 g replicate
21 of beans was treated for a theoretical surface area of 127.08 cm² (based on a
22 grand mean number of beans of 53.65 ± 0.092 (S.D.) with a grand mean bean mass
23 of $0.37 \text{ g} \pm 0.0027$ (S.D.)). This procedure aided in the conservation of the oil
24 by facilitating the calculation of concentrations to be tested in beans based on

1 the same units as those used in the initial acute toxicity study on filter paper.
2 This reduced the number of experiments needed to yield data that indicated
3 partial inhibition of the insect population.

4 2.5 Treatment of beans with essential oil

5 *T. riparia* essential oil is insoluble in water. Emulsions were prepared
6 in distilled, de-ionized water using 0.5% Triton X-100® to better approximate
7 actual field usage, rather than using solutions prepared in a volatile organic
8 solvent. Five experiments were conducted which used 1.0 ml aliquots of these
9 emulsions to deliver the appropriate concentration of essential oil to 20 g of
10 Pinto beans (14.1% equilibrium moisture content) in a 10 cm Petri dish lid. The
11 liquid was uniformly applied to the surface of the beans by gentle agitation
12 using 20 semi-circular arm motions. The water was then evaporated for 2 hr in the
13 open container, after which the bioassay was initiated.

14 2.6 Evaluation of protectant potential of freshly-applied oil against adults.

15 Two such experiments were conducted. Concentrations of 0, 5.4, 26.8, 53.6,
16 267.8, 535.5, and 1071 μg of essential oil per cm^2 of Pinto bean surface were
17 used in the initial experiment, but the 53.6 - 267.8 $\mu\text{g}/\text{cm}^2$ range nearly
18 completely spanned those treatments that could cause partial suppression of the
19 resulting insect population. Therefore, a second experiment using concentrations
20 of 0, 71.4, 142.8, 214.2, 285.6 and 357 μg of *T. riparia* oil per cm^2 of Pinto
21 beans were prepared as described above to yield 20 g of treated beans. The
22 treated beans were transferred to a 10 dram (2.5 cm internal diameter X 8.0 cm
23 height) plastic snap-cap vial immediately after H_2O evaporation and ten adult *Z.*

1 *subfasciatus* (5♂ and 5♀, 0-1 days post-adult emergence) were added. The
2 fecundity of females and fertility of eggs laid was assessed at 25 days and the
3 number of emergent F₁ adult progeny determined at 55 days. The bioassay was
4 conducted at 27 ± 1°C, 65 ± 5% RH, with a 12:12 (L:D) photoperiod. There were
5 ten replicates of all concentrations and controls.

6 2.7 Evaluation of the ovicidal activity of freshly-applied oil.

7 Cohorts of 10♂ and 10♀ adult *Z. subfasciatus* (0-1 days post-adult
8 emergence) were added to 20g of equilibrated (as above) Pinto beans and allowed
9 to mate and oviposit for 24 hr prior to removal. The eggs were allowed to harden
10 for 24 hr after which the beans and the eggs attached to them were treated.
11 Concentrations of 0, 5.4, 26.8, 53.6, 267.8, 535.5, and 1071 µg of essential oil
12 per cm² of Pinto bean surface were initially used, but again the 53.6 - 267.8
13 µg/cm² range nearly completely spanned those treatments that could cause partial
14 suppression of the resulting insect population. Therefore, a second experiment
15 using concentrations of 0, 71.4, 142.8, 214.2, 285.6, and 357 µg of essential oil
16 per cm² of Pinto bean was conducted. Disturbance of the beans during treatment
17 resulted in mortality due to egg destruction, so an additional undisturbed
18 control was included to measure this. Egg destruction during treatment made
19 accurate counts of the number of treated eggs and subsequent determination of
20 fertility impossible (even careful attempts to pre-count eggs with minimal
21 handling were found to significantly alter fertility). Therefore, number of F₁
22 adult progeny at 55 days was used to measure ovicidal activity of the essential
23 oil relative to that of the disturbed controls (H₂O + emulsifier only and H₂O
24 only). The bioassay was conducted at 27 ± 1°C, 65 ± 5% RH, with a 12:12 (L:D)

1 photoperiod in plastic 12 dram (2.9 cm internal diameter X 7.0 cm height) snap-
2 cap vials in which the treated material was placed immediately after H₂O
3 evaporation. There were ten replicates of all concentrations and controls.

4 2.8 Evaluation of the larvicidal activity of freshly-applied oil.

5 Cohorts of 10♂ and 10♀ adult *Z. subfasciatus* (0-1 days post-adult
6 emergence) were added to 20g of equilibrated (as above) Pinto beans and allowed
7 to mate and oviposit for 24 hr prior to removal. The eggs were allowed to mature
8 with subsequent larval penetration under the controlled conditions (described
9 above) for a period of seven additional days. At this point the beans were
10 treated with concentrations of 0, 5.4, 26.8, 53.6, 267.8, 535.5, and 1071 µg of
11 essential oil per cm² of Pinto bean surface. This procedure once again made
12 accurate assessment of initial egg number and associated fertility impossible.
13 F₁ adult progeny were counted at 55 days and adult emergence was again determined
14 at 85 days. The bioassay was conducted at 27 ± 1°C, 65 ± 5% RH, with a 12:12
15 (L:D) photoperiod in plastic 12 dram (2.9 cm internal diameter X 7.0 cm height)
16 snap-cap vials in which the treated material was placed immediately after H₂O
17 evaporation. There were ten replications of all concentrations and controls.

18 2.9 Evaluation of the protectant potential of oil against adults
19 at 210 days post-application in oviposition choice and no-choice tests.

20 Beans treated with 0, 5.4, 26.8, 53.5, 267.8, 535.5, and 1071 µg of
21 essential oil per cm² of surface were stored in an incubator under bioassay
22 conditions for 210 days. At this time, three treated and three control beans

1 (both at 14.2% equilibrium moisture content) were mixed and placed in a 20 dram
2 plastic snap-cap vial (4.8 cm internal diameter X 6.2 cm height). Beans were
3 identified using a small colored ink dot on both treated and control beans
4 (colors were exchanged among replicates, red and green were used). Two male and
5 two female adult *Z. subfasciatus* (0-1 days post-adult eclosion) were added and
6 allowed to oviposit until death. At 25 days the number of eggs laid and eggs
7 hatched on the treatments and the control were determined, no accurate counts
8 could be conducted subsequently due to the potential for cannibalism among
9 conspecifics within the beans. However, the relative activity of the material
10 against oviposition could be readily assessed. The bioassay was conducted at 27
11 $\pm 1^{\circ}\text{C}$, $65 \pm 5\%$ RH, with a 12:12 (L:D) photoperiod. There were four replications
12 of each concentration. The no-choice experiment was conducted exactly as in the
13 choice test except that only two beans were used and both had been treated 210
14 days previously. There were four replications of each concentration.

15 2.10 Evaluation of germination of Pinto beans

16 treated with essential oil at 180 days post-treatment.

17

18 Beans treated with 0, 5.4, 26.8, 53.6, 267.8, 535.5, and 1071 μg of
19 essential oil per cm^2 of surface were stored in an incubator under bioassay
20 conditions for 180 days. At this time five beans for each concentration were
21 placed in equally spaced, randomized locations on each of four isocratic (27.0
22 $\pm 0.6^{\circ}\text{C}$) thermal gradient bars (15.2 cm wide by 106.7 cm long). The bar was
23 covered with Whatman #1 chromatography paper that was thoroughly wetted with
24 distilled H_2O and maintained moistened by wicking from reservoirs containing
25 distilled H_2O at the ends of the bar. Humidity was controlled by covering the

1 apparatus with acrylic boxes to maintain near 100% humidity. Germination was
2 evaluated at 24 hr intervals and the natural photoperiod was approximately
3 13L:11D. The experiment was replicated twice.

4 2.11 Statistical analysis

5 The bioassay procedures used yielded data which indicated no, partial or complete
6 suppression of the insect population. Suppression data for developing
7 populations is difficult to describe precise cause and effect relationships for,
8 because the reduced numbers in the following generation at a given time are
9 caused by parental mortality, parental oviposition suppression, and mortality or
10 developmental delay in the immature stages. Therefore, the best illustration of
11 the reproducibility of the experiments that were conducted more than once and the
12 best description of the concentration-dependant effects can be accomplished for
13 most of the experiments using the logistic dose-response transition equation
14 $y = a + b / (1 + (x/c)^d)$, where $x = \log_{10}(\text{concentration} + 1)$ and $y =$ either a proportion, p ,
15 transformed using $\sin^{-1}(p^{0.5})$ or a unit measure of productivity, z , transformed
16 using $(z + 0.375)^{0.5}$ (ANSCOMBE 1949). The specific parameters describe different
17 portions of the data set. These are: a - the lower plateau of the data set, b -
18 the height of the transition of the data set, c - the center of the transition
19 portion of the data set, and d - the steepness of the transition in the data set.
20 This procedure was conducted using PROC NLIN (SAS 1988). A three parameter power
21 function, $y = a + bx^c$, was used to describe the data resulting from one experiment,
22 again using PROC NLIN (SAS 1988). Linear regression was conducted when
23 appropriate using PROC REG (SAS 1988). The data collected from these experiments
24 follow a many Y for each X pattern. Thus, it is difficult to determine how well

1 the regression equation describes the data with its innate variability. This is
2 an important and measurable component of the percentage variation explained (R^2)
3 and can be used to calculate the maximum R^2 possible; that is, the greatest
4 amount of the total variation that can be explained by any model given the
5 inherent variation, or pure error, in the data. The equation used is Max R^2
6 possible = $(SS_{\text{Corrected Total}} - SS_{\text{Pure Error}}) / SS_{\text{Corrected Total}}$ (DRAPER and SMITH 1981). This equation
7 has been suggested for the percentage variation explained for linear equations,
8 for which it is exact, but it also provides useful descriptive information for
9 the approximate percentage variation explained for non-linear equations. ET_{50}
10 and EC_{50} values were estimated using probit-transformed percent incapacitation
11 or suppression regressed against log10-transformed time (hr X 40) or log10-
12 transformed concentration (FINNEY 1971).

13 3 Results and Discussion

14 Chemical composition of the oil. The chemical analysis indicated that the
15 essential oil of *T. riparia* was quite complex and contained many compounds
16 present in relatively low proportions (figure 1). Most of the compounds showed
17 fragment ion patterns characteristic of terpenoids (data not shown). There
18 appeared to be little gross qualitative difference between the spectra of the
19 essential oil used in this study and the spectra previously published for a
20 different oil sample (VAN PUYVELDE 1988), nor was there any noticeable change in
21 the spectra of the oil samples injected at the initiation of these trials and
22 those stored in the freezer until the end of the trials eight months later (data
23 not shown).

24 Toxicity evaluation. The evaluation of the insecticidal activity of the
25 essential oil indicated limited toxicity to *Z. subfasciatus* with the EC_{50} value

1 for the larger females being 1.5 times that for the males at 24 hr (figure 2).
2 The temporal component of the bioassay showed that time to incapacitation was
3 concentration-dependant (figure 3) with the ET_{50} for the larger females being
4 approximately 1.35 times that for the males at the $1583 \mu\text{g}/\text{cm}^2$ concentration and
5 2.5 times that for males at the extremely high $3165 \mu\text{g}/\text{cm}^2$ concentration (figure
6 3).

7 This toxicity data indicated that treatment with this material would not
8 provide high mortality against adults of this bruchid species at any reasonable
9 dosage. However, adult bruchids are very short-lived and adequate control of a
10 developing population may be achieved by any combination of sub-lethal
11 physiological and purely behavioral effects that effectively reduce progeny
12 production. Thus, the cumulative effect of these potential non-catastrophic
13 effects of this oil on developing populations was determined.

14 3.1 Protectant potential of the essential oil

15 Adults added immediately after application of the oil to the surface of beans.
16 The freshly-applied oil was active at a concentration range beginning at
17 approximately $55 \mu\text{g}/\text{cm}^2$ of bean and caused complete suppression at approximately
18 $250 \mu\text{g}/\text{cm}^2$ of bean (figure 4). It was evident that the first experiment (figure
19 4- ●), spanned the concentrations which induced partial suppression of the F_1
20 adult progeny numbers. The second experiment, (figure 4- ▲), yielded a better
21 concentration-dependant response. The EC_{50} value of $72 \mu\text{g}/\text{cm}^2$ for figure 4- ▲ was
22 not very different from the c parameter for both experiments (figure 4- ● and ▲,
23 described in table 1) which gave a value of $121 \mu\text{g}/\text{cm}^2$ for the midpoint of the
24 transition of the data. The water plus emulsifier (origin of the regression line

1 at log concentration = 0, figure 4) appeared to have a negative impact on the
2 potential population compared to the controls. Surprisingly, gently mixing the
3 beans with water and evaporating prior to addition of the adults (figure 4- ■)
4 had no more effect on progeny production than mixing the dry beans prior to
5 adding the adults (figure 4- □), despite the visible enlargement and loosening
6 of the testa that was induced by this treatment.

7 Oil application on eggs deposited on the surface of beans. The 1-2 day old
8 eggs attached to the beans surface were slightly more sensitive to the mixing
9 treatment with the oil than were the adults to the presence of freshly-applied
10 oil on the beans surface. The suppression commenced at approximately $27 \mu\text{g}/\text{cm}^2$
11 and was complete at $125 \mu\text{g}/\text{cm}^2$. Again, the initial experiment (figure 5- ●)
12 generally missed the concentration range inducing partial suppression of the F_1
13 adult progeny and a second experiment (figure 5- ▲) yielded better concentration-
14 dependant data. The EC_{60} of $50 \mu\text{g}/\text{cm}^2$ for figure 5- ▲ was not very different
15 from the $63 \mu\text{g}/\text{cm}^2$ predicted c parameter of the logistic dose-response transition
16 equation (table 1) for both experiments (figure 5- ● and ▲). The addition of
17 water (figure 5- ■) and water plus emulsifier (origin of the regression line at
18 log concentration = 0, figure 5) both had increasingly suppressive effects when
19 compared with the dry agitated control (figure 5- □). In addition, the process
20 of agitation itself reduced F_1 adult progeny to 42% of that for an undisturbed
21 control (data not shown), clearly demonstrating that the mode of action of the
22 essential oil against the hardened eggs might be closely linked to the physical
23 treatment of the eggs. Therefore the EC_{60} or c parameter units for the
24 concentration dependant activity of the oil are also the amount required to
25 reduce a developing population to 21% of that achieved in the controls.

26 Oil application against beans containing developing larvae. The efficacy

1 of the essential oil against the larvae within the beans was greatly reduced.
2 In particular, the number of F₁ progeny emerged at 55 days was only slightly
3 decreased by the three highest concentrations, and the response to the highest
4 concentration was quite variable (figure 6a). The overall distribution of the
5 data suggested a weak but valid power function (table 1), and this distribution
6 was very distinct from that for either the treatment of the eggs or the exposure
7 of the adults. Again, the emulsifier (origin of the regression line at log
8 concentration = 0, figure 6a) appeared to have a slight effect on the number of
9 emerged F₁ progeny at 55 days, compared with the agitation + water control
10 (figure 6a- ■). The EC₅₀ value for this treatment is approximately 3980 μg/cm².

11 The experiment was followed to determine the relative emergence of adults
12 during the next 30 days. These adult progeny may be either developmentally
13 delayed F₁ individuals or normally developing F₂ adults. These data followed a
14 stronger power function distribution (table 1), with a clear inhibition of
15 cumulative emergence at the higher concentrations (figure 6b). The EC₅₀ of the
16 treatment at this point was 104 μg/cm². This number is very similar to that for
17 the freshly-applied oil against adults and suggests that the dominant effect
18 against the developing population at this point is suppression of potential F₂
19 progeny. This was further evaluated by dividing the progeny emerging from day
20 55-85 by the F₁ females present at 55 days. This data again follows a power
21 function (figure 6c; table 1) although not as strongly as when the number was not
22 corrected by using the number of F₁ females present at 55 days. However, the EC₅₀
23 value for this treatment is 121 μg/cm², although the χ^2 test indicated the data
24 were too heterogeneous to determine confidence intervals for this value. This
25 EC₅₀ value is in good agreement with the value obtained for cumulative
26 uncorrected emergence (figure 6b) and for the suppression of F₁ progeny which

1 resulted from placing adults on beans immediately after treatment with oil. It
2 is important to note that the test variables in figures 6b and 6c represent two
3 different treatments of the same data. The uncorrected cumulative emergence data
4 (figure 6b) represents any proportion of F₁ and F₂ individuals (including only
5 F₁ or only F₂ progeny) whereas the corrected value (figure 6c) assumes that all
6 are F₂ progeny. The fact that the data distributions (figures 6b; 6c) and EC₅₀
7 values were quite similar suggests that the dominant effect was on the number of
8 F₂ progeny. Therefore, it might be appropriate to assume that the emerging F₁
9 females are responding to the 55-85 day old oil in a similar way as did the
10 females placed on beans immediately post-treatment with oil.

11 Protection against adult infestation at 210 days post-treatment. The
12 reduced scale no-choice experiment conducted to assess oviposition on beans
13 treated 210 days previously and incubated at 27°C and 65% r.h. indicated that the
14 data for both eggs laid and eggs hatched again displayed distributions best
15 described by the logistic dose response equation (figure 7a; 7b). In addition,
16 all four equation parameters were virtually identical for both eggs laid and eggs
17 hatched (table 1) and both regressions lines fit within the confidence limits for
18 each other (figure 7a and 7b). Therefore, there was no concentration-dependant
19 effect on fertility at the amounts of oil used, nor was there any significant
20 infertility at any dose tested. Surprisingly, the EC₅₀ values are lower for the
21 210 day incubated oil than for the fresh oil (23 and 27 $\mu\text{g}/\text{cm}^2$ for hatched eggs
22 and eggs laid, respectively, figure 7a, 7b, although there were not enough
23 partial suppression data points to accurately predict confidence limits for
24 either parameter). However, these lower values are also corroborated by the c
25 parameter of the logistic dose response equation which gives values of 36 and 40
26 $\mu\text{g}/\text{cm}^2$ for the two variables, respectively (table 1). The experiment used was

1 greatly reduced in scale, but the number of newly hatched larvae per female
2 (approximately 25) in the control (origin of the regression line at log
3 concentration = 0, figure 7b) certainly is biologically compatible with the
4 approximately 18 F₁ adults per female for the agitated control for the experiment
5 using oil newly-applied on beans against adults (figure 4).

6 Evaluation of the behavioral activity of the 210 day incubated oil in a
7 choice test indicated that the 53.5 $\mu\text{g}/\text{cm}^2$ treatment reduced the oviposition on
8 the treated beans to nearly zero with an EC_{60} of only 10 $\mu\text{g}/\text{cm}^2$ (figure 8a). The
9 c parameter for the midpoint of the transition was more conservative, giving a
10 value of 17 $\mu\text{g}/\text{cm}^2$ (table 1). The volatiles that were released from the surface
11 of the beans treated 210 days previously exerted a subtle concentration-dependant
12 influence on oviposition upon the untreated beans (figure 8b, 8c). The slopes
13 for the effect on both eggs laid and eggs hatched are identical, indicating no
14 concentration-dependant infertility, but the each line does not fit within
15 confidence limits for the other, so there is a significant and relatively
16 constant proportion of unhatched eggs (table 1; figure 8b, 8c). The numbers for
17 both variables at the highest concentration used (1071 $\mu\text{g}/\text{cm}^2$) are approximately
18 70% of that for the control (figure 8b, 8c). However, because the value for the
19 slope of each line was small (table 1), the EC_{60} for both variables was greater
20 than 19 mg/cm^2 (49 μl per bean). Again, this is a small-scale test, but the
21 control numbers are very similar to those for the no-choice experiment and are
22 also biologically plausible compared to the untreated control in figure 4.

23 3.2 Effect of the essential oil on bean germination.

24 The germination of Pinto beans that had been stored for 180 days under
25 bioassay conditions was rapid and no concentration dependant effects were
26 evident. At four days, $56.1 \pm 4.1\%$ (mean \pm S.E.) of the beans had germinated

1 across all treatments. The regression line was y (proportion of beans
2 germinated) = $-0.038x$ (log concentration [$\mu\text{g}/\text{cm}^2$] + 1) + 0.627. Neither the
3 slope of the line ($t = -0.93$, d.f. = 54) nor the regression ($F = 0.865$, d.f. =
4 1, 54) were significant at $\alpha = 0.05$. At seven days, $94.3 \pm 1.6\%$ (mean \pm S.E.)
5 of the beans had germinated across all treatments. The regression line was y
6 (proportion of beans germinated) = $-0.022x$ (log concentration [$\mu\text{g}/\text{cm}^2$] + 1) +
7 0.981. Again, neither the slope of the line ($t = -1.41$, d.f. = 54) nor the
8 regression ($F = 1.997$, d.f. = 1, 54) were significant at $\alpha = 0.05$.

9 3.3 Comparison of *T. riparia* essential oil

10 with other natural products active against bruchids.

11 The 250 $\mu\text{g}/\text{cm}^2$ (1600 ppm) concentration of *T. riparia* oil should provide
12 excellent protection against developing populations of *Z. subfasciatus* on Pinto
13 beans, under most circumstances. Complete control will not be immediately
14 achieved for beans already infested with developing larvae, but this
15 concentration should reduce the number of adult progeny emerging 55-85 days post-
16 treatment to 65% of that for the untreated control. This may be quite
17 significant, given the apparent increase in activity caused by degradation of the
18 oil with the passage of time. However, more research is required to determine
19 the long-term effects of oil exposure on developing populations when infested by
20 larvae prior to treatment. An evaluation of the activity of this material for
21 longer storage periods should also be undertaken, particularly as a protectant
22 against infestation by field adults.

23 To readily compare the units used in this paper with those used in others,
24 a multiplication of $\mu\text{g}/\text{cm}^2$ by 6.37 gives ppm (wt/wt). Where possible, ppm
25 (wt/wt) units will be calculated for other reports where these have not been

1 used. Oil densities have arbitrarily been assigned a value of 0.90 g/ml if
2 volume/mass units have been given, and density has not been given. In addition,
3 EC_{50} estimates have been determined when necessary, and, if possible. The intent
4 is to make comparisons of the relative efficacy of this material with those
5 already evaluated, not to rigorously determine alternative units to those already
6 published.

7 The most effective natural treatment against bruchids on a commodity
8 reported thus far is the essential oil of *Acorus calamus* L. Complete suppression
9 of *Callosobruchus maculatus* (F.) progeny was achieved at 1000 ppm in black-eyed
10 peas immediately post-treatment, with an EC_{50} of approximately 160 ppm (SU
11 1991a). This activity was significantly influenced by acute toxicity to females
12 (SU 1991a). After seven days of exposure to 500 ppm *A. calamus* oil on black-eyed
13 peas there was 86.7% mortality compared to no mortality in controls, with an LC_{50}
14 of approximately 303 ppm (SU 1991a). This confirmed an earlier report by YADAVA
15 (1971) of high topical toxicity of this oil to *Callosobruchus chinensis* L. This
16 acutely toxic mode of action is distinct from that which we observed in this
17 study. SU (1984) found that three peppercorn extracts at 2000 ppm gave > 90%
18 control of F_1 progeny of *C. maculatus*. The EC_{50} values were: black pepper (*Piper*
19 *nigrum* L.) acetone extract- 391 ppm, green peppercorn (*Piper nigrum* L.) acetone
20 extract- 609 ppm, and West African pepper (*Piper guineense* Schumach and Thonn.)
21 hexane extract- 642 ppm (SU 1984). Clearly these extracted compounds are
22 suppressive to bruchids. However, a *Piper cubeba* L. hexane extract at 2000 ppm
23 suppressed only 43% of the F_1 progeny relative to the controls (SU 1990),
24 indicating that closely related species may differ greatly in efficacy.
25 *Chenopodium ambrosioides* L. essential oil at 2000 ppm in black-eyed peas caused
26 only 13.3% mortality to female *Callosobruchus maculatus* after seven day exposure,

1 but reduced adult F₁ progeny to 3.3 % of the control with an EC₅₀ = 822 ppm (Su
2 1991b). This very closely parallels the effective concentration and type of
3 activity we have found here for *T. riparia* essential oil. There are several
4 other reports of relatively toxic natural products to bruchids in bioassays, but
5 do not include treatment of a commodity. For example, SINGH and RAO (1985) found
6 that *Cedrus deodora* Roxburgh essential oil was acutely toxic to *C. chinensis* at
7 221 µg/cm₂ of Petri dish surface after 24 hours. SU and HORVAT (1987) found that
8 a topical application of a hexane extract of lemon peel at 35 µg/insect caused
9 40% mortality in adult *C. maculatus* after 5 days.

10 Vegetable oils are quite effective against *Z. subfasciatus* (SCHOONHOVEN
11 1978). In trials initiated 1 day post treatment with 1 ml of a series of
12 vegetable oils per kg of beans (910 ppm), adult F₁ progeny were 1.5% - 10.3% of
13 the number for the untreated control, while trials initiated at 75 days post
14 treatment with the same concentrations suppressed adult progeny from 2.1% - 36.3
15 % of the control number (SCHOONHOVEN 1978). A concentration of 4550 ppm of
16 African palm oil completely protected beans infested at 75 days post-treatment
17 (SCHOONHOVEN 1978). These treatments had less effect against the larvae within
18 the beans with the 4550 ppm treatment of crude cottonseed oil reducing the
19 numbers of F₁ progeny by approximately 60% (SCHOONHOVEN 1978) which is similar
20 to the EC₅₀ value reported here for control of larvae within the beans by *T.*
21 *riparia* essential oil. These vegetable oils had no effect on overall germination
22 or the ability of the beans to absorb water, which again parallels our findings
23 here, although the potential rancidity of the more refined oils may impede
24 consumption (SCHOONHOVEN 1978). Similarly, peanut oil at 5 ml/kg (4550 ppm) was
25 found to completely protect cowpeas from *C. maculatus* after 6 months (SINGH et
26 al. 1978). It is interesting to note that SCHOONHOVEN (1978) found that the

1 primary activity of African palm oil against *Z. subfasciatus* was to decrease
2 oviposition by significant acute toxicity, while SINGH et al. (1978) found that
3 groundnut oil worked ovicidally and larvicidally against *C. maculatus*, with weak
4 oviposition suppression and limited acute toxicity.

5 It is readily apparent that *T. riparia* has a protectant activity which is
6 quite comparable with most other active natural products tested on bruchids. It
7 is interesting to note that the non-toxic oviposition suppression we observed for
8 the crushed or milled dried leaves of this species (WEAVER et al. In Press) can
9 be mimicked using its essential oil. Clearly, the preparation of approximately
10 1.7 liters of oil to treat a tonne of dried beans would be fairly laborious and
11 expensive. It also could not be recommended that such a treatment be planned for
12 foodstuffs since the concentrations used are fairly large and both palatability
13 and health may be endangered by attempted consumption of treated beans. However,
14 given the long term activity we have observed for this material suggests that it
15 could be used effectively as a strategic treatment for beans destined for
16 planting in upcoming crops.

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13

Table 1. Equations, parameters and analyses of concentration dependent effects of *T. riparia* oil applied against different life stages on population growth variables for *Z. subfasciatus*.

3 4 52-	1- Insect Stage Treated in Beans. Variable Evaluated.	Parameter ^a	Value . S.E.	z^b	95% confidence limits	$\frac{F_{L^c}}{F_{Reg^d}}$	R^2	% of maximum R^2 possible ^e
6	1- 0-24 hr post-	a	0.58 ± 0.11	5.25*	(0.36, 0.80)	N/A		
7	emergent adults. 2-	b	3.1 ± 0.16	19.8*	(2.77, 3.39)	221 _{3, 126} *	0.84	96
8	F ₁ adults emerged at	c	2.1 ± 0.033	63.9*	(2.02, 2.15)	529 _{4, 126} *		
9	55 d. Fig. 4.	d	21 ± 5.7	3.90*	(10.2, 31.1)			
101-	24-48 hr old eggs.	a	0.69 ± 0.043	16.0*	(0.60, 0.77)	N/A		
11	2- F ₁ adults emerged	b	1.5 ± 0.069	21.5*	(1.35, 1.63)	209 _{3, 126} *	0.83	97
12	at 55 d. Fig. 5.	c	1.8 ± 0.025	72.4*	(1.76, 1.86)	752 _{4, 126} *		
		d	16 ± 4.8	3.56*	(7.01, 24.5)			
13	1- 7-8 d old larvae	a	3.2 ± 0.10	31.8*	(3.00, 3.41)	N/A		
14	within beans. 2- F ₁	b	-0.0013 ± 0.0045	0.297	(-0.0104, 0.0769)	11.3 _{2, 67} *	0.25	79
15	adults emerged at	c	5.9 ± 3.1	1.93*	(-0.209, 12.0)	624 _{3, 67} *		
16	55 d. Fig 6a.							
17	1- 7-8 d old larvae	a	13 ± 0.39	32.7*	(12.0, 13.6)	N/A		
18	within beans. 2-	b	-0.20 ± 0.13	-1.56*	(-0.458, 0.0557)	133 _{2, 67} *	0.80	98
19	Adults emerged from	c	3.5 ± 0.58	6.09*	(2.36, 4.66)	717 _{3, 67} *		
20	55-85 d. Fig 6b.							
21	1- 7-8 d old larvae	a	2.0 ± 0.079	25.0*	(1.81, 2.12)	N/A		
22	within beans. 2-	b	-0.021 ± 0.025	-0.850	(-0.0700, 0.0282)	40.6 _{2, 67} *	0.58	95
23	Adults emerged from	c	3.6 ± 1.1	3.37*	(1.46, 5.69)	461 _{3, 67} *		
24	55-85 d per F ₁ ♀							
25	at 55 d. Fig. 6c.							

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1	1- 0-24 hr post-	a	1.1 ± 0.27	4.00°	(0.528, 1.65)	N/A		
2	emergent adults in a							
3	no-choice test with	b	4.4 ± 0.43	10.3°	(3.53, 5.31)	41.6 _{3,24} °		
4	oil residue at						0.84	96
5	240 d. 2- Eggs laid	c	1.6 ± 0.061	26.6°	(1.49, 1.74)	106 _{4,24} °		
6	per parental ♀.							
7	Fig. 7a.	d	14 ± 5.3	2.61°	(2.89, 24.6)			
8	1- 0-24 hr post-	a	1.0 ± 0.26	3.89°	(0.468, 1.54)	N/A		
9	emergent adults in a							
10	no-choice test with	b	4.1 ± 0.41	10.2°	(3.31, 4.98)	40.0 _{3,24} °		
11	oil residue at						0.83	96
12	240 d. 2- Hatched	c	1.6 ± 0.061	25.6°	(1.44, 1.69)	99.1 _{4,24} °		
13	eggs per parental ♀							
14	at 25 d. Fig 7b.	d	13 ± 4.8	2.61°	(2.62, 22.4)			
15	1- 0-24 hr post-	a	-1.2 ± 4.0	-0.302	(-9.40, 7.00)	N/A		
16	emergent adults in a							
17	choice test with oil	b	45 ± 6.0	7.56°	(33.0, 57.8)	47.6 _{3,24} °		
18	residue at 240 d.						0.86	95
19	2- Percentage of eggs	c	1.3 ± 0.13	9.59°	(0.99, 1.54)	67.8 _{4,24} °		
20	on treated beans.							
21	Fig. 8a.	d	3.8 ± 1.3	2.81°	(1.01, 6.55)			
22	1- 0-24 hr post-	a	6.1 ± 0.21	29.4°	(5.70, 6.56)	0.834 _{6,21}		
23	emergent adults in a							
24	choice test with oil							
25	residue at 240 d.					10.4 _{1,28} °	0.29	71
26	2- Eggs laid per	b	-0.33 ± 0.10	-5.22°	(-0.548, -0.121)	10.4 _{1,28} °		
27	parental ♀.							
28	Fig. 8b.							

130

1	1- 0-24 hr post-							
2	emergent adults in a	a	5.7 ± 0.20	29.3*	(5.33, 6.15)	0.201 _{5,21}		
3	choice test with oil							
4	residue at 240 d. 2-					11.9 _{1,28} *	0.31	91
5	Hatched eggs per							
6	parental ♀ at 25 d.	b	-0.33 ± 0.10	-3.44*	(-0.534, -0.135)	11.9 _{1,28} *		
7	Fig. 8c.							

Parameters for regression equations. The four parameter equation is for the logistic dose response transition, $y = a + b / (1 + (x/c)^d)$; the three parameter power equation is $y = a + bx^c$; and the two parameter equation is linear ($y = a + bx$). All experiments were commenced with 0-1 day post-adult eclosion parental cohorts that oviposited until senescence. Experimental incubation was always at $27 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH and 12:12 L:D. All proportion data were transformed using $\sin^{-1}(x^{0.5})$ and all biological productivity variables were transformed using $(x+0.375)^{0.5}$. Concentration units are transformed using $\log_{10}(x+1)$. Values of \underline{z} marked with an "*" denote that the parameter is significantly greater than zero, $\underline{p} \leq 0.2$.

$\chi^2_{L} = (SS_{\text{Lack-of-Fit}} / df_{\text{Lack-of-Fit}}) + (SS_{\text{Pure Error}} / df_{\text{Pure Error}})$. This test is required for data in which no biologically meaningful equation is readily apparent. Many variables evaluated here are clearly appropriately evaluated using the logistic dose-response transition or a negative power function, and a lack-of-fit test is not required.

$F_{\text{Int}} = [(SS_{\text{Corrected Total}} - SS_{\text{Residual}}) / (df_{\text{Corrected Total}} - df_{\text{Residual}})] \div (SS_{\text{Residual}} / df_{\text{Residual}})$. *F is significant, $\underline{p} \leq 0.005$.

$F_{\text{Reg}} = (SS_{\text{Regression}} / df_{\text{Regression}}) + (SS_{\text{Residual}} / df_{\text{Residual}})$. *F is significant, $\underline{p} \leq 0.005$.

% of maximum R^2 possible = $R^2 / [(SS_{\text{Corrected Total}} - SS_{\text{Pure Error}}) \div SS_{\text{Corrected Total}}]$. Note: for non-linear equations both this quantity and R^2 are approximate.

1 **Figure captions**

2 Figure 1. Relative ion chromatogram obtained from the composite sample of the
3 essential oil of *Tetradenia riparia* used in all experiments. The chromatogram
4 was obtained using gas chromatography - electron impact mass spectroscopy.

5 Figure 2. Regressions of probit-transformed percentage incapacitation for male
6 and female *Zabrotes subfasciatus* (0-1 day post-adult eclosion) against log-
7 transformed concentration of *T. riparia* oil at 24 hr. Ten replicates containing
8 five males and five females were used. Bioassay conditions were $23 \pm 2^\circ\text{C}$, $65 \pm$
9 5% RH with a photoperiod of 14L:10D.

10 Figure 3. Regressions of probit-transformed percentage incapacitation for male
11 and female *Zabrotes subfasciatus* (0-1 day post-adult eclosion) against log-
12 transformed (hr X 40) at two high concentrations of *T. riparia* essential oil.
13 Ten replicates containing five males and five females were used. Bioassay
14 conditions were $23 \pm 2^\circ\text{C}$, $65 \pm 5\%$ RH with a photoperiod of 14L:10D.

15 Figure 4. Regression of transformed adult F_1 progeny/ female against log-
16 transformed concentration of *T. riparia* oil freshly applied to Pinto beans as a
17 protectant against infestation by 0-1 day post-emergent adults. Incubator
18 conditions were $27 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH with a photoperiod of 12L:12D. The equation
19 is the logistic dose-response transition equation (see table 1 for parameters and
20 statistics). Two experiments are plotted, one with a broad range of
21 concentrations (\bullet), and a second with a narrower concentration range (\blacktriangle) suitable
22 for probit analysis. Both data sets are regressed for the logistic dose-response

1 equation and the dashed lines indicate the 95% confidence interval. The 95%
2 prediction interval is indicated by the dotted lines. Probit analysis on (▲)
3 gives $y = 4.06x - 2.53$, $\chi^2 = 1.8$, d.f. = 4. The EC_{60} is $72 \mu\text{g}/\text{cm}^2$ with 95%
4 confidence limits of 65, 80. The χ^2 value is not significantly heterogenous at
5 $\alpha = 0.5$.

6 Figure 5. Regression of transformed adult F, progeny/ female against log-
7 transformed concentration of *T. riparia* oil freshly applied to Pinto beans
8 infested with 0 - 1 day old eggs laid by 0-1 day post-emergent adults. Incubator
9 conditions were $27 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH with a photoperiod of 12L:12D. The equation
10 is the logistic dose-response transition equation (see table 1 for parameters and
11 statistics). Two experiments are plotted, one with a broad range of
12 concentrations (●), and a second with a narrower concentration range (▲) suitable
13 for probit analysis. Both data sets are regressed for the logistic dose-response
14 equation and the dashed lines indicate the 95% confidence interval. The 95%
15 prediction interval is indicated by the dotted lines. Probit analysis on (▲)
16 gives $y = 3.62x - 1.14$, $\chi^2 = 1.7$, d.f. = 4. The EC_{60} is $50 \mu\text{g}/\text{cm}^2$ with 95%
17 confidence limits of 44, 57. The χ^2 value is not significantly heterogenous at
18 $\alpha = 0.5$.

19 Figure 6a. Regression of transformed adult F, progeny/ female against log-
20 transformed concentration of *T. riparia* oil freshly applied to Pinto beans
21 infested with 7 - 8 day old larvae from 0-1 day post-emergent adults. Incubator
22 conditions were $27 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH with a photoperiod of 12L:12D. The equation
23 is the three parameter power equation (see table 1 for parameters and statistics)
24 and the dashed lines indicate the 95% confidence interval. The 95% prediction

1 interval is indicated by the dotted lines. Probit analysis gives $y = 0.28x -$
2 4.00 , $\chi^2 = 3.6$, d.f. = 5. The EC_{50} is $3980 \mu\text{g}/\text{cm}^2$ with 95% confidence limits of
3 1370 , $11,500$. The χ^2 value is not significantly heterogenous at $\alpha = 0.5$.

4 Figure 6b. Regression of transformed adult F_2 progeny emerged during days 55-85/
5 F_1 female present at 55 days against log-transformed concentration of *T. riparia*
6 oil freshly applied to Pinto beans initially infested with 7 - 8 day old larvae
7 from 0-1 day post-emergent adults. Incubator conditions were $27 \pm 1^\circ\text{C}$, $65 \pm 5\%$
8 RH with a photoperiod of 12L:12D. The equation is the three parameter power
9 equation (see table 1 for parameters and statistics) and the dashed lines
10 indicate the 95% confidence interval. The 95% prediction interval is indicated
11 by the dotted lines. Probit analysis gives $y = 0.97x + 3.04$, $\chi^2 = 12.4$, d.f. =
12 5. The EC_{50} is $104 \mu\text{g}/\text{cm}^2$ with 95% confidence limits of 79, 136. The χ^2 value
13 is not significantly heterogenous at $\alpha = 0.025$.

14 Figure 6c. Regression of transformed adult F_2 progeny emerged during days 55-85
15 against log-transformed concentration of *T. riparia* oil freshly applied to Pinto
16 beans initially infested with 7 - 8 day old larvae from 0-1 day post-emergent
17 adults. Incubator conditions were $27 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH with a photoperiod of
18 12L:12D. The equation is the three parameter power equation (see table 1 for
19 parameters and statistics) and the dashed lines indicate the 95% confidence
20 interval. The 95% prediction interval is indicated by the dotted lines. Probit
21 analysis gives $y = 0.97x + 2.97$, $\chi^2 = 21.1$, d.f. = 5. The EC_{50} is $121 \mu\text{g}/\text{cm}^2$
22 without 95% confidence limits. The χ^2 value is significantly heterogenous at α
23 = 0.001.

24 Figure 7a. Regression of transformed eggs laid/ female against log-transformed
25 concentration of *T. riparia* oil applied 210 days previously to Pinto beans and

1 newly infested with 0 - 1 day old post-emergent adults in a small-scale no-choice
2 experiment. Incubator conditions were $27 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH with a photoperiod
3 of 12L:12D for the entire duration of exposure. The equation is the logistic
4 dose-response transition equation (see table 1 for parameters and statistics) and
5 the dashed lines indicate the 95% confidence interval. The 95% prediction
6 interval is indicated by the dotted lines. Probit analysis gives $y = 1.31x +$
7 3.13 , $\chi^2 = 28.2$, d.f. = 5. The EC_{50} is $27 \mu\text{g}/\text{cm}^2$ without 95% confidence limits.
8 The χ^2 value is significantly heterogenous at $\alpha = 0.001$.

9 Figure 7b. Regression of transformed eggs hatched/ female against log-transformed
10 concentration of *T. riparia* oil applied 210 days previously to Pinto beans and
11 newly infested with 0 - 1 day old post-emergent adults in a small-scale no-choice
12 experiment. Incubator conditions were $27 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH with a photoperiod
13 of 12L:12D for the entire duration of exposure. The equation is the logistic
14 dose-response transition equation (see table 1 for parameters and statistics) and
15 the dashed lines indicate the 95% confidence interval. The 95% prediction
16 interval is indicated by the dotted lines. Probit analysis gives $y = 1.31x +$
17 3.23 , $\chi^2 = 21.4$, d.f. = 5. The EC_{50} is $23 \mu\text{g}/\text{cm}^2$ without 95% confidence limits.
18 The χ^2 value is significantly heterogenous at $\alpha = 0.001$.

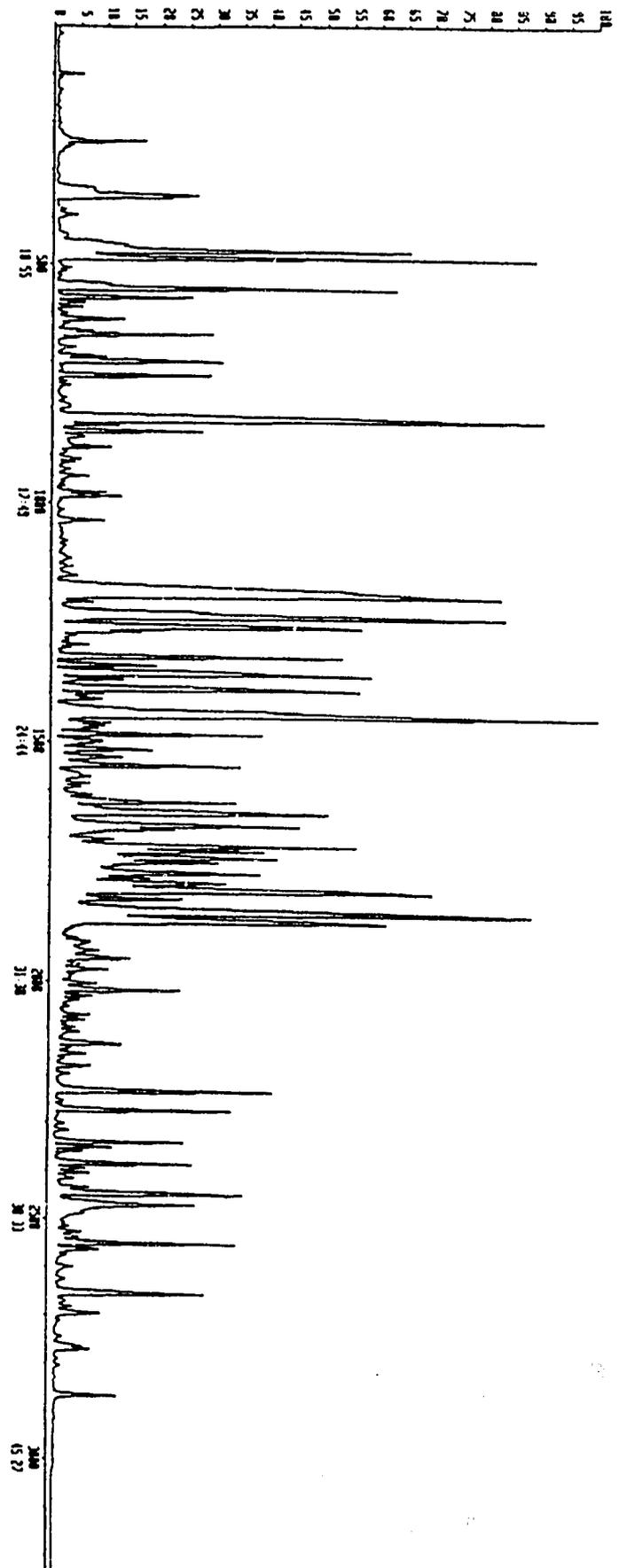
19 Figure 8a. Regression of transformed proportion of eggs laid on treated beans
20 against log-transformed concentration of *T. riparia* oil applied 210 days
21 previously to Pinto beans and newly infested with 0 - 1 day old post-emergent
22 adults in a small-scale choice experiment. Incubator conditions were $27 \pm 1^\circ\text{C}$,
23 $65 \pm 5\%$ RH with a photoperiod of 12L:12D for the entire duration of exposure.
24 The equation is the logistic dose-response transition equation (see table 1 for
25 parameters and statistics) and the dashed lines indicate the 95% confidence

1 interval. The 95% prediction interval is indicated by the dotted lines. Probit
2 analysis gives $y = 1.56x + 3.44$, $\chi^2 = 9.4$, d.f. = 5. The EC_{50} is $10 \mu\text{g}/\text{cm}^2$ with
3 95% confidence limits of 8, 12. The χ^2 value is not significantly heterogenous
4 at $\alpha = 0.1$.

5 Figure 8b. Regression of transformed eggs laid/ female against log-transformed
6 concentration of *T. riparia* oil applied 210 days previously to Pinto beans and
7 newly infested with 0 - 1 day old post-emergent adults in a small-scale choice
8 experiment. Incubator conditions were $27 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH with a photoperiod
9 of 12L:12D for the entire duration of exposure. The equation is linear (see
10 table 1 for parameters and statistics) and the dashed lines indicate the 95%
11 confidence interval. The 95% prediction interval is indicated by the dotted
12 lines. Probit analysis gives $y = 0.33x + 3.49$, $\chi^2 = 10.8$, d.f. = 5. The EC_{50} is
13 $42,000 \mu\text{g}/\text{cm}^2$ with 95% confidence limits of 13,000, 140,000. The χ^2 value is not
14 significantly heterogenous at $\alpha = 0.05$.

15 Figure 8c. Regression of transformed eggs hatched/ female against log-transformed
16 concentration of *T. riparia* oil applied 210 days previously to Pinto beans and
17 newly infested with 0 - 1 day old post-emergent adults in a small-scale choice
18 experiment. Incubator conditions were $27 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH with a photoperiod
19 of 12L:12D for the entire duration of exposure. The equation is linear (see
20 table 1 for parameters and statistics) and the dashed lines indicate the 95%
21 confidence interval. The 95% prediction interval is indicated by the dotted
22 lines. Probit analysis gives $y = 0.34x + 3.56$, $\chi^2 = 5.8$, d.f. = 5. The EC_{50} is
23 $20,000 \mu\text{g}/\text{cm}^2$ with 95% confidence limits of 6700, 57,000. The χ^2 value is not
24 significantly heterogenous at $\alpha = 0.1$.

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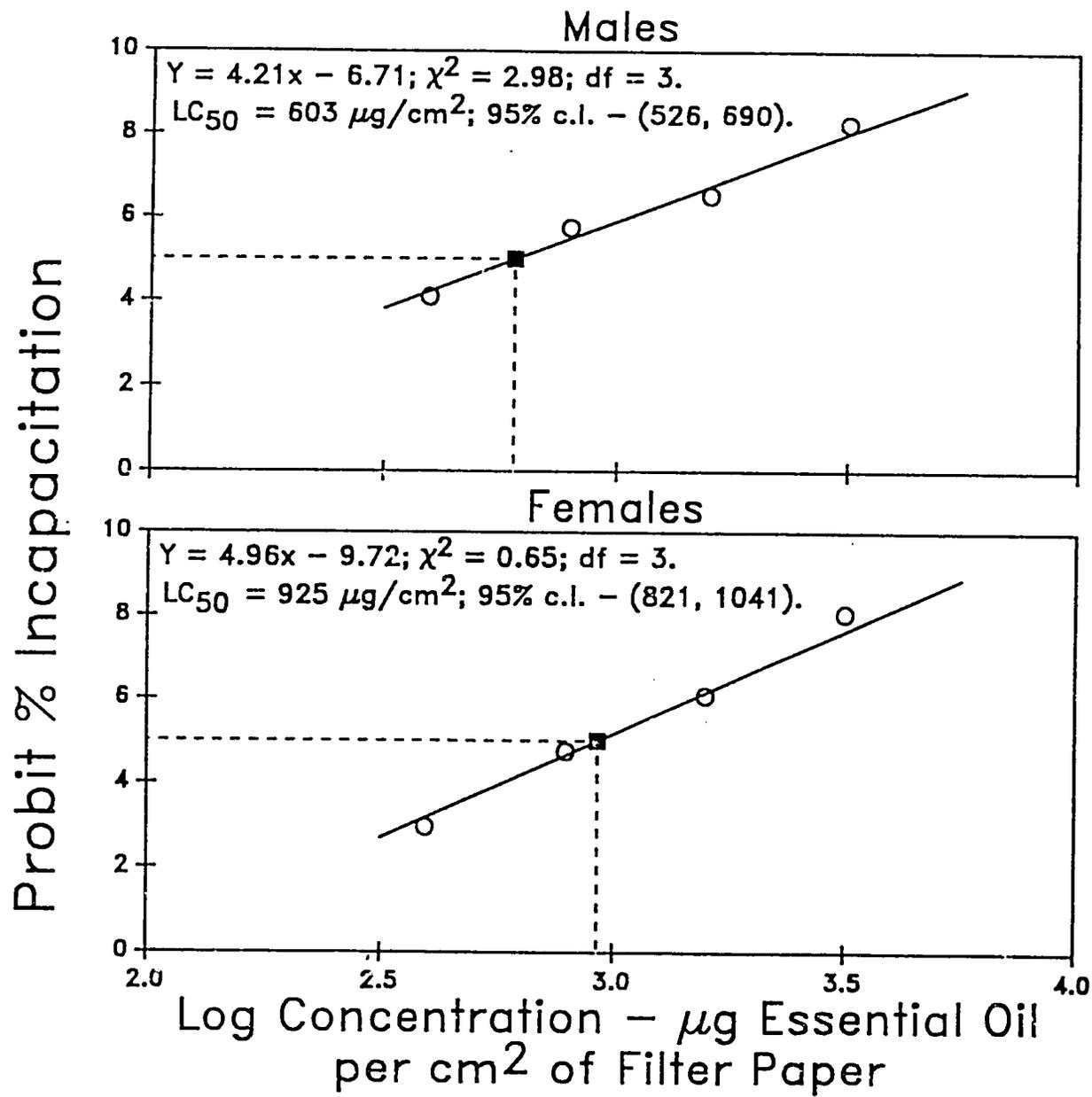


Fig. 2

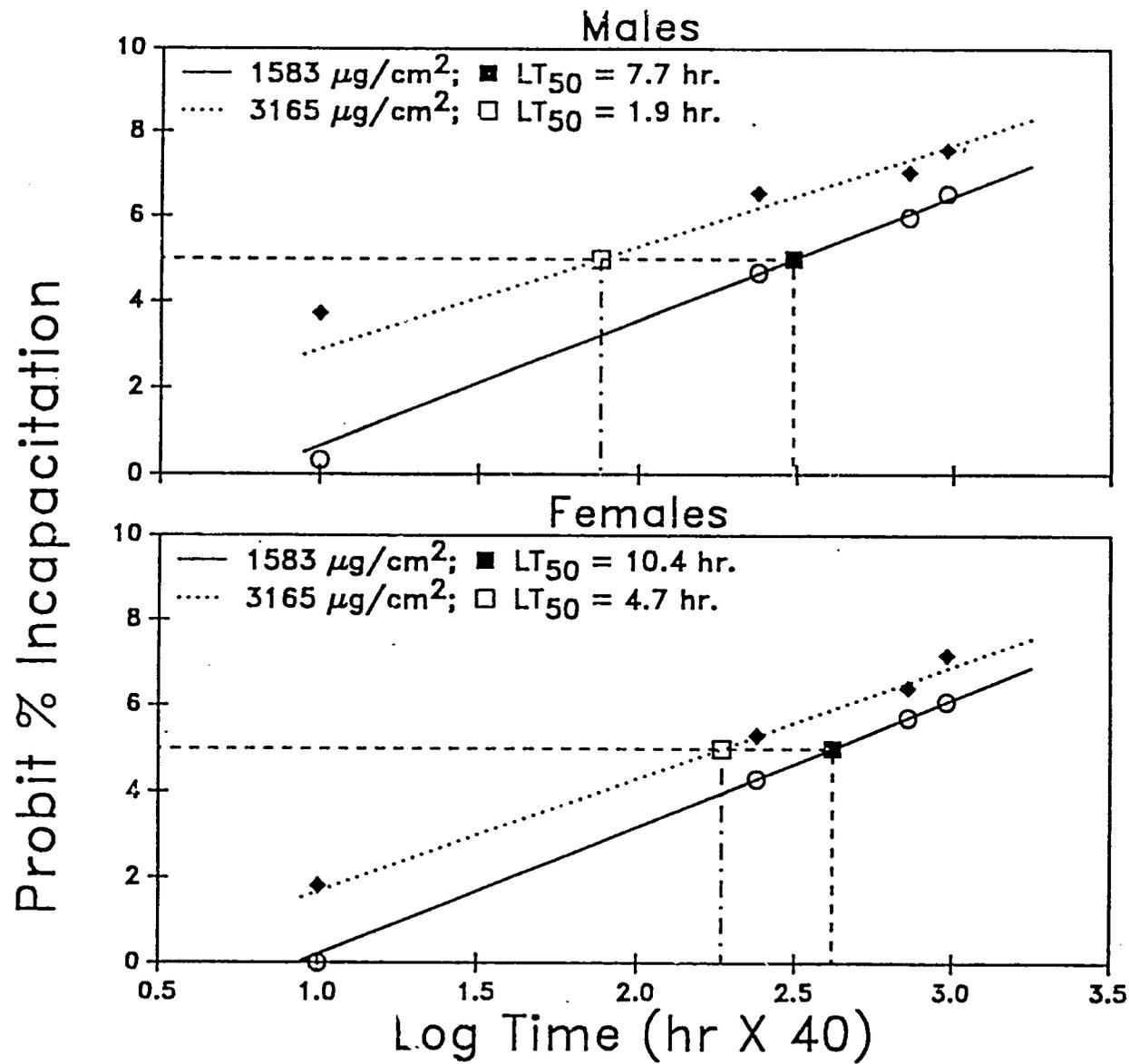
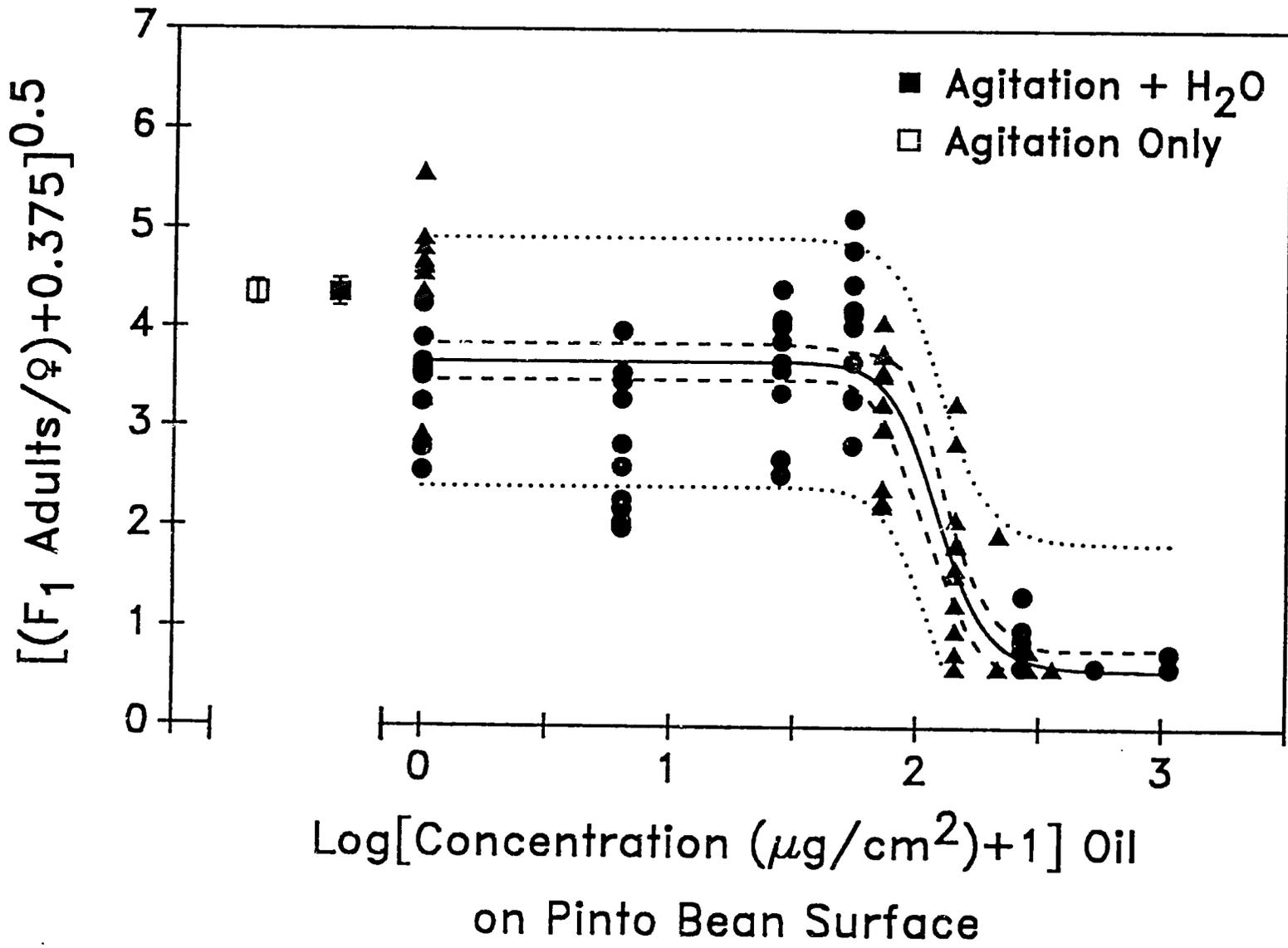
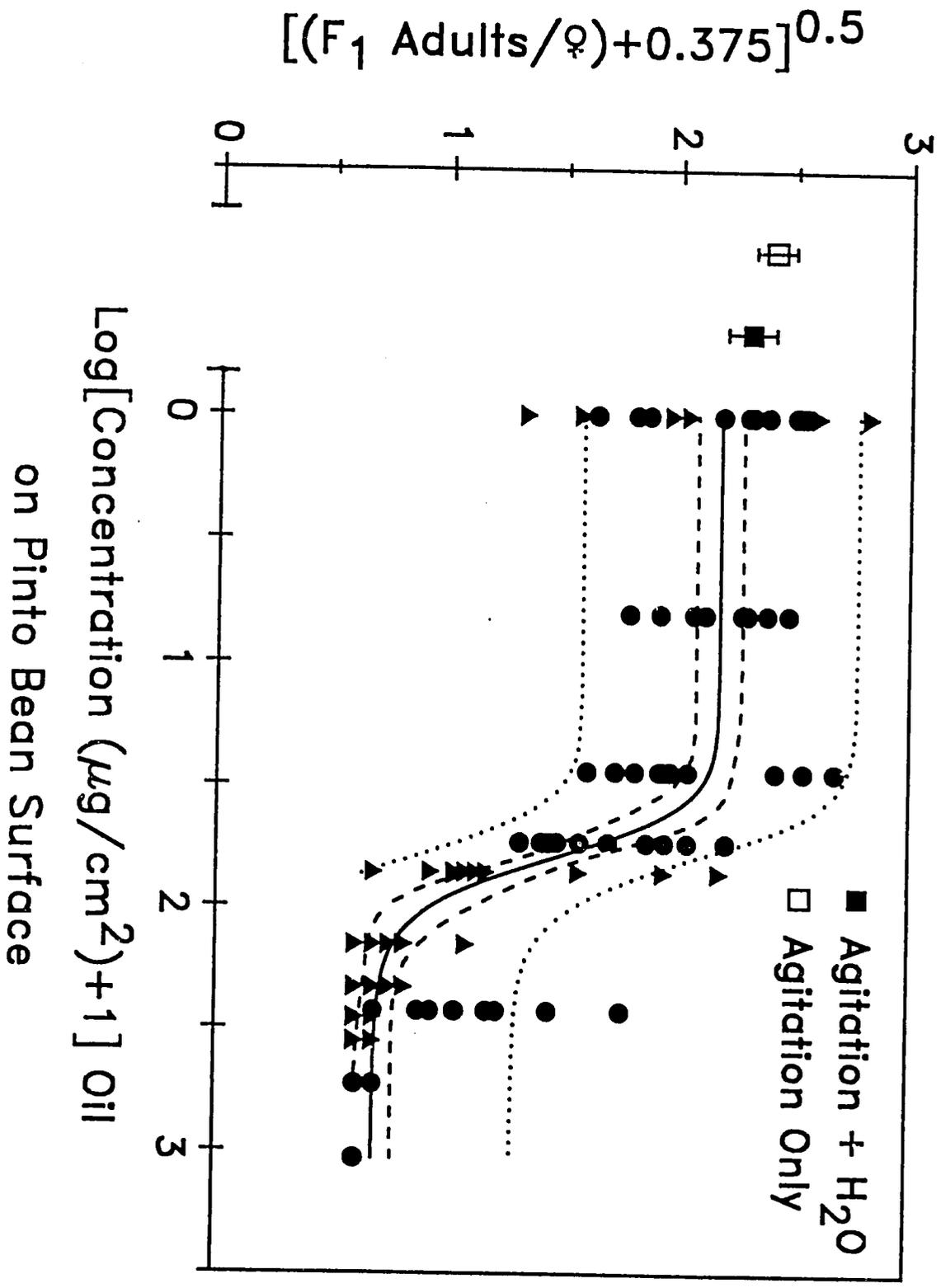
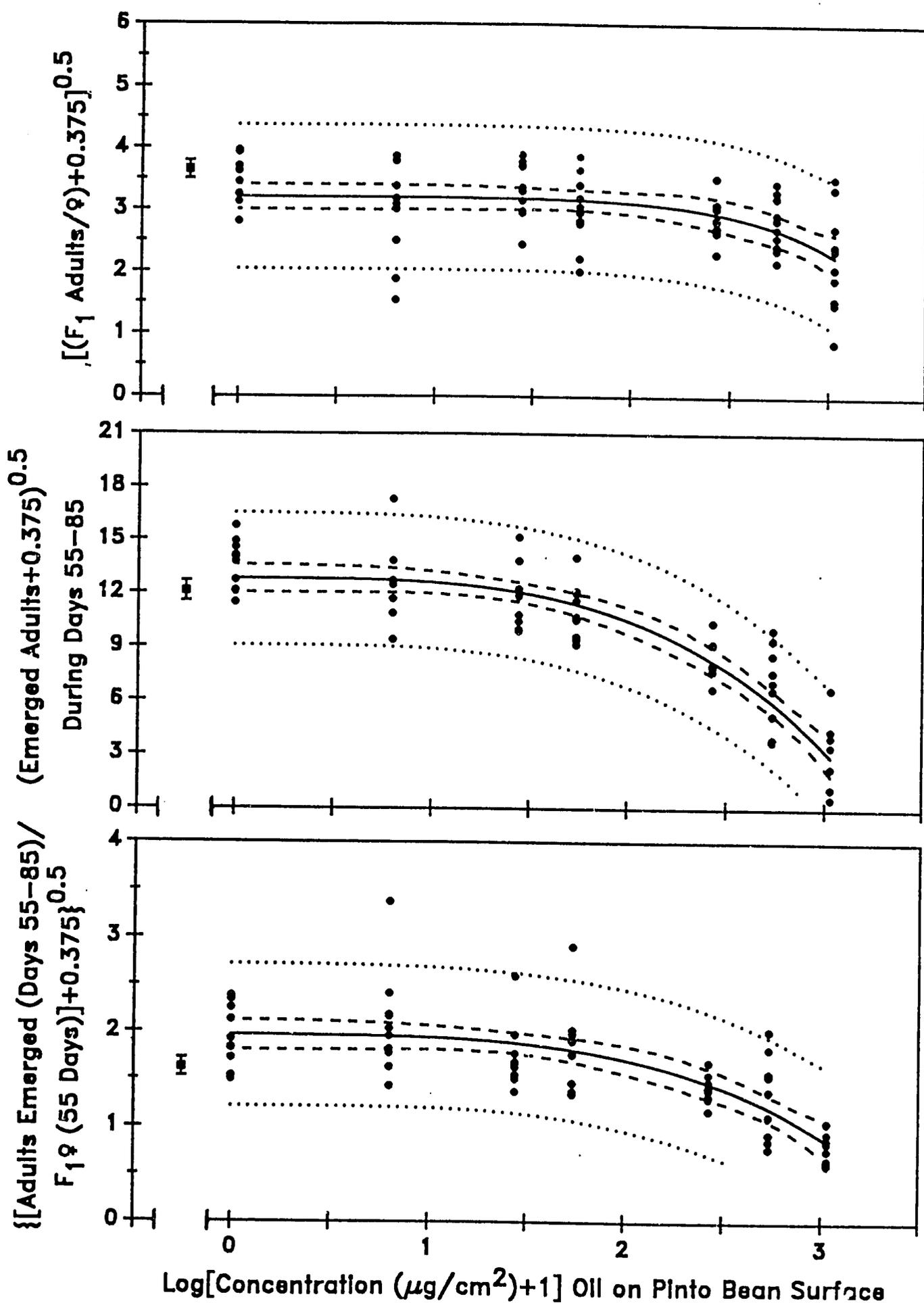
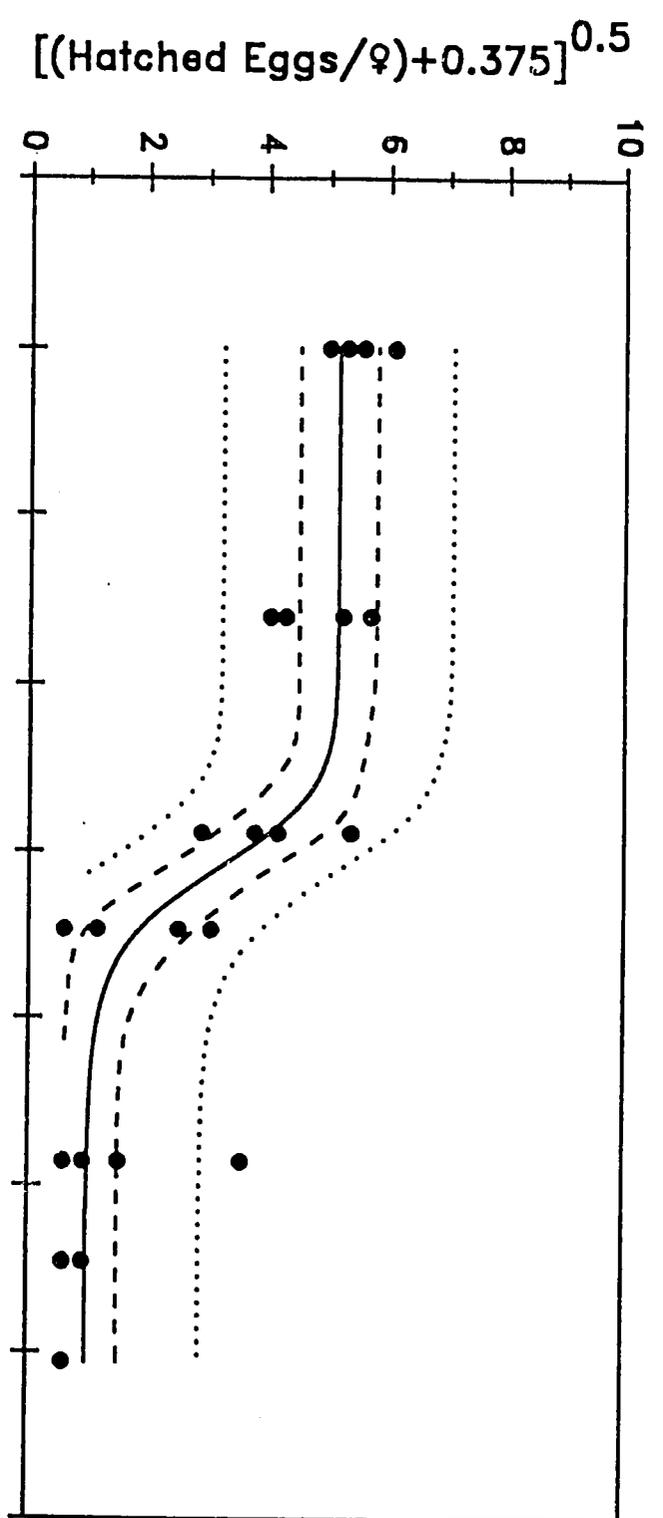
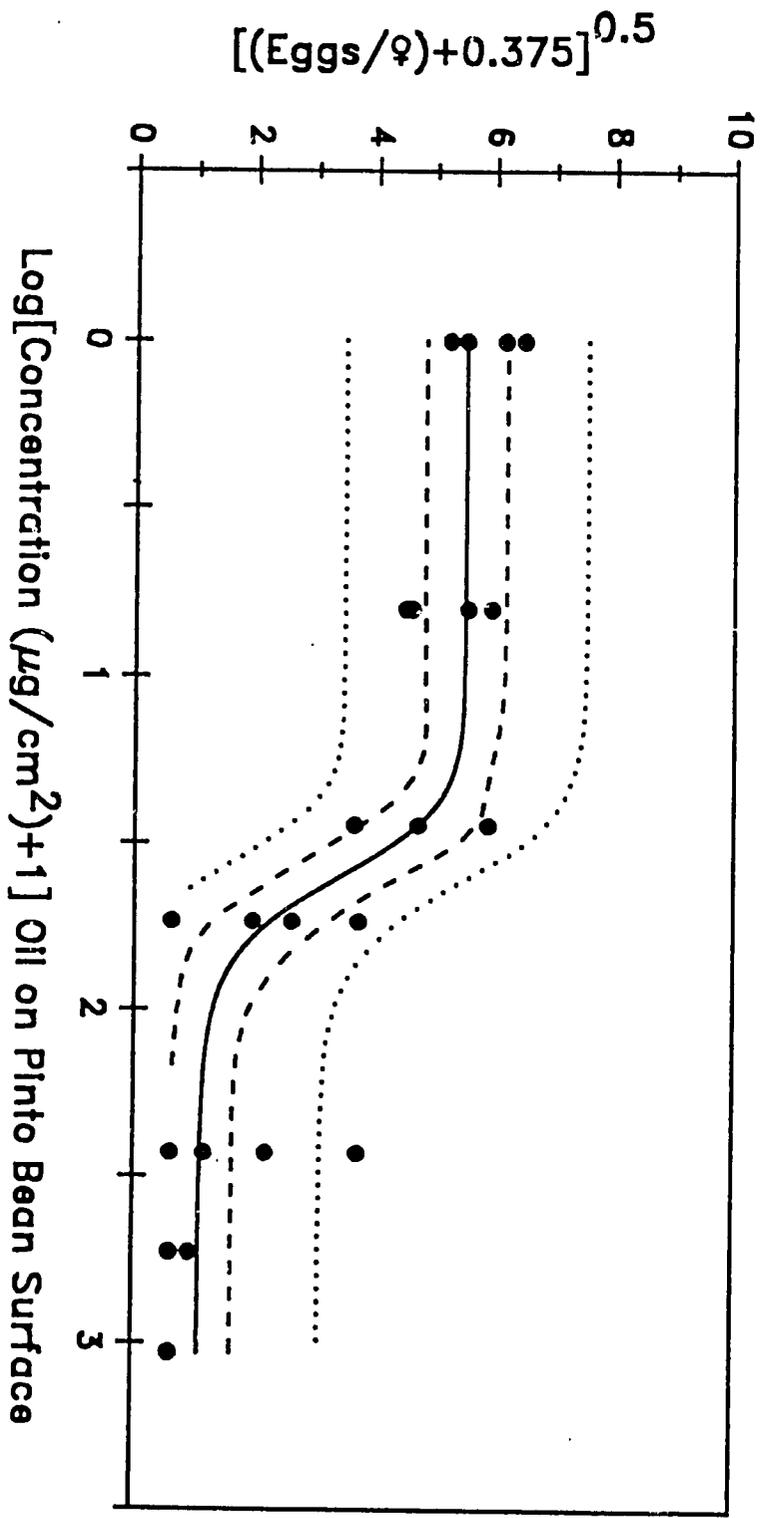


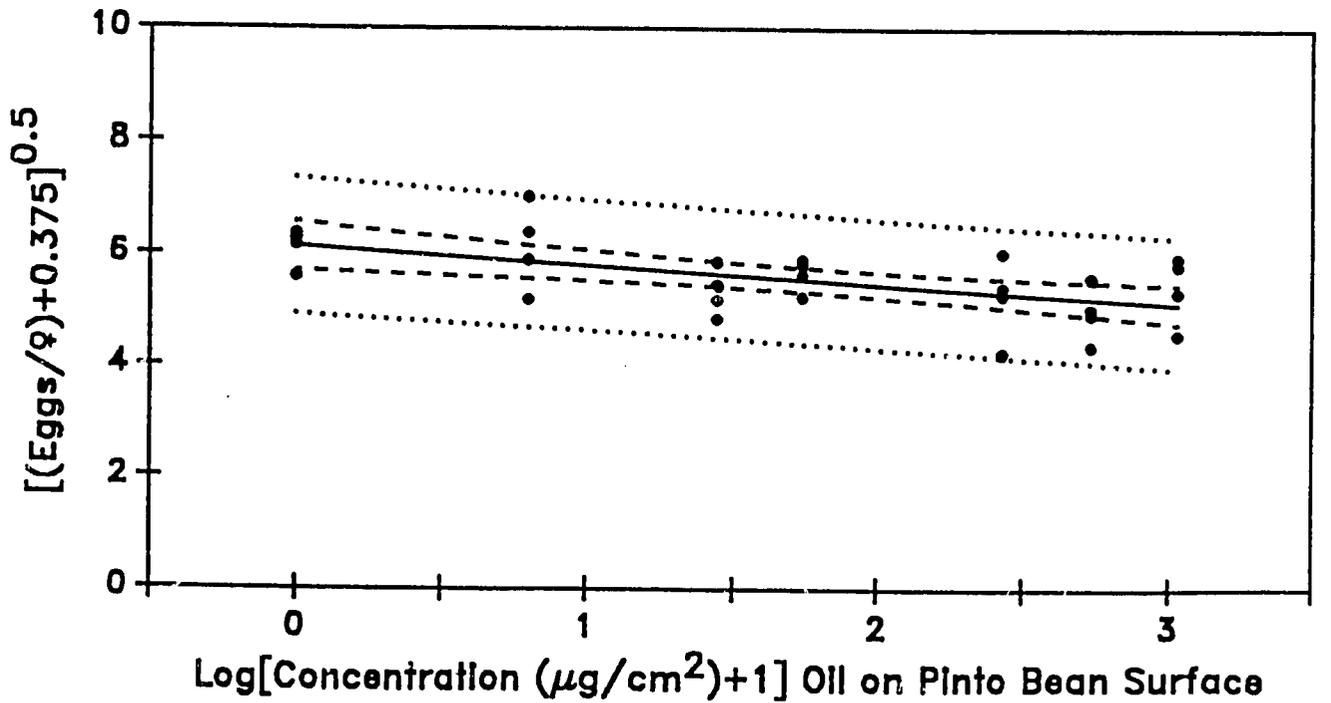
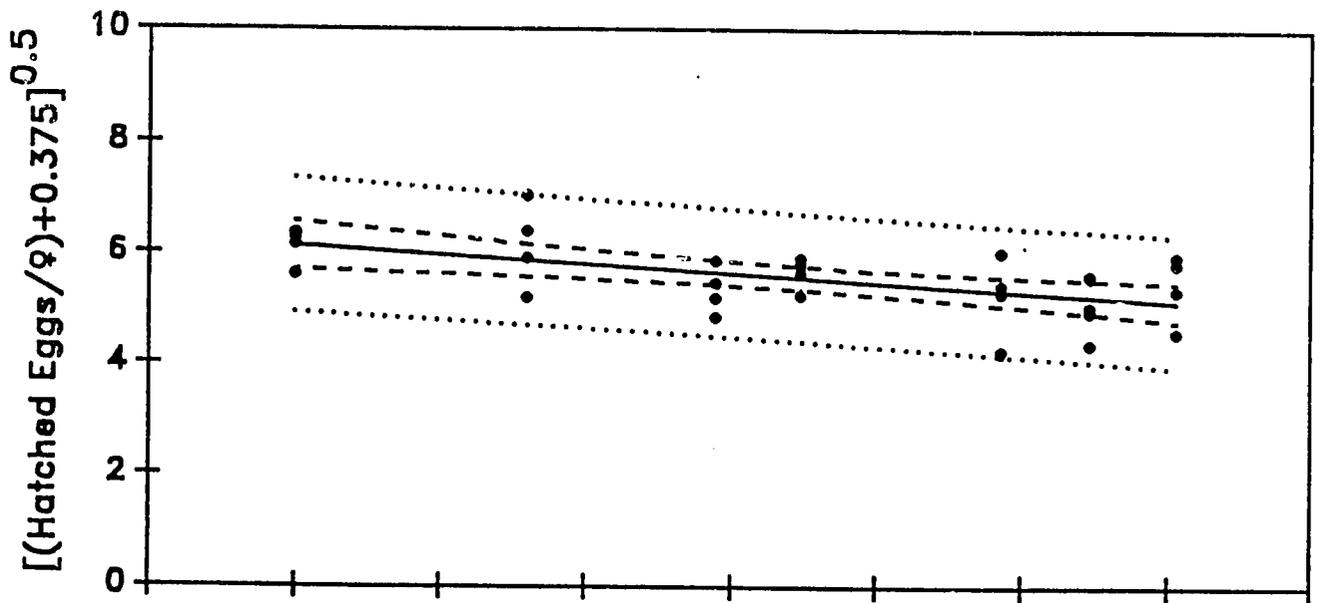
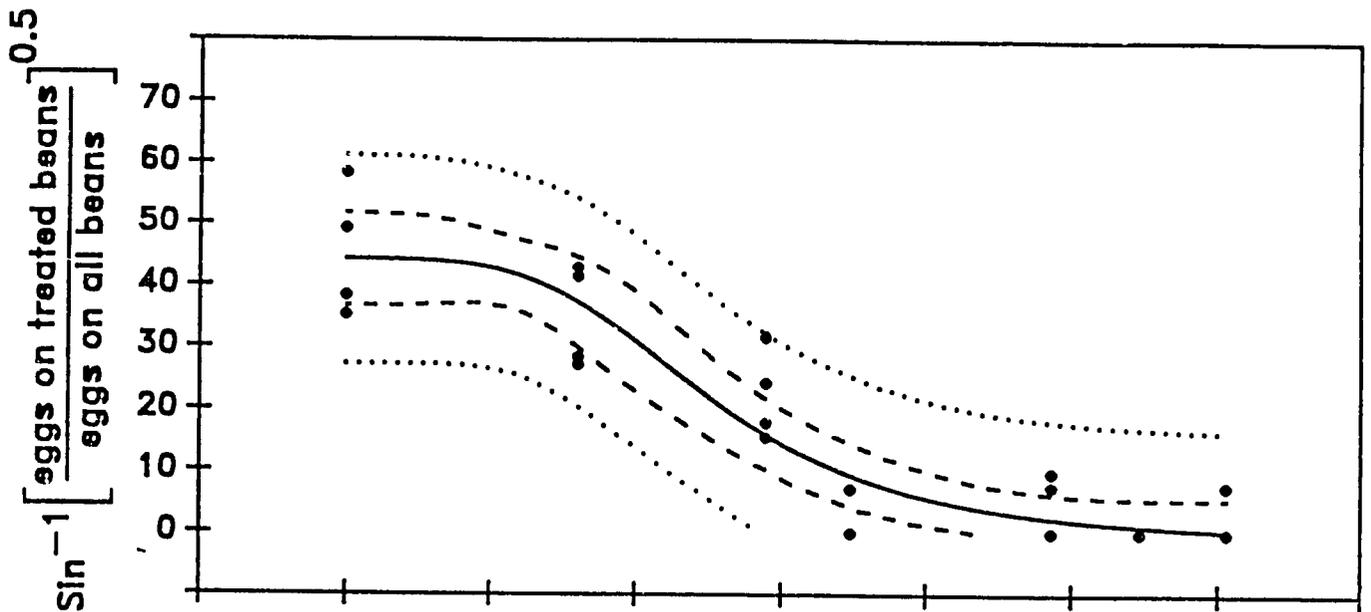
Fig. 2











Appendix 5. Refereed journal article in review. "Contact and fumigant toxicity of milled Ocimum canum Sims to Zabrotes subfasciatus (Boheman) adults." to be submitted to J. Stored Prod. Res. in USDA-ARS and MSU review.

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Contact and Fumigant Toxicity of milled *Ocimum canum* Sims
to *Zabrotes subfasciatus* (Boheman) adults.

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1 Abstract- The efficacy of milled and intact dried leaves of *Ocimum canum* Sims
2 for control of adult *Zabrotes subfasciatus* (Boheman) in dried Pinto beans was
3 determined. The finely-milled dried leaves suppressed oviposition completely
4 at 2% w/w, with an EC_{50} of 0.45% w/w. Increasing concentration of intact
5 dried leaves caused a weak but significant increase in fecundity. At 5%
6 intact leaves (w/w) the number of eggs, hatched eggs and adult F₁ progeny were
7 110, 110 and 111% percent of those for the control, respectively. Additional
8 experiments determined that 1% milled leaves (w/w) in Pinto beans caused 100%
9 mortality in adult *Z. subfasciatus* at 48 hours. An assessment of the fumigant
10 toxicity of milled *O. canum* leaves indicated that 24 g/liter had an ET_{50} of 75
11 hours, whereas neat linalool at 207 mg/liter (the amount extractable from 24 g
12 of milled *O. canum* leaves) had an ET_{50} of 20 hours. Linalool sorbed onto oat
13 proteinaceous microparticles (0.85% w/w- the amount extracted from milled *O.*
14 *canum* leaves) at 1% w/w in beans caused only $24 \pm 10\%$ and $56 \pm 7\%$ mortality in
15 female and male adult *Z. subfasciatus* at 48 hours, respectively. However,
16 fumigation trials with this preparation at 24 g/liter had an ET_{50} of 73 hours,
17 which is statistically similar to that for the milled leaves. Thus, the
18 fumigant toxicity of milled *O. canum* leaves can be completely explained by the
19 gradual release of linalool from the milled material, but the contact toxicity
20 must involve other chemical constituents. In addition to linalool present at
21 8.6 ± 0.9 mg/g (already reported), the milled leaves of this supply of *O.*
22 *canum* also contained β -caryophyllene at 0.80 ± 0.10 mg/g and α -bergamotene at
23 3.41 ± 0.43 mg/g dry weight. These three compounds represented $97.2 \pm 0.3\%$ of
24 the material extracted from this supply of leaves.

INTRODUCTION

The use of locally-available plant materials to limit insect damage in stored foodstuffs is common in low technology surroundings. In Rwanda, sprigs of *Ocimum canum* Sims are sometimes added to stored foodstuffs, particularly in sealed traditional storages called imboho (Dunkel *et al.*, 1986).

Documentation of the usage of this plant species for insect control indicates that it has been used to limit insect damage in storage in several African countries (Irvine, 1955; Prevett, 1962; Watt and Breyer-Brandwijk, 1962, Giles, 1964, Golob and Webly, 1980). *O. canum* (= *O. americanum* L.) was also reported to repel mosquitoes (Watt and Breyer-Brandwijk, 1962).

A previous study with the groundnut bruchid, *Caryedon serratus* (Olivier), indicated that powdered *O. canum* leaves were ineffective at 2.5% w/w, although this plant species was locally used in subsistence systems (Delobel and Malonga, 1987). Weaver *et al.* (1991) found that the milled, dried leaves of *O. canum* had significant topical toxicity to adult *Z. subfasciatus* and that linalool, the major constituent of hexane-isopropanol extracts of these leaves, was a moderately toxic insecticide for several major stored-products pests. The first objectives of this study were to determine the effective concentration of milled *O. canum* leaves for control of *Z. subfasciatus* in beans, and if intact leaves, as traditionally used, had any effect on population development. Subsequently, we wished to determine the relative importance of the fumigative and topical activities of this preparation against this insect species. Lastly, it was necessary to determine if the insecticidal activity observed was simply a function of the large amount of linalool in the leaves, or were other larger secondary plant compounds also involved in the toxicity observed.

1 MATERIALS AND METHODS

2 *Insect rearing.* *Z. subfasciatus* were reared on dried Pinto beans
3 (*Phaseolus vulgaris* L.) at 13.9% equilibrium moisture content (oven dry
4 method: 110 ± 1°C for 24 hr). The incubator was at 27 ± 1°C, 65 ± 5% r.h.
5 with a 12:12 light:dark photoperiodic regime. The mass cultures were
6 maintained in 0.95 liter glass jars containing 0.5 liter of equilibrated
7 beans. Beans were inoculated with approximately 300 adults, and culture diet
8 was discarded after emergence of two successive generations. This stock
9 culture had been laboratory maintained for many years.

10 *Quantification of secondary plant compounds.* The collection, supply and
11 storage of plant material are identical to those described in Weaver *et al.*
12 (1991). Chromatography and mass spectroscopy are also identical to those
13 described in Weaver *et al.* (1991).

14 *Milling procedure.* The intact leaves were removed from a freezer and
15 milled for two minutes in a glass Waring Blendor. The resulting particles had
16 a mode particle size of 0.1- 0.4 mm. These particles were then mixed with the
17 beans and immediately bioassayed.

18 *Concentration-response experiment in beans.* Two experiments were used.
19 A preliminary, cursory experiment indicated that milled leaves prevented
20 oviposition at greater than 2% w/w, while crushed leaves had no effect. A
21 larger scale experiment was conducted using both milled and crushed leaves at
22 0.5, 1, 2, 3 and 5% w/w in 20 g of Pinto beans (13.9% equilibrium moisture
23 content). All experimental incubation was similar to that used for culture
24 maintenance. Treatments were replicated 10 times and 10 control replicates
25 were prepared for each preparation. Parental insect cohorts consisted of 5
26 male and 5 female *Z. subfasciatus* (0-24 hr post-adult emergence) that were

1 placed in 10 dram plastic snap cap vials (2.5 cm internal diameter X 8.0 cm
2 height) with perforated lids for gas exchange. Efficacy was evaluated by
3 counting hatched and unhatched eggs at 25 days and emerged adult progeny at 55
4 days. These counts give reliable measures of fecundity, fertility and F₁
5 adult emergence under normal development (Weaver et al., In Press).

6 *Determination of fumigant toxicity.* Glass 42.5 ml screw cap vials with
7 teflon cap liners were used as fumigation chambers. Inside each chamber a 7.2
8 ml glass vial was supported on the head of a 2.5 cm stainless steel screw
9 standing obliquely at the bottom of the vial. Individual female *Z.*
10 *subfasciatus* (0-18 hr post-adult emergence) were placed in the small vial and
11 sealed with nylon mesh secured by a rubber band, thus exposing the insect to
12 the volatile components of the system only. The test material and test
13 insects were deposited into the fumigation chambers within a 27 ± 2°C, 65 ± 6%
14 r.h. room with a 12:12 light:dark photoperiodic regime to ensure that the
15 initial atmospheric humidity within the fumigation chambers was equivalent to
16 that within the rearing incubator. Three such experiments were conducted.
17 The first experiment used stock synthetic linalool (Sigma Chemical Co., St.
18 Louis MO, U.S.A.) volumes of 0.5, 1, 2, 5, and 10 µl per vial (1 µl/vial =
19 20.7 mg/l assuming all the material volatilized). Ten replicates of each
20 treatment and ten replicates of a no linalool control were prepared and
21 mortality counts were made 15 times during the next 188 hours. The second
22 experiment used the finely-milled leaves of *O. canum* at 1 and 1.5 g per vial
23 (1 g/vial = 205 mg/l linalool assuming complete volatilization or 10 µl of
24 pure linalool per vial). Ten replicates of each treatment and ten replicates
25 of a no linalool control were prepared and mortality counts were made 6 times
26 during the next 120 hours. The third experiment used linalool sorbed onto a

1 proteinaceous oat microparticles (Nurture®- sequestering grade, Basic Bio
2 Systems, Inc., Missoula MT, U.S.A.) at 0.85% w/w. Treatments of 1 and 1.5 g
3 per vial of this preparation were used (1 g/vial = 205 mg/l linalool assuming
4 complete volatilization or 10 µl of pure linalool per vial), as well as two
5 types of controls, one consisting of untreated particles and the other of no
6 material added to the vial containing the insect. Ten replicates of
7 treatments and controls were prepared and mortality counts were made 6 times
8 during the next 120 hours.

9 *Determination of contact toxicity.* These experiments followed the same
10 protocol as the dose-response experiment using leaves, although the materials
11 added were different. Materials added in the first experiment were finely-
12 milled leaves of *O. canum* at 1% and 2% w/w per 20 g of equilibrated Pinto
13 beans. Five replicates of each treatment were prepared as well as five
14 control replicates where no material was added prior to inoculating the beans
15 with insects. The second experiment used linalool sorbed on sequestering grade
16 oat microparticles at 0.85% w/w. This preparation was added to 20 g of Pinto
17 beans at 1%, 2.5% and 5% w/w. Controls used consisted of untreated oat
18 particles at 2% w/w and Pinto beans only. Five replicates of each preparation
19 were used. Live insects were added and mortality assessed at 24 and 48 hours.

20 *Statistical analysis.* The dose-response experiment yielded data which
21 were subjected to regression analysis. All models were evaluated for validity
22 using lack-of-fit tests because the percentage variation explained (r^2) values
23 were very low for certain of the regressions used (Draper and Smith, 1981).
24 Some of the data for intact leaves were subjected to linear regression
25 ($y=a+bx$) using PROC REG (SAS, 1988). Other data for intact leaves were
26 subjected to non-linear regression using either a two-parameter positive

1 asymptotic regression equation, $y=a+be^x$, or a two parameter negative
2 exponential decay equation, $y=a+be^{-x}$. This procedure was performed using PROC
3 NLIN (SAS, 1988). The data for the finely-milled leaves suggested an
4 asymmetric transition and was evaluated using the logistic dose response
5 transition equation $y=a+b/(1+(x/c)^d)$, where $x=\log_{10}(\text{concentration}+1)$ and $y=$ a
6 measure of biological productivity (eggs per female, hatched eggs per female
7 or F, adult progeny at 55 days per female) transformed using $(y+0.375)^{0.6}$
8 (Anscombe, 1948). This procedure was conducted using PROC NLIN (SAS, 1988).
9 The specific parameters describe different portions of the data set. These
10 are : a- the lower plateau of the data set, b- the height of the transition of
11 the data set, c- the center of the transition portion of the data set and d-
12 the steepness of the transition in the data set. The data collected from
13 these experiments follow a many Y for each X pattern. Thus, it is difficult
14 to determine how well the regression equation describes the data with its
15 innate variability. This variability has been termed pure error (Draper and
16 Smith, 1981) or replications error (Bates and Watts, 1988). This is a
17 significant and measurable component of the percentage variation explained
18 (r^2) and can be used to calculate the maximum r^2 possible: that is, the
19 greatest amount of the total variation that can be explained by any model
20 given this inherent variation in the data. The equation used is $\text{max } r^2$
21 $\text{possible} = (SS_{\text{Corrected Total}} - SS_{\text{Pure Error}}) / SS_{\text{Corrected Total}}$ (Draper and Smith, 1981). This
22 equation has been suggested for linear equations, for which it is exact, but
23 it also provides useful descriptive information for the approximate percentage
24 variation explained for non-linear equations.

25 LT_{50} and LC_{50} values were estimated using probit-transformed percent
26 mortality regressed against time and regressed against \log_{10} -transformed

1 concentration using PROC PROBIT (SAS, 1988).

2 The contact toxicity data were transformed using arcsin(proportion
3 killed)^{0.5} to stabilize variances. A one-way analysis of variance (ANOVA) was
4 used to determine if treatment had a significant effect on transformed
5 proportion killed. If treatment was significant in the ANOVA, individual
6 treatments were compared to controls with Dunnett's one-tailed \underline{t} tests to
7 determine if transformed proportion killed for treatments was higher than for
8 the controls. The level of significance was set at $\alpha = 0.01$. This procedure
9 was conducted using PROC GLM (SAS, 1988) and untransformed means are tabulated
10 along with the differences between the transformed treatment and transformed
11 control means. The 99% confidence limits for the differences between the
12 transformed means are also reported.

13 RESULTS

14 β -caryophyllene was present in the milled dried, leaves of
15 *O. canum* at 0.80 ± 0.10 mg/g ($6.0 \pm 0.7\%$ of the extracted compounds) and α -
16 bergamotene was present at 3.41 ± 0.43 mg/g ($25.4 \pm 3.2\%$ of the extracted
17 compounds).

18 The milled leaves of this aromatic mint completely suppressed
19 oviposition of *Z. subfasciatus* at 2% w/w (Figure 1a). The "c" parameter
20 (comparable to an LC₆₀ value) for this treatment was 0.45% w/w (Table 1). The
21 eggs that were laid at the 0.5% and 1% w/w concentrations showed a high
22 percent hatch because all four transition equation parameters for hatched eggs
23 are virtually identical to those for fecundity (Figure 1a and 1b; Table 1).
24 Similarly, F₁ adult progeny emerged at 55 days also reflect good developmental
25 survivorship for eggs that hatched with the slight decrease in the "c"
26 parameter (0.43%) reflecting either a slight developmental delay or a slight

1 increase in mortality for only a very small proportion of the hatched eggs in
2 the 0.5% w/w cohort (Figure 1c; Table 1).

3 In contrast, a weak but significant linear concentration-dependant
4 increase in fecundity and fertility occurred for the intact, dried leaves
5 (Figure 1a and 1b). Adult F₁ progeny present at 55 days followed a positive
6 asymptotic regression for the intact, dried leaves (Figure 1c). These models
7 show no significant lack-of-fit although the r² values are low (Table 1). The
8 number of beans oviposited on decreased (despite an increase in fecundity) at
9 the highest concentrations for the intact leaves, thus displaying a weak
10 negative exponential decay (Figure 2). This is probably the cause for the
11 asymptotic pattern in the F₁ progeny, i.e., more eggs hatched on less beans
12 increased internecine activity within the larval cohorts enough to create the
13 asymptote.

14 The fumigation experiment with synthetic linalool indicated that the
15 10.4 and 20.7 mg/liter concentrations caused little acute toxicity for female
16 *Z. subfasciatus*, although chronic exposure resulted in small decreases in
17 longevity (Table 2). The 104 and 207 mg/liter concentrations caused
18 significant acute toxicity, with the 41.4 mg/liter concentration having
19 intermediate toxicity (Table 2). In all cases, the full volume of linalool
20 volatilized indicating that the atmosphere was not saturated.

21 The fumigation experiment with the milled leaves of *O. canum* gave
22 significant concentration-dependant toxicity, although the LT₅₀ for the 1 g
23 treatment was 3.5 times greater than that for synthetic linalool at the amount
24 present in 1.0 g of milled *O. canum* (Table 2). This suggested that the
25 extractable linalool might be adsorbed by the leaf particles following
26 trichome rupture during the milling process. This was evaluated using

1 synthetic linalool adsorbed on proteinaceous oat microparticles at the amount
2 extractable from milled *O. canum* leaves. The LT_{50} for the 1 g treatment of
3 this preparation was 1.03 times that for the milled *O. canum*, suggesting that
4 it was very likely that the adsorption of linalool on the leaf particles was
5 responsible for the decrease in efficacy relative to neat linalool.

6 The data for the contact toxicity experiment in beans indicated that the
7 milled *O. canum* leaves killed all adult *Z. subfasciatus* at 48 hr for both the
8 1% and 2% w/w preparation (Table 3). In contrast, the 5% oat microparticle
9 w/w formulation (containing linalool at the concentration extractable from
10 milled *O. canum* leaves- 0.85%) caused only 44% mortality in females and 56%
11 mortality in males (Table 3). The 1% (w/w) oat microparticle formulation
12 containing 0.85% linalool (w/w) caused only 24% mortality in females and 56%
13 mortality in the males at 48 hours (Table 3).

14 DISCUSSION

15 It is apparent that the milled leaves of *O. canum* could serve as both
16 contact and fumigant preparations to control *Z. subfasciatus* in stored beans.
17 The potency as a contact treatment in low technology applications is more
18 compelling since there is no requirement for airtight storage containers or a
19 longer duration of exposure. However, as was noted earlier (Weaver *et al.*,
20 1991), it is evident that these fumigative properties represent an inevitable
21 loss of linalool in an unsealed system. Therefore, the remaining protectant
22 potential of finely-milled leaves must be carefully evaluated over longer
23 storage periods, if that is their ultimate intended use. Similarly, the rapid
24 increase in loss of volatiles that occurs with milling also makes it
25 imperative that this preparation be used immediately, or the results may again
26 be less reliable. Also, it is exceedingly important to note that extracted

1 compounds or essential oils frequently show great differences in activity for
2 different stored-product insect pests, so it is exceedingly important that
3 concentrations required for control be determined for each pest insect species
4 prior to usage. This is certainly necessary for powdered *O. canum*, because
5 Delobel and Malonga (1987) found it to be ineffective against the groundnut
6 bruchid, *Caryedon serratus* (Olivier), at 2.5% w/w in groundnuts. However,
7 Ntezurubanza (1987) stated that the taxonomic status of *O. canum* was
8 uncertain, so it is possible that reports of differing activities may be based
9 on different plant species. In addition, there are several chemotypes of *O.*
10 *canum* reported from Rwanda and these are characterized by the concentration of
11 linalool present in their essential oils (Ntezurubanza, 1987). The *O. canum*
12 used in these experiments would be classified as low linalool content-type
13 (about 60%), although the proportion of α -bergamotene extracted (about 25%) is
14 greater than the expected 10% (Ntezurubanza, 1987). The amount of β -
15 caryophyllene (about 6%) agrees with the amount found for low-linalool
16 content-type *O. canum* (Ntezurubanza, 1987). It appears that the combination
17 of these three secondary plant metabolites is more effective than linalool
18 alone in a topical treatment.

19 Our data for fumigation with linalool may suggest that *Z. subfasciatus*
20 is considerably more resistant than other more economically important pests.
21 Shaaya et al. (1991) found that linalool at 15 μ l/ liter of air caused greater
22 than 75% mortality in *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.), and
23 *Oryzaephilus surinamensis* (L.) in 24 hours, whereas the LT_{50} for *Z.*
24 *subfasciatus* with 238 μ l linalool per liter of air in our study was 20.1
25 hours. However, our study was much more conservative than that used by these
26 authors. Our insects were caged above a droplet of linalool clinging to the

1 glass surface at the bottom of a vial and dissolution was passive, whereas
2 Shaaya *et al.* (1991) applied linalool to a small piece of filter paper
3 suspended near the caged insects and aided dissolution with a magnetic stirrer
4 at the base of the vessel. In addition, the bioassay temperature was not
5 reported for this study (Shaaya *et al.*, 1991), so direct comparison with the
6 results we report are impossible. The important point is that we have found
7 that the gas equilibria of relatively large amounts of linalool is sufficient
8 to kill insects above a point source of volatilization, thus suggesting that
9 this heavier-than-air gas may penetrate a stored-product mass efficiently
10 enough to kill free-living insect pests. In addition, β -caryophyllene was
11 found to be relatively non-toxic as a fumigant in this same study (Shaaya *et*
12 *al.*, 1991) which is consistent with our current results. β -caryophyllene has
13 been implicated as a growth inhibitor for certain phytophagous insects
14 (Gunasena *et al.*, 1988; Farrar and Kennedy, 1990).

15 The intact, dried leaves of *O. canum* had no beneficial effect on the
16 fecundity or F_1 adult progeny numbers at the amounts tested, but the highest
17 concentration did have a subtle effect on the number of beans oviposited on.
18 Rwandan consumers base their usage of beans on the presence of bruchid
19 emergence holes, with a criteria of beans with more than a single bruchid
20 emergence hole being unsuitable for consumption (Dunkel *et al.*, 1986).
21 Therefore, although this preparation actually increases insect numbers, the
22 number of damaged beans was reduced at 55 days relative to the control. This
23 might help account for the continued usage of such materials by farmers
24 although their "efficiency is low" and certainly is "more of a token of
25 allegiance to tradition than an effective control measure" (Delobel and
26 Malonga, 1987). In addition, the freshly-harvested *O. canum* sprigs are

1 generally mixed with beans immediately after harvest (Dunkel et al., 1986),
2 presumably releasing volatiles when bruised during the mixing process.
3 Therefore, the true effectiveness of this plant as traditionally used in
4 Rwanda has yet to be evaluated, although the activity of the milled, dried
5 leaves suggests that its effectiveness can be greatly enhanced by such
6 relatively simple processing.

7
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20 (1991) The efficacy of linalool, a major component of freshly-milled
21 *Ocimum canum* Sims (Lamiaceae), for protection against postharvest damage
22 by certain stored product Coleoptera. *J. stored Prod. Res.* 27: 213-220.

Table 1. Parameter values and statistical verification of the regression equations used for data obtained from the concentration-response experiment using milled and intact leaves. Data and regressions are shown in Fig. 1 and Fig. 2.

*Parameters for each regression equation. The four parameter equation is the logistic dose response transition, $y=a+b/l+(x/c)^d$ and is used for milled leaves in Fig. 1a, Fig. 1b, and Fig. 1c; the two parameter linear equation is $y=a+bx$ and is used for intact leaves in Fig. 1a, 1b; the two parameter positive asymptotic equation is $y=a+be^x$ and is used for intact leaves in Fig. 1c; and the two parameter negative exponential decay equation, $y=a+be^x$, is used for intact leaves in Fig. 2. All data were obtained from parental cohorts of 5 male and 5 female *Z. subfasciatus*. There were 10 replicates (n=10) of each of 5 concentrations of both milled and intact leaves. Controls for the two treatments of the leaves (milled and intact) were statistically similar and thus pooled (n=20).

^bAll values of \underline{z} are significantly greater than zero at $\underline{p} \leq 0.05$.

^c $\underline{F}_L = (SS_{Lack\text{-}Of\text{-}Fit}/df_{Lack\text{-}Of\text{-}Fit}) + (SS_{Pure\text{-}Error}/df_{Pure\text{-}Error})$. None of the reported values of \underline{F} indicate a significant lack of fit at $\underline{p} \leq 0.05$.

^d $\underline{F}_{Int} = [(SS_{Corrected\text{-}Total} - SS_{Residual}) / (df_{Corrected\text{-}Total} - df_{Residual})] + (SS_{Residual}/df_{Residual})$. All values of \underline{F} are significant at $\underline{p} \leq 0.05$.

^e $\underline{F}_{Reg} = (SS_{Regression}/df_{Regression}) + (SS_{Residual}/df_{Residual})$. All values of \underline{F} are significant at $\underline{p} \leq 0.05$.

^f% of maximum r^2 possible = $r^2 / [(SS_{Corrected\text{-}Total} - SS_{Pure\text{-}Error}) + SS_{Corrected\text{-}Total}]$. Note: for non-linear equations both this quantity and r^2 are approximate.

1- Treatment. 2- Variable Evaluated.	Parameter ^a	Value ± S.E.	\bar{z}^b	95% confidence limits	$\frac{F_{L^c}}{F_{Res^a}}$ $\frac{F_{Int^d}}{F_{Res^a}}$	r^2	% of maximum r^2 possible ^f
1- Intact leaves.	a	4.9 ± 0.057	86.1	(4.8, 5.0)	1.41 _{4, 64}		
2- Eggs laid per female. Fig. 1a.	b	0.056 ± 0.024	2.30	(0.0075, 0.10)	5.31 _{1, 68}	0.07	49
					5.31 _{1, 68}		
1- Milled leaves.	a	0.60 ± 0.092	6.47	(0.41, 0.78)	0.0147 _{2, 64}		
	b	4.4 ± 0.14	32.2	(4.1, 4.7)	412 _{3, 68}	0.95	100
2- Eggs laid per female. Fig. 1a.	c	0.45 ± 0.024	18.9	(0.40, 0.50)	716 _{4, 66}		
	d	3.3 ± 0.87	3.74	(1.5, 5.0)			
1- Intact leaves.	a	4.9 ± 0.057	84.9	(4.8, 5.0)	1.50 _{4, 64}		
2- Hatched eggs per female at 25 days. Fig 1b.	b	0.055 ± 0.024	2.26	(0.0065, 0.10)	5.13 _{1, 68}	0.07	47
					5.13 _{1, 68}		
1- Milled leaves.	a	0.60 ± 0.92	6.53	(0.42, 0.78)	0.0124 _{2, 64}		
	b	4.3 ± 0.14	31.9	(4.1, 4.6)	402 _{3, 66}	0.95	100
2- Hatched eggs per female at 25 days. Fig 1b.	c	0.45 ± 0.024	19.0	(0.41, 0.50)	701 _{3, 67}		
	d	3.3 ± 0.91	3.66	(1.5, 5.1)			
1- Intact leaves.	a	4.8 ± 0.067	72.6	(4.7, 5.0)	2.26 _{4, 64}		
2- F ₁ adults emerged at 55 days per female. Fig. 1c.	b	-0.36 ± 0.11	-3.27	(-0.58, -0.14)	10.7 _{1, 68}	0.14	56
					5690 _{2, 68}		
1- Milled leaves.	a	0.60 ± 0.083	7.20	(0.43, 0.76)	0.0241 _{2, 64}		
	b	3.9 ± 0.12	32.6	(3.7, 4.2)	433 _{3, 66}	0.95	100
2- F ₁ adults emerged at 55 days per female. Fig. 1c.	c	0.43 ± 0.028	15.1	(0.37, 0.48)	777 _{4, 66}		
	d	3.0 ± 0.82	3.61	(1.3, 4.6)			

1- Intact leaves.	a	0.89 ± 0.015	59.9	(0.86, 0.92)	0.366 _{4.64}		
2- Proportion of beans oviposited on. Fig. 2.	b	-0.00082 ± 0.00026	-3.14	(-0.0013, -0.00030)	9.83 _{1.68}	0.13	87
					2170 _{2.68}		

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Table 2. Probit analyses of mortality data obtained from fumigation bioassays with neat linalool, milled *Ocimum canum* leaves and linalool sorbed onto a proteinaceous carrier composed of oat microparticles.

*Airtight 42 ml vials were used. Ten replicates of 0 - 18 hr post-adult eclosion females were used for each treatment. The mg/vial quantity represents the amount of each material added and the mg/liter of air quantity represents the amount of linalool present that could theoretically volatilize. In the case of the neat linalool, all of the material added did volatilize.

*All regressions of probit transformed data against time were statistically valid. χ^2 test indicates no significant heterogeneity at $P < 0.1$.

Treatment	*Amount (mg/vial) [mg/liter of air]	^b Slope (± SEM)	LT ₅₀ (hr)	95% fiducial limits	LT ₉₅ (hr)	95% fiducial limits
Neat linalool	0.435 [10.4]	0.014 ± 0.0032	175	150, 227	289	234, 423
	0.870 [20.7]	0.015 ± 0.0030	165	142, 209	279	228, 393
	1.74 [41.4]	0.012 ± 0.0022	96	65, 125	236	192, 325
	4.35 [104]	0.22 ± 0.10	29	11, 37	36	32, 147
	8.70 [207]	0.12 ± 0.03	20	14, 25	34	28, 48
<i>Ocimum canum</i> milled leaves (0.86% linalool w/w)	1000 [205]	0.033 ± 0.0073	73	58, 88	122	103, 164
	1500 [307]	0.052 ± 0.013	58	46, 70	90	76, 123
Linalool sorbed onto oat microparticles (0.85% linalool w/w)	1000 [202]	0.021 ± 0.0053	75	51, 102	148	116, 229
	1500 [304]	0.032 ± 0.0071	48	28, 64	98	79, 138

Table 3. Mean percentage mortality (\pm SEM) and statistical analysis for contact toxicity evaluations of milled *Ocimum canum* leaves and for linalool sorbed onto a proteinaceous carrier composed of oat microparticles at 24 hr and 48 hr in 20 g of Pinto beans. Bioassays were conducted in 10 dram plastic vials with perforated lids.

^aPercent mortality is untransformed. Five replicates ($n=5$) for each treatment and each control contained 5 male and 5 female *Z. subfasciatus* (0-1 day post-adult eclosion).

^bDifference between arcsine-transformed treatment and control means. Each ANOVA (for all treatment and sex combinations) was significant. All values of F were greater than 10 for the milled *O. canum* leaves ($df = 2, 12; p \leq 0.002$) and all values of F were greater than 5 for the linalool sorbed onto the proteinaceous oat carrier ($df = 4, 20; p \leq 0.005$). Differences indicate that transformed means for the treatment are significantly greater than transformed means for the control at $\alpha = 0.01$, Dunnett's one-tailed test. Values in [] compare transformed means for linalool sorbed onto carrier to transformed means for untreated carrier at 2.0% (w/w) in Pinto beans, whereas values not in parentheses are for comparisons of transformed treatment means to the transformed mean for a control consisting of untreated beans.

^c99% confidence limits for the differences between transformed treatment and transformed control means.

^dSecond control is untreated 2.0% w/w proteinaceous oat microparticles in Pinto beans.

Treatment	Amount (% w/w)	^a Female mean % mortality (± SEM)	^b Difference between means	^c Confidence limits (99%)	^a Male mean % mortality (± SEM)	^b Difference between means	^c Confidence limits (99%)
<i>Ocimum canum</i> milled leaves (0.86% linalool w/w) at 24 hr	0.0	4.0 ± 4.0	N/A	N/A	0.0	N/A	N/A
	1.0	28 ± 10	23	-3.6, 50	48 ± 8	43*	30, 58
	2.0	56 ± 7	43*	16, 70	72 ± 5	58*	44, 72
Linalool sorbed onto oat microparticles (0.85% linalool w/w) at 24 hr	0.0	4.0 ± 4.0	N/A	N/A	0	N/A	N/A
	0.0 ^d	0	-5.3	-33, 22	0	0	-14, 14
	1.0	20 ± 11	15 [21]	-12, 43 [-6.9, 48]	44 ± 10	42* [42]*	28, 55 [28, 55]
	2.5	32 ± 8	29* [34]*	1.0, 56 [6, 62]	48 ± 5	44* [44]*	30, 58 [30, 58]
	5.0	28 ± 8	24 [29]*	-4, 51 [1.2, 57]	44 ± 4	42* [44]*	28, 55 [28, 55]
<i>Ocimum canum</i> milled leaves (0.86% linalool w/w) at 48 hr	0.0	4.0 ± 4.0	N/A	N/A	0.0	N/A	N/A
	1.0	100	67*	21, 110	100	90*	90, 90
	2.0	100	85*	39, 130	100	90*	90, 90
Linalool sorbed onto oat microparticles (0.85% linalool w/w) at 48 hr	0.0	4.0 ± 4.0	N/A	N/A	0	N/A	N/A
	0.0 ^d	8.0 ± 5.0	5.3	-21, 31	16 ± 7.0	18	-2.1, 39
	1.0	24 ± 10	21 [16]	-5.3, 47 [-11, 42]	56 ± 7.0	46* [28]*	26, 67 [9.5, 51]
	2.5	32 ± 8.0	29* [23]	2.5, 55 [-2.8, 49]	52 ± 8.0	48* [30]*	28, 69 [7.4, 48]
	5.0	44 ± 7.0	36* [31]*	10, 62 [4.6, 57]	56 ± 4.0	49* [30]*	28, 69 [9.7, 51]

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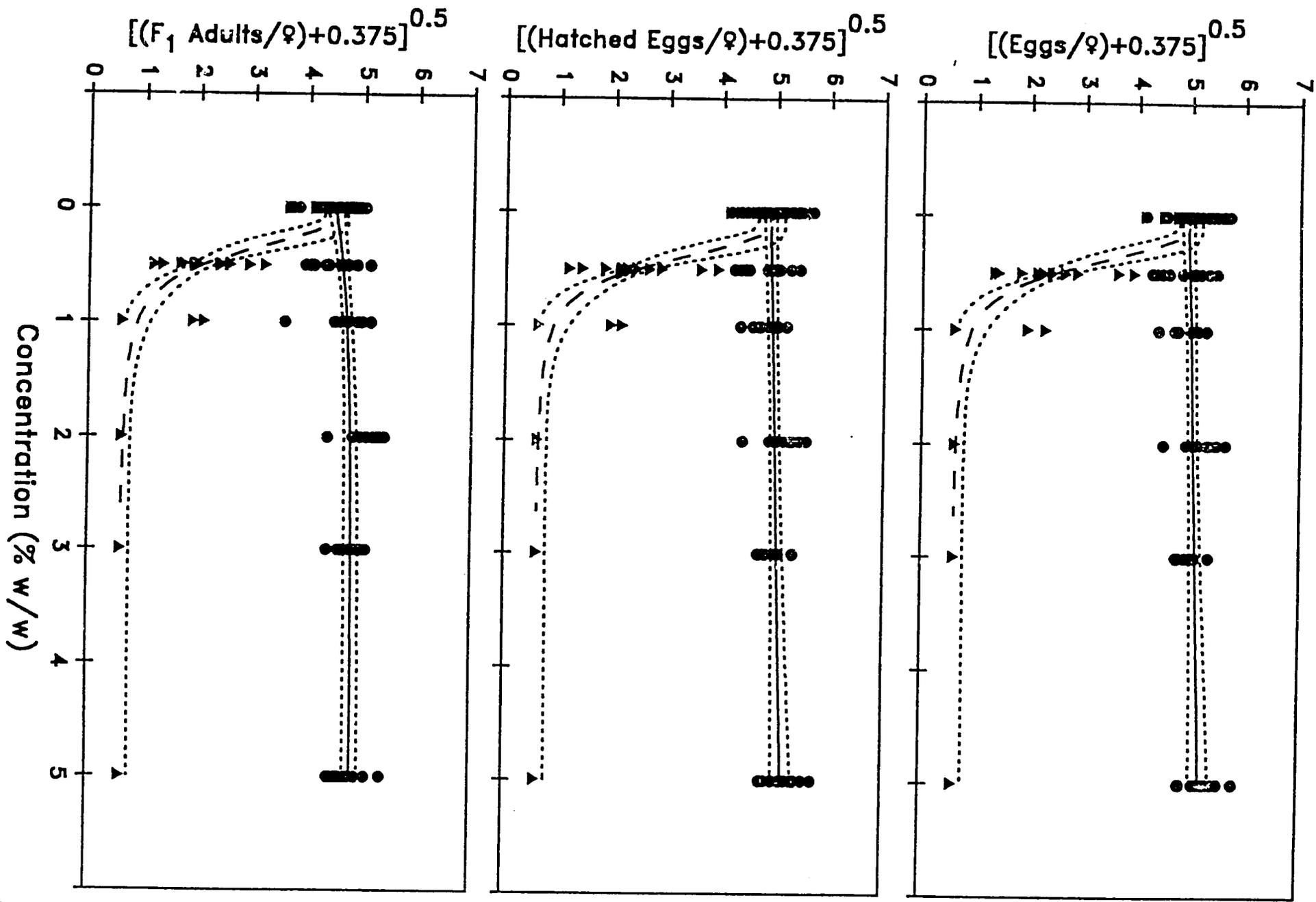
167

Figure 1. Plots of data and regression lines for concentration-response experiment using milled and intact leaves of *O. canum* in Pinto beans. Top portion (1a) is for eggs laid per female; middle portion (1b) is for hatched eggs per female at 25 days; bottom portion (1c) is for F₁ adult progeny per female at 55 days.

See Table 1 for equations, parameters and statistical analyses. All treatments used parental cohorts consisting of 5 male and 5 female *Z. subfasciatus* (0-1 day post-adult eclosion). Incubation was $27 \pm 1^\circ\text{C}$, $65 \pm 5\%$ with a 12 hr light:12 hr dark diel period. Data for milled leaves are (\blacktriangle) with a long dashed regression line. Data for intact leaves are (\bullet) with a solid regression line. Short dashed lines indicate the 95% confidence intervals for all regressions. All measures of maternal productivity have been square root transformed.

Figure 2. Arcsine-transformed proportion of beans oviposited on regressed against concentration of intact leaves (w/w) in 20 g of Pinto beans. Data follow a negative exponential decay.

See Table 1 for equation, parameters and statistical analysis. Parental cohorts consisted of 5 male and 5 female *Z. subfasciatus* (0-1 day post-adult eclosion). Incubation was $27 \pm 1^\circ\text{C}$, $65 \pm 5\%$ with a 12 hr light:12 hr dark diel period. The solid line is the regression line and the short dashed lines indicate the 95% confidence interval.



Appendix 6. Published article from proceedings of international conference. "Toxicity of R,S-linalool to four species of storage Coleoptera as influenced by volatilization and degradation." Proc. Fifth Internat. Conf on Stored Product Protection. Sept. 1990. Vol III, pp. 1609-1617.

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TOXICITY OF R,S-LINALOOL TO FOUR SPECIES OF STORAGE COLEOPTERA AS INFLUENCED BY DEGRADATION AND VOLATILIZATION

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Abstract

Linalool was present at 8.59 ± 0.92 S.D. (standard deviation) mg/g in the dried leaves of Ocimum canum Sims, an annual mint used in Rwanda to protect against postharvest insect damage. The essential oil of O. canum contains up to 90% linalool. Direct exposure of adults of Zabrotes subfasciatus Bohem. to milled, dried Ocimum leaves resulted in 100% mortality of males and 50% mortality of females after 48 hours. Dose-response curves for linalool with adult Z. subfasciatus Bohem., Acanthoscelides obtectus (Say), Rhyzopertha dominica (F.), and Sitophilus oryzae (L.) were completed. A filter paper bioassay system was used to obtain the LC₅₀ values which ranged between 412 and 430 ug/cm². These species all have a narrow dosage between those doses causing 100% mortality and those causing 0% mortality. Concomitant chemical analyses of the treated filter papers at bioassay count times indicated time-dependent quantitative and qualitative changes in the chemical composition of the treated substrates. These results are discussed in terms of the efficacy of using O. canum for the protection against loss due to insects in the traditional food storage systems of Rwanda.

Introduction

In Rwanda, some farmers store dry edible beans, Phaseolus vulgaris L., in traditional closed structures (imboho). Sprigs of whole leaves of Ocimum canum Sims are usually added to the stored foodstuff to prevent insect damage within these structures (Dunkel *et al.*, 1988). Linalool is a major component of the essential oil of this annual mint, representing 60% to 90% of the total volatiles collected (Ntezurubanza, 1987). Linalool (3,7-dimethyl-1,6-octadien-3-ol) is a common component of floral scents and is an olfactory cue in the seeking of host plants by numerous phytophagous invertebrates. It is an oxygenated monoterpenoid which acts as a reversible competitive inhibitor of acetylcholinesterase (Ryan and Byrne, 1988)

and has been suggested as an alternative to conventional insecticides in controlling all life stages of the cat flea, Ctenocephalides felis (Bouche), (Hink et al., 1988). The acute oral LD₅₀ for rats is 2.8 g/kg and the acute dermal LD₅₀ for rabbits is 5.6 g/kg (Opdyke, 1979). The LC₅₀ value for adult C. felis is 39 ug/cm² (Hink et al., 1988). The LC₅₀ value for adult red flour beetles, Tribolium castaneum (Herbst) is 2.5 X 10⁴ ppm (Ryan and Byrne, 1988).

At the national government warehouses of Rwanda (OPROVIA = Office National pour le Developpement et la Commercialisation des Produits viviers et des Protections Animales), a search is underway to identify preparations (Dunkel et al. 1990a,b) or procedures that will replace their sole insect grain/bean protectant, actellic (pirimiphos methyl) which has been used prophylactically since 1983 (Dunkel et al. 1988). Populations of A. obtectus and S. oryzae with a significantly increased resistance to actellic have been identified in OPROVIA Warehouses (Sriharan et al. 1990).

This article describes the quantification of linalool from dried leaves of O. canum Sims and experiments using milled dried leaves against adults of Z. subfasciatus Bohem. This basic information is followed by an evaluation of the toxicity of linalool to adults of stored-products insect species. The degradation and volatilization of linalool from treated filter papers is subjected to insect bioassay and quantitative chemical analysis. The use of this information in the postharvest systems, on farm and in national (OPROVIA) warehouses, of Rwanda is discussed.

Materials and Methods

Insect rearing- All four species were reared at 27±1°C and 65±5% relative humidity under a photoperiodic regime of 12:12 light:dark. Z. subfasciatus and A. obtectus were reared on a diet of dried Pinto beans. R. dominica and S. oryzae were reared upon a diet of 96:2:2 (w/w) soft white wheat:whole wheat flour:brewers yeast.

Quantification of linalool from Ocimum canum- Leaves of O. canum were collected from the Butare prefecture in Rwanda, air-dried according to traditional practice, and express-shipped to Montana State University where they were stored in a -20°C freezer prior to bioassay and chemical analysis. The leaves (1g- 4 replicates) were milled in a Waring blender and extracted immediately in 1:4 isopropanol:hexane (containing decane as an internal standard) in an 125 ml Erlenmeyer flask. The flasks were covered to prevent photodegradation and occasionally agitated. An aliquot of the resulting solution was directly injected into a gas chromatograph for quantitative analysis. Narrow-bore capillary gas chromatography was performed on a Varian Model 3700 equipped with a flame-ionization detector containing a 50m X .20mm (i.d.) HP-1 column with 0.11um film thickness; carrier gas velocity was 31cm³/s (200°C- helium). Temperature program- initial temperature 60°C, initial hold 8 min, temperature increase 4°C/ min, final temperature 260°C.

The linalool peak was tentatively identified using narrow-bore capillary GC-MS. The gas chromatograph was a Varian Model 3700 equipped with a flame ionization detector and a 30.0 m X 0.25 mm (i.d.) DB5 column with 0.25u film thickness: Column conditions used were: He carrier gas velocity- 30cm³/s (220°C); temperature

programming- initial temperature 50°C, initial hold 4.0 min, temperature increase 5.0°C, final temperature 280°C, final hold 10 min; injector temperature 260°C; detector temperature 290°C. The electron impact mass spectra were obtained on a VG Analytical model VG 70EHF mass spectrometer operating at 70eV with a source temperature of 200°C.

Leaf exposure bioassay- Ten adult *Z. subfasciatus*, 5 male and 5 female (0-3 days post-adult eclosion), were added to 5.5 cm glass petri dishes in which 1.0 g of milled dried leaves of *Q. canum* had been distributed evenly. Ten replicates of the treatment and ten replicates of a control consisting of a petri dish containing no plant material were also conducted. Bioassays were evaluated by viewing mortality at 24 and 48 hours. The bioassay conditions were $28 \pm 1^\circ\text{C}$ at $65 \pm 5\%$ R.H. with a 12:12 light:dark photoperiodic regime.

Linalool dose-response bioassays- A 9.0 cm diam. Whatman #1 filter paper was placed in a 10cm diam. glass Corning petri dish. A 1.0 ml aliquot of the appropriate dilution of R,S linalool to the filter paper in 1.0 ml of absolute ethanol. The ethanol was allowed to evaporate for 20 minutes prior to the addition of the insects. Mortality was evaluated if the insect was immobile and did not react to a probing with a blunt dissecting probe three times. Moribundity was assessed by viewing those insects that were on their backs and ambulating very weakly. These insects were subsequently righted and viewed carefully. Those that immediately fell onto their backs again as a result of intoxication were classified as moribund. At higher dosages all moribund insects subsequently died with the passage of time. Recovery occurred rarely at lower dosages. The bioassay conditions for this and the following bioassay were $27 \pm 2^\circ\text{C}$ at $65 \pm 8\%$ R.H. with a 12:12 light:dark photoperiodic regime. *Z. subfasciatus* were sexed with 5 males and 5 females used in each replicate; 10 adults of unknown sex were used for the other species. Counts of mortality/moribundity were conducted at 24 hours.

Bioassay of linalool with increasing duration of air exposure- The protocol was similar to that for the dose-response bioassays. The ethanol in an aliquot delivering 500 ug/cm^2 on the filter paper was allowed to evaporate for 20 minutes and ten replicates of *Z. subfasciatus* (5 male, 5 female; 0-1 days post-adult eclosion) were added immediately. Other replicates were covered though they contained no insects and ten *Zabrotes* (as above) were added at times of 0.25 hr, 6 hr, 18 hr and 24 hr post-ethanol evaporation. Mortality /moribundity were determined as above and evaluated at 24 hr after the introduction of the insects into each trial. Ten replicates of an ethanol control were conducted simultaneously for each trial. The bioassay conditions were $28 \pm 1^\circ\text{C}$ at $65 \pm 5\%$ R.H. with a 12:12 light:dark photoperiodic regime.

Quantitative chemical analysis of linalool-treated substrates with increasing duration of air exposure- The air exposure bioassay procedure (above) included four additional replicates. At the time of insect introduction each filter paper for these replicates was handled with forceps, cut into ca. 0.5 cm^2 pieces and transferred into 1:4 isopropanol:hexane (containing decane as an internal standard) in a 250 ml Erlenmeyer flask for 24 hours. The flasks were covered to prevent photodegradation

and occasionally agitated. The resulting solution was directly injected into a gas chromatograph for quantitative analysis and GC-MS for identification as per above.

Statistical analysis- The linalool dose-response bioassay data were subject to probit analysis (Matsumura, 1975) after Abbott's formula (Abbott, 1925) was used to adjust for control mortality. Since the dominant response of the moribund insects was to die, moribundity and mortality were pooled for statistical analysis.

Results

Extraction and quantitative gas chromatographic analysis indicated that linalool is present in milled, air-dried leaves of Q. canum at 8.59 ± 0.92 S.D. (standard deviation) mg/g. Figure 1 shows the gas chromatogram of the solvent extract of milled Q. canum leaves; the peak at the retention time of 11.73 is decane (internal standard) and the peak at 15.89 is linalool (Weaver et al., submitted).

The results of the leaf exposure bioassay (Table I) indicated 100% mortality of male Z. subfasciatus at 24 hours and only 50% mortality of the females at 48 hours (Weaver et al., submitted).

The linalool dose-response bioassays (Figure 2) indicated that the LC_{50} values for all four species were similar (Weaver et al., submitted). The LC_{50} values for each species were: Z. subfasciatus- 429.3 $\mu\text{g}/\text{cm}^2$; A. obtectus- 412.1 $\mu\text{g}/\text{cm}^2$; R. dominica- 430.2 $\mu\text{g}/\text{cm}^2$; S. oryzae- 426.7 $\mu\text{g}/\text{cm}^2$ (Table II).

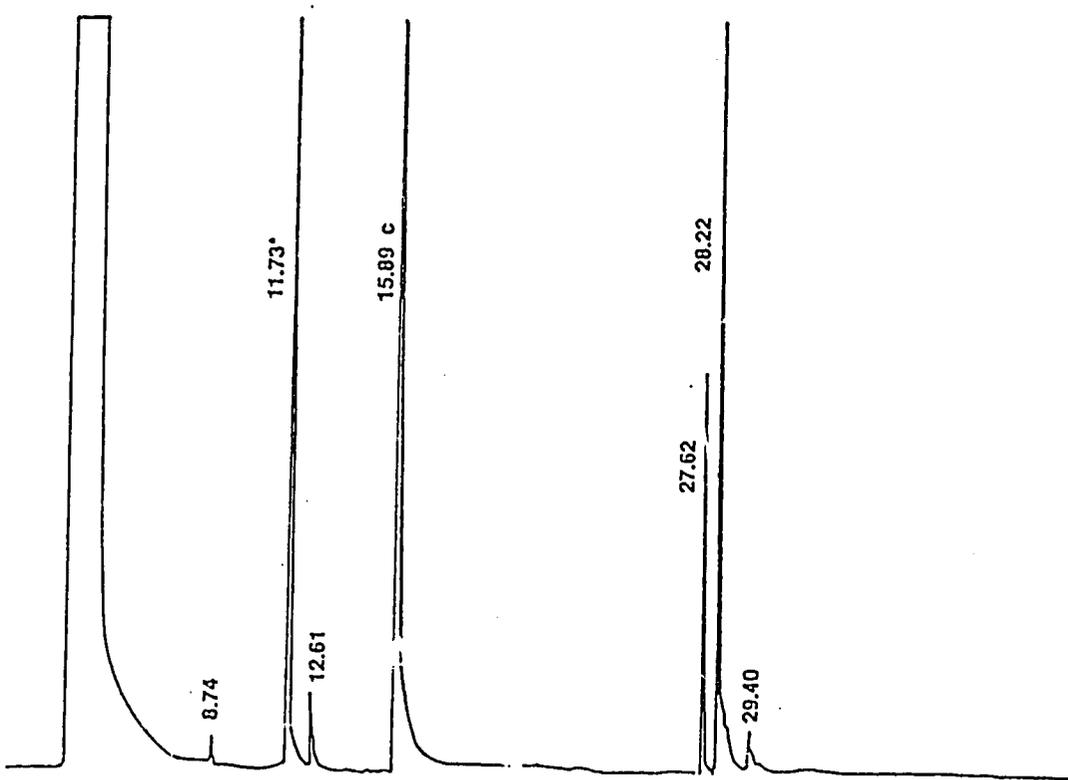
Increased duration of air exposure of linalool treated substrates had differing effects upon male and female Z. subfasciatus susceptibility (Table III). The susceptibility of females was halved at fifteen minutes post-ethanol evaporation, whereas the susceptibility of males was not noticeably decreased until eighteen hours post-ethanol evaporation (Weaver et al., in preparation).

The quantitative analysis indicated that the decrease in susceptibility of females at 15 minutes post-solvent evaporation is correlated to a decrease of 50 $\mu\text{g}/\text{cm}^2$ (approximately 10% of the initial aliquot) of linalool at this time (Table IV). No further increase in the amount of linalool volatilized is evident, nor is any consistent change in the proportions of linalool relative to degradation products evident (Weaver et al., in preparation).

The amount of linalool in the stock solution decreases most dramatically when the stock solution is delivered on filter paper. After the introduction to filter paper, the next major decrease occurs 15 minutes after evaporation of the ethanol solvent is begun. No further changes in quantity of linalool on the filter papers occurs after 6, 18, or 24 hours of air-exposure on Whatman # 1 filter paper, either qualitatively or quantitatively.

Discussion

The large amount of linalool in the dried leaves of Q. canum corroborates the earlier reported high percentage of linalool found in the essential oil of this species (Ntezurubanza, 1987). The milled leaves of this species are quite toxic to Zabrotes males, however the amount of linalool present does not correlate with the filter paper dose-response trials (Table II). This is due to two factors relating to methodology. The first factor is that the insects in the milled plant trials are rapidly coated with



c Tentative identification: R,S-linalool.

* decane — internal std.

Figure 1. Gas chromatogram of 1g of freshly-milled leaves of air-dried Qcimum canum extracted in 1:4 isopropanol:hexane for 24 hours. Retention times: 11.73- decane (internal standard); 15.89- R,S-linalool. Gas chromatography described in Materials and Methods.



Table I. Acute mean mortality (\pm std. dev.) of *Zabrotes subfasciatus* Bohem. (0–3 days post-adult eclosion) exposed¹ to 1g of milled dried leaves of *Ocimum canum* Sims (Lamiaceae). 10 replicates; 5 ♀, 5 ♂ per rep.

Treatment	Mortality			
	(24 hr)		(48 hr)	
	♀	♂	♀	♂
<i>O. canum</i>	1.8 \pm 1.2	5.0 \pm 0.0	2.5 \pm 1.4	5.0 \pm 0.0
control	0	0	0	0

¹ Temperature -- 28 \pm 1°C. Relative humidity — 65 \pm 5%.
Photoperiod — 12:12 light:dark.

Table II. Acute mean mortality (\pm standard deviation) and LC50 from petri dish bioassay of adult stored product insects to R,S-Linalool (3,7-dimethyl-1,6-octadien-3-ol). (n=10; 10 insects/rep; 27 \pm 2° C; 65 \pm 8% relative humidity; 12:12:light:dark).

Zabrotes subfasciatus Bohem. (0–1 day post adult eclosion; 5♂ + 5♀)

Dose (μ g Linalool/cm ² filter paper)								LC50
0	250	300	350	400	450	500	750	(95% confidence interval)
0	0	0.20 \pm 0.42	0.40 \pm 0.84	2.40 \pm 2.17	5.80 \pm 2.57	9.80 \pm 0.63	10	428.30 (417.91, 438.94)

Acanthoscelides obtectus (Say) (0–1 day post adult eclosion; unsexed)

Dose (μ g Linalool/cm ² filter paper)								LC50
0	250	300	350	400	450	500	750	(95% confidence interval)
0	0.10 \pm 0.32	0.60 \pm 0.97	2.50 \pm 1.18	4.00 \pm 1.49	6.60 \pm 2.76	9.90 \pm 0.32	10	412.15 (401.64, 422.92)

Rhyzopertha dominica (F.) (0–6 days post adult eclosion; unsexed)

Dose (μ g Linalool/cm ² filter paper)								LC50
0	250	300	350	400	450	500	750	(95% confidence interval)
0.40 \pm 0.69	0.50 \pm 0.71	1.40 \pm 1.27	2.30 \pm 1.49	3.40 \pm 1.96	5.30 \pm 3.90	8.40 \pm 3.06	10	430.19 (417.74, 443.02)

Sitophilus oryzae (L.) (0–6 days post adult eclosion; unsexed)

Dose (μ g Linalool/cm ² filter paper)								LC50
0	250	300	350	400	450	500	750	(95% confidence interval)
0.10 \pm 0.32	0.70 \pm 1.25	0.90 \pm 1.60	1.80 \pm 1.55	1.80 \pm 0.79	5.30 \pm 4.24	9.50 \pm 1.27	10	426.66 (412.75, 441.05)

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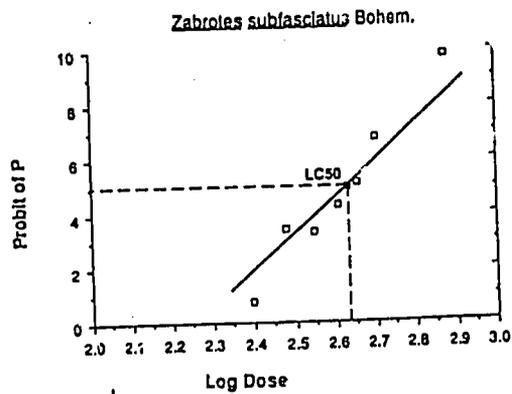
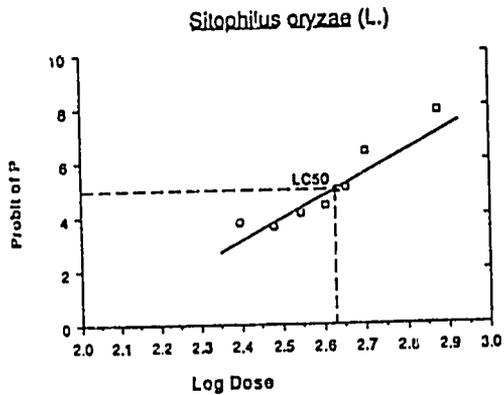
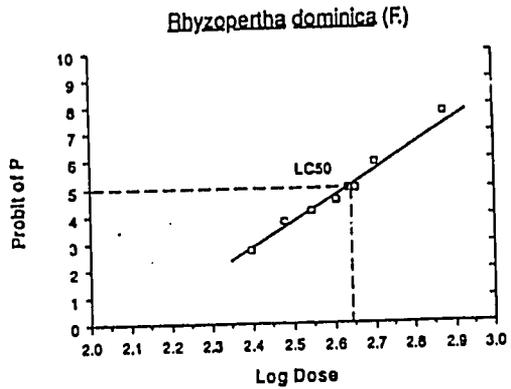
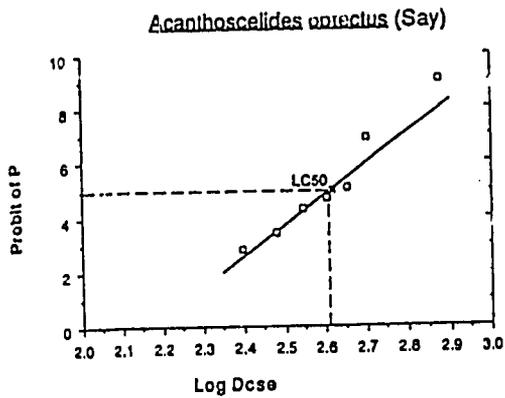


Figure 2. Linalool-induced dose-response curves for four species of stored-products Coleoptera in filter paper bioassays ($n = 10$; 10 insects/rep; $27 \pm 2^\circ\text{C}$; $65 \pm 8\%$ relative humidity; 12L:12D). *Zabrotes subfasciatus* and *Acanthoscelides obtectus*- 0-1 day post-adult eclosion; *Rhyzopertha dominica* and *Sitophilus oryzae*- 0-6 days post-adult eclosion.

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Table III. Acute mean mortality (\pm std. dev.) of *Zabrotes subfasciatus* Bohem. (0-1 day post-adult eclosion) exposed¹ to linalool-treated Whatman #1 filter papers with increasing pre-bioassay interval duration. R,S-linalool (aliquot to yield 500 $\mu\text{g}/\text{cm}^2$) applied in ethanol. 10 replicates, 5 σ and 5 σ each. Mortality and morbidity data after 24 hours of exposure. Ethanol was allowed to evaporate 20 min in all trials including the control.

Treatment	Sex	Pre-exposure interval ¹				
		0 hrs	0.25 hrs	6 hrs	18 hrs	24 hrs
linalool	σ	5.0 \pm 0.0	4.9 \pm 0.2	4.8 \pm 0.4	0.6 \pm 0.7	1.2 \pm 1.2
	σ	3.8 \pm 1.9	1.7 \pm 1.7	2.0 \pm 1.1	0.3 \pm 0.5	0.1 \pm 0.3
control	σ	0	0	0	0	0
	σ	0	0	0	0	0

¹ Temperature — 28 \pm 1°C. Relative humidity — 65 \pm 5%.
Photoperiod — 12:12 light:dark.

Table IV.

Amount ($\mu\text{g}/\text{cm}^2$) (\pm std. dev.) and proportions (\pm std. dev.) of R,S-linalool and degradation products extracted from a Whatman #1 filter paper with increasing duration of air exposure. Linalool delivered at 500 $\mu\text{g}/\text{cm}^2$; four replicates. Extractions were concomitant with introduction of insects for bioassay of the same protocol (Table 3).

Compound ¹	Amount ($\mu\text{g}/\text{cm}^2$)						
	O ⁽¹⁾	O ⁽²⁾	O ⁽³⁾	15 minutes*	6 hours*	18 hours*	24 hours*
14.6 a	0.634 \pm 0.158	0.548 \pm 0.170	1.133 \pm 0.234	1.181 \pm 0.277	1.344 \pm 0.123	1.335 \pm 0.138	1.213 \pm 0.282
15.2 b	1.124 \pm 0.115	1.217 \pm 0.329	1.648 \pm 0.264	1.622 \pm 0.195	1.362 \pm 0.322	1.531 \pm 0.316	1.664 \pm 0.383
15.8 c	432.974 \pm 11.693	402.528 \pm 19.533	437.128 \pm 37.059	337.447 \pm 25.411	384.286 \pm 18.080	335.114 \pm 22.627	306.502 \pm 20.076
17.3 d	2.990 \pm 1.415	4.729 \pm 0.404	2.524 \pm 0.170	2.379 \pm 1.455	2.768 \pm 2.062	2.741 \pm 1.255	1.349 \pm 0.629

Compound ¹	Proportion (≤ 1.0)						
	O ⁽¹⁾	O ⁽²⁾	O ⁽³⁾	15 minutes*	6 hours*	18 hours*	24 hours*
14.6 a	0.001 \pm 0.000	0.002 \pm 0.000	0.002 \pm 0.000	0.003 \pm 0.001	0.003 \pm 0.000	0.003 \pm 0.000	0.003 \pm 0.001
15.2 b	0.002 \pm 0.000	0.003 \pm 0.001	0.004 \pm 0.001	0.004 \pm 0.001	0.003 \pm 0.001	0.005 \pm 0.001	0.004 \pm 0.001
15.8 c	0.991 \pm 0.003	0.987 \pm 0.005	0.988 \pm 0.001	0.986 \pm 0.005	0.986 \pm 0.008	0.986 \pm 0.003	0.986 \pm 0.001
17.3 d	0.006 \pm 0.003	0.011 \pm 0.000	0.006 \pm 0.000	0.012 \pm 0.002	0.007 \pm 0.005	0.007 \pm 0.003	0.003 \pm 0.002

O⁽¹⁾ Aliquot delivered directly into extraction flask.

O⁽²⁾ Aliquot delivered upon filter paper and immediately extracted.

O⁽³⁾ Aliquot delivered upon filter and ethanol evaporated (20 min).

* After ethanol evaporation.

Air exposure at 28 \pm 1°C; 55 \pm 5% relative humidity; 12:12 light:dark.

a Tentative identification: beta-myrcene.

b Tentative identification: d-limonene.

c Tentative identification: R,S-linalool.

d Tentative identification: 3,7 dimethyl 1-octen-3-ol.

1 Tentative identification based on gas chromatography — mass spectroscopy

sticky particles (due to their own movement) that adhere to them and receive a topical chemical and physical treatment whereas the filter paper trials provide little direct topical treatment initially. After the insects become disoriented and fall upon their dorsal surfaces frequently they may also become coated with residual linalool from the surface of the filter paper. The second factor is that the active surface area of the filter paper is much greater than its two dimensional area, so the actual amount per cm^2 is much lower than that reported.

The data indicate a surprising degree of similarity in LC_{50} values for the four species. However, the slopes of the probit lines vary, the lesser slopes indicating that the responses of R. dominica and S. oryzae are more heterogeneous (Figure 2). This may be due to greater genetic variability in the susceptibility of the populations of these species or to the greater age range of individuals used for these two species (0-6 days). The Z. subfasciatus and A. obtectus were 0-1 day old.

The LC_{50} values reported differ greatly from the 39 ug/cm^2 reported for Ctenocephalides felis adults (Hink *et al.*, 1988). However, this cat flea bioassay involved saturated papers treated with linalool in a water/Tween 80 solution, which was not evaporated, thus allowing greater potential contact (and subsequent topical coating) than the bioassay method used in the present study. The LC_{50} values do compare very favorably with the $2.5 \times 10^4 \text{ ppm}$ (526 ug/cm^2) reported for an insecticide-susceptible strain of Tribolium castaneum (Ryan and Byrne, 1988). This bioassay was very similar to the one used in the present study, except that the solvent was acetone and was evaporated in one minute.

The decrease in the amount of linalool extracted from the filter papers with increasing air exposure appears to be directly related to volatilization. Immediately after ethanol evaporation the odor of linalool is quite apparent, whereas after several hours it is much less apparent. The logical assumption is that the amount of linalool present has decreased below an odor threshold. The extractions indicate that this decrease may be due to the linalool molecules associating with the substrate strongly enough to prevent volatilization, but not liquid solvation. It is interesting that the males have higher susceptibility to both O. canum and air-exposed linalool, though this may be simply due to their smaller size.

In the Rwandan grain and bean storage system, insecticidal plants such as O. canum are used only at the farm level. Of the farmers surveyed throughout Rwanda, only 2.1% ($n=94$) use insecticidal plants in their bean storage, and 2.6% of the farmers use insecticidal plants in their sorghum storage ($n=39$) (Dunkel *et al.*, 1988). Based on our results, it would be efficacious for more Rwandan farmers to grow O. canum and to use finely crushed, dried leaves of the plant for on farm protection of beans and grain. The methodology that the farmers would need to practice would make traditional baskets with open tops ineffective with this preparation. The O. canum will be more efficacious if used with the imboho, the plastic sack, or the metal drum. Other studies (Dunkel *et al.*, 1988) indicate that these structures were used by 17% of the farmers for June harvest beans, 10 % of the farmers for January harvest beans and 23% of the farmers for sorghum. Rwandan farmers also use earthen pots and gourds for storage. If these are used with the O. canum preparation, our results suggest that these structures should be covered and used for long term storage or re-sealed immediately when beans or grain are used prior to the end of the storage period.

At national (OPROVIA) warehouses in Rwanda, there is a critical need for alternatives to actellic (pirimiphos methyl) as a long term protectant against insects.

Results of this study indicate that use of the entire plant or a preparation that contained a high percentage of linalool would be more efficacious in structures or containers that prevent reentry by insects. At OPROVIA warehouses, the plastic bags already in use would be preferable. At cooperative storehouses, the concrete silo structures would be ideal for use of this preparation. Although more efficacy testing is required before actual use of the preparation, this promises to be a useful material which does not require foreign currency to produce.

The studies on O. canum and linalool reported here indicate that freshly milled or very finely crushed leaves of O. canum have the potential for protection in closed structures such as the imboho, above ground sealed storage, individual plastic bags, metal drums, and below ground storages.

Conclusion

The efficacy of O. canum in providing protection against postharvest insect damage may be partly due to the concentration of linalool in its leaves. Linalool, as a component of O. canum, or when purchased as a synthetic preparation, appears to have its effect in a relatively short time frame, similar to that of a fumigant. Control, however, may be achieved by behavioral effects such as repellency or oviposition deterrence which require lower dosages of chemicals. We are currently investigating these phenomena.

The short effective period suggests that the leaves of Ocimum canum provide the greatest protection against insect damage when initially added to the stored foodstuff in a finely milled preparation. Protection over many months would require that the grain or other food commodity be sealed and airtight. With minor modifications in the current on farm storage system in Rwanda, farmers could make better use of this linalool-producing plant which can be produced by the farmers without the expenditure of local currency. At the national (OPROVIA) warehouses, linalool, as a component of O. canum, could be adapted as a fumigant in plastic, not jute, bags. For OPROVIA, this preparation could be obtained within the country without the use of foreign exchange. Use of the entire plant or a preparation which contained a high percentage of the linalool component would be especially useful in other areas of the world where underground and/or sealed storage is a traditional practice.

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Appendix 7. Published article from proceedings of international conference. "Growth regulatory effects of a Rwandan medicinal plant: Tetradenia riparia (Lamiaceae) on stored grain and bean insects." Proc. Fifth Internat. Conf on Stored Product Protection. Sept. 1990. Vol III. pp. 1609-1617.

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POPULATION SUPPRESSION EFFECTS OF RWANDAN MEDICINAL PLANT,
TETRADENIA RIPARIA (HOCHST.) CODD (LAMIACEAE)
ON STORED GRAIN AND BEAN INSECTS

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ABSTRACT

Tetradenia riparia (Hochst.) Codd (Lamiaceae) is a perennial mint whose dried leaves are used as an infusion in Rwandan folk medicine for many conditions in which microorganisms play a major role. Laboratory studies by others have confirmed the antimicrobial properties of this plant against Candida albicans, Shigella dysenteriae, and Streptococcus pyogenes. Since antimicrobial preparations also often have insecticidal or population regulatory properties, the leaves of this plant were examined as a possible insect protectant in wheat and dry edible beans (Phaseolus vulgaris L.). Multigenerational growth and development bioassays were completed for the two main causes of loss in dry edible beans, the bean weevil, Acanthoscelides obtectus Say and the Mexican bean weevil, Zabrotes subfasciatus Bohem with concentrations (w/w) of 0, 0.1, 0.5, 1.0, 2.0, 3.0, 4.0, and 10.0. At the highest concentration (10.0%) there was a significant decrease in the number of eggs laid. At $\geq 2.0\%$, there was a significant decrease in the proportion of beans used for oviposition and subsequent emergence. Beans stored with T. riparia were also subjected to human sensory evaluation by a trained panel in Rwanda. The panel found beans stored with T. riparia as acceptable as those stored with conventional methods (1% actellic), which is currently used as the sole commercial protectant against storage insects in Rwanda.

INTRODUCTION

Traditional medicine and traditional insect management in most cultures are a rich source of ideas for sustainable pest management. These practices are a focus in our laboratory for two reasons. First, within the culture that developed the practice, these traditional on-farm procedures may require only minor changes to be used successfully on a large scale. Second, these traditional practices may be a source of novel techniques and of genetic material that codes for defensive phytochemicals which may be adapted for insect management in the USA. Laboratory studies need to determine if, indeed, these traditional products and techniques are efficacious and environmentally safe. This study was based on both a search for plant products we can use in the USA and ideas that can be adopted by farmers and managers of large scale warehouses in Rwanda.

Pest management solutions for a developing country can often be found within the borders of the country itself. One possible source of such solutions is traditional medicine (antimicrobial treatments) used by the people (Dunkel 1988). The most important plant in Rwandan pharmacopoea is a perennial mint, umuruvumba, *Tetradenia riparia* (Hochst.) Codd. Most farmsteads contain one or more of these shrubs (Dunkel, personal observation). In Rwanda, umuruvumba is used against malaria, yaws, helminths, gastroenteritis, dental abscesses, and other disorders (Van Puyvelde et al. 1975; Van Puyvelde 1976). An infusion is made of the leaves and it is taken orally or applied as moist leaves under the tongue. The whole leaf contains hundreds of compounds, including 8(14),15-sandaracopimaradiene-7 α ,18-diol, a diterpenol. In laboratory tests it was shown to have good antimicrobial activity for fungi, bacteria, and protozoa of medical importance (Van Puyvelde et al., 1986). A leaf fraction containing this compound is currently being mass produced in Rwanda at Centre de Recherche sur la Pharmacopée Médecine Traditionnelle (CURPHAMETRA). The question was posed by CURPHAMETRA as to the possibility of other fractions or the essential oil having insect control properties.

The main sources of protein and calories for the people of Rwanda are dry beans (*Phaseolus vulgaris* L.). The main grain is sorghum. In 1984-1986, we conducted an extensive national survey of these commodities, on-farm, in cooperative silos and hangars, and at OPROVIA warehouses (Dunkel et al. 1988a). This survey indicated that the most common storage insects causing loss in Rwanda were the bean bruchid, *Acanthoscelides obiectus* Say and the two grain insects, the lesser grain borer, *Rhyzopertha dominica* (F.) and the rice weevil, *Sitophilus oryzae* (L.). The main insecticide used is actellic (pirimiphos methyl). It is used by 50% of the farmers for beans and by 28% of the farmers for sorghum (Dunkel et al. 1988). At the national government warehouses of Rwanda (=OPROVIA = Office National pour le développement et la Commercialisation des Produits Viviers et des Productions Animales), actellic has been used prophylactically, since 1983. The survey also indicated populations of these two grain insects at OPROVIA warehouses may already show significant resistance to actellic, the main insecticide used in Rwanda. In 1988, populations of *A. obiectus* and *S. oryzae* with significantly increased resistance to actellic were identified in OPROVIA Warehouses (Sriharan et al. 1990).

Due to the suspected resistance problem, it was recommended that alternative, more sustainable insect protectant procedures be developed for the postharvest system in Rwanda (Dunkel et al. 1988). A search is underway to identify low input, sustainable storage structures (Hanegreefs et al. in press) or plant preparations which are or can be locally produced (Dunkel et al. 1990; Weaver et al. 1990, 1991). One of these plants, *Ocimum canum* Sims, is used by a small percentage of Rwandan farmers for protection against storage insects (Dunkel et al. 1988a). Laboratories indicate that it has easily volatilized, strongly insecticidal properties (Weaver et al. 1990, 1991).

We tested the general hypothesis that the most common traditional antimicrobial in Rwanda can play a role in storage insect management in Rwanda. We tested the specific hypothesis that: *T. riparia* has population suppression effects on Rwandan stored grain and bean insects and these effects can be used as an economical, sensory acceptable alternative to actellic in Rwandan warehouses.

MATERIALS AND METHODS

Plant material preparation- Leaves of *T. riparia* were collected from the area surrounding Butare, Rwanda and dried in a 40°C oven for 24 hr. The dried leaves were then crushed and shipped via courier to Montana State University. Upon receipt, the plant material was stored at -20°C until used. Two lots of plant material were used in the following experiments. The first was collected and shipped in February, 1990 (Lot A) and the second in June, 1990 (Lot B). All experiments were begun within 14 days of the receipt of the plant material. Plant material was received in a crushed condition (mode particle size 3-5mm, particle size range 0.75-15.0 mm). Freshly milled preparations were prepared in an electric coffee grinder for 40s just prior to initiation of experiment (mode particle size 0.3-6mm, particle size range 0.15-1.1mm).

Essential oil was prepared using a large scale Clevenger-type steam distillation apparatus at CURPHAMETRA (Center for Ethnopharmacology) in Butare, Rwanda. The yield of this procedure was 0.07% from fresh leaves. Shipping and storing conditions were as for the leaves.

Insect rearing and experimental conditions- All four species were reared at $27 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ relative humidity under a photoperiodic regime of 12:12 light:dark. Experiments were conducted under similar conditions. The Mexican bean weevil, *Zabrotes subfasciatus* Bohem and *A. obtectus* were reared on a diet of Pinto beans (*Phaseolus vulgaris* L.). *Z. dominica*, and *S. oryzae* were reared on a diet of 96:2:2 w/w (weight/weight) soft white wheat:whole wheat flour:brewers yeast.

Gas chromatographic/mass spectroscopy analysis- Relative ion chromatograms of the essential oil from leaves of *T. riparia* were obtained under the following conditions: Initial T = 50°C ; Initial hold = 4 min; Ramp = $5^\circ\text{C}/\text{min}$; Final T = 280°C ; Final hold = 10 min; Column = 30 m DB5 (J and W Scientific Folsom CA), 0.25 mm internal diameter, 0.25 μm film thickness; 260°C injection port; 290°C detector T. EI-MS were obtained on VG Analytical VG 70EHF operating at 70 eV with a source T of 200°C .

Oviposition and egg development studies- Leaves of *T. riparia* were added to ten replicates of 20g dried Pinto beans (approximately 50 beans; 16.07% moisture content determined by oven dry at 110°C) at dosages of 0.1, 0.5, 1.0 and 10.0% w/w or dosages of 1.0, 2.0, 3.0, 4.0 and 10.0% w/w. Ten control replicates, without *T. riparia*, were also prepared for each experiment for both the milled and crushed preparations. The plant material was placed in a 10 dram plastic shell vial and mixed by shaking. Five male and five female *Z. subfasciatus* (0-3 da after emergence from bean) were added to each vial, and it was sealed with a perforated lid. Adults were allowed to oviposit until death, which occurred within 17 da for all individuals. At 25 days, individual beans were examined to count the number of hatched and unhatched eggs.

For *A. obtectus*, a reduced scale experiment was required because this species did not affix its eggs directly to the bean pericarp as *Z. subfasciatus* does, but lays them loose among the plant material and beans. Dosages were prepared as percentage by weight of three beans only, to which four male and four female *A. obtectus* (1-2 da after adult emergence from bean) were added. Insects were sexed according to Halstead (1963). Preliminary experiments with our stock culture indicated that this number of beans had no negative effect on oviposition of four mating pairs when compared to a larger quantity of beans. Insects were removed from the oviposition chamber after 6 da. Hatched and unhatched eggs were counted at 15 da. The 6 da oviposition period encompassed the peak oviposition period of *A. obtectus*, 2-5 da after adult eclosion in our preliminary experiment. Females lived approximately 13 da, but laid less than 25% of their eggs after 6 da post-emergence. Plant material and insects were placed in a 3 ml glass vial sealed with perforated filter paper taped to the sides of the vial with transparent tape. Ten replicates of each dosage and of a control were used.

Essential oil bioassays- A 9.0 cm diam. Whatman #1 filter paper was placed in a 10 cm diam. glass Corning petri dish. A 1.0 ml aliquot of the appropriate dilution of the essential oil of *T. riparia* was delivered to the filter paper. The control was diluant (absolute ethanol) only. The ethanol was allowed to evaporate for 20 minutes prior to the addition of the insects. Mortality was evaluated if the insect was immobile and did not react to probing with a blunt dissecting probe. Moribundity was assessed by viewing those insects that were on their backs and ambulating very weakly. These insects were subsequently righted and viewed carefully. Those that immediately fell on their backs again as a result of intoxication, were classified as moribund. At higher dosages all moribund insects subsequently died with the passage of time. Recovery rarely occurred at lower dosages. Five males and five females were used in each replicate with *Z. subfasciatus*; 10 adults of unknown sex were used for the other species. Counts of mortality/moribundity were conducted at 24 hrs. To obtain data for a probit analysis of the effect on *S. oryzae*, a count was also required at 48 hrs.

Sensory evaluation- A trained panel of twenty Rwandans (13 males and 7 females ages 24 to 47) evaluated the cooked samples in standard evaluation booths. Beans were stored for two weeks with crushed *T. riparia* leaves or other insecticidal plant preparation. Control beans were obtained from OPROVIA and therefore had been treated with actellic (pirimiphos methyl 1%). Prior to cooking, the insecticidal plant material was removed. Beans were cooked in the traditional manner with water and salt. After 3 hrs of cooking, the beans were served to the panel with a score card.

Statistical analysis- Data were subjected to two way analysis of variance. Comparisons of paired means were made with t-tests. If the two-way analysis of variance (ANOVA) indicated no significant interaction occurred between preparation and dose, a one way analysis of variance was conducted and individual dosages were compared to the control if dosage was significant in the ANOVA. Count data were normalized by square root transformation and proportion/percent data were normalized by arcsine transformation prior to analysis (Sokal and Rohlf, 1981). Possible linear correlations were determined using regression analysis (Sokal and Rohlf, 1981). All statistical analyses were conducted using MSUATAT Version 4.12 (Lund, 1988)

The essential oil dose-response bioassay data were subject to probit analysis (Matsumura, 1975) after Abbott's formula was used to adjust for control mortality (Abbott, 1925). Since the dominant response of the moribund insects was to die, moribundity and mortality were pooled for statistical analysis.

RESULTS

The gas chromatogram of the essential oil which we used for the bioassays has 206 distinct peaks (Figure 1). One of these we recognize as linalool which is a readily volatilized compound (Weaver et al. in press). The GC-MS analysis indicated negligible variability between essential oil samples at the initiation and completion of the experiments. Similarly, there was little variability between the lot of essential oil used for bioassay with *S. oryzae* exclusively and the one used for the three other species. The slopes of the probit lines were: *Z. subfasciatus* $Y=4.2748X + -0.4514$; *A. obtectus* $Y=3.0726X + 0.7829$; *R. dominica* $Y=2.0227X + 2.1048$; *S. oryzae* $Y=5.0079X + -5.3060$ (Figure 2). This indicates the two bruchid populations tested were relatively homogeneous and the grain insects, particularly *S. oryzae*, were more heterogeneous. The LC50 for these species showed wide variation between species (Table 1). The most sensitive species was *Z. subfasciatus*. The LC50 of *S. oryzae* was six times that of *Z. subfasciatus*.

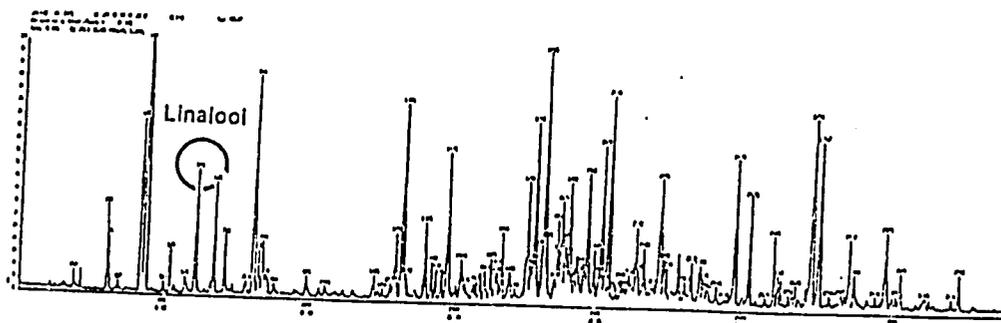


Figure 1. Relative ion chromatogram of the essential oil obtained by steam distillation from leaves of *Tetradenia riparia* (Hochst.) Codd grown in Rwanda (Prefecture Butare) and obtained September 1989 after 6 months in a crushed condition at ambient temperatures. Peak number 615 is linalool.

Oviposition studies indicated that beans to which 2.0% w/w *T. riparia* leaves had been added, there was a significant decrease in the number of eggs laid by *A. obtectus* (Figure 3) with

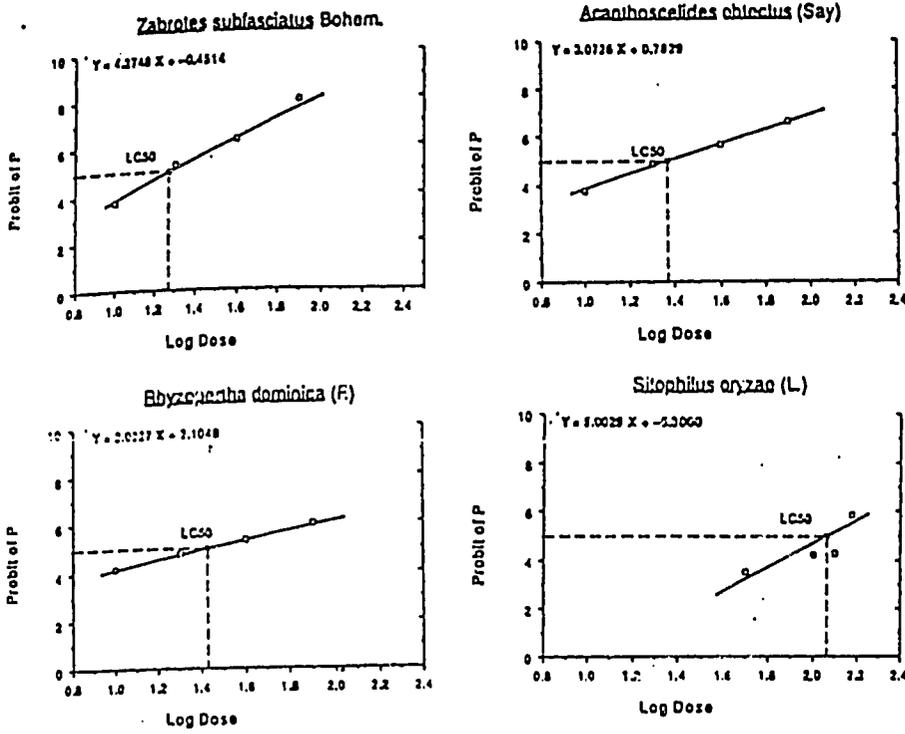


Figure 2. Dose-response curves induced by the essential oil of *Tetradenia riparia* (Hochst.) Codd for four species of stored product Coleoptera in filter paper bioassays (n=10; 10 insects/rep; 27±2°C; 65±8% relative humidity; 12L:12D). *Zabrotes subfasciatus* Bohem., *Acanthoscelides oblectus* Say, and *Sitophilus oryzae* (L.) were 0-2 da post-adult eclosion. *Rhyzopertha dominica* (F.) were 0-3 da post-adult eclosion.

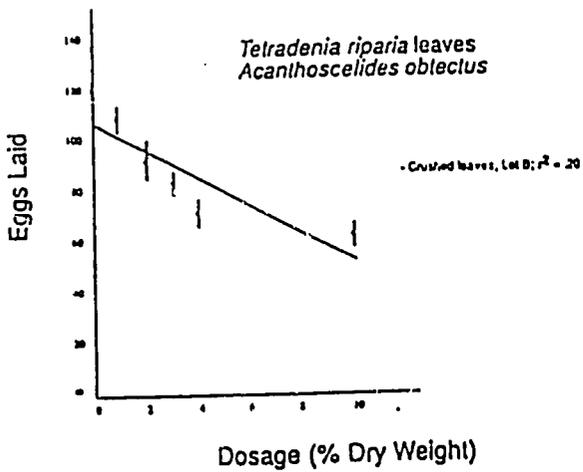


Figure 3. Eggs laid (mean ± standard deviation) by *Acanthoscelides oblectus* Say exposed to dosages of dry, crushed leaves of *Tetradenia riparia* (Hochst.) Codd (n=10; 4 males + 4 females 0-2 da post-adult emergence; 6 da oviposition period).

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a similar effect at 4.0% for *Z. subfasciatus* (Table 2). Analysis of egg development with *Z. subfasciatus* indicated only at 10% w/w did the plant leaves significantly decrease the proportion of eggs hatched (Table 2). The number of beans oviposited on by *Z. subfasciatus*, however, was significantly reduced at the 2% dose of *T. riparia* (Figure 4). At 10% w/w *T. riparia*, the number of beans oviposited on was decreased by 71.5%. There was some variation between plant lots, but within plant lot (only Lot A was tested), there was no significant difference between whether the material was milled or crushed.

In sensory evaluation studies, 55% of the panel preferred beans that had been stored with *T. riparia* versus 45% of the panel that preferred beans stored the conventional way with actellic (Table 3). When asked to evaluate properties that contributed to positive and negative aspects of the samples, the scores were similar to the conventional insecticide (actellic) treated beans. Texture and odor were more positive than the conventional beans.

DISCUSSION

Insect management materials used for these experiments are either grown in most farmsteads (the leaves) or prepared in Butare, Rwanda in a pilot scale facility (the essential oil) for producing a traditional medicine. Both products, therefore, are available to Rwandans without the expenditure of foreign exchange. The essential oil has potential for use as a protective coating applied to beans and sorghum stored in government warehouses. Target studies with this material are presently underway in our laboratory. Nontarget studies are planned.

In the Rwandan open marketing system and in the household as the beans are prepared for cooking or planting, we found in an earlier study (Dunkel et al. 1988b) that the number of emergence holes in an individual bean is important. Those with more than one emergence hole are discarded and therefore represent a complete loss. The leaf preparations dramatically decreased this type of loss. *Tetradenia* did not prevent development of bruchid populations, even at the highest concentrations used. The efficacy of the leaf material in concentrating the reproductive effort of the insects, however, will significantly decrease loss in the marketing system and in the triage process in the household.

These low input, sustainable methods would be unimportant if the beans would lose their acceptability after cooking or be harmful to consumers. Clearly, after a short storage period, protection with *T. riparia* leaves did not negatively affect sensory properties of the consumed product. Studies are underway to evaluate the product after longer term storage with the leaf preparations.

Studies on leaves of *T. riparia* indicate that the crushed leaves would be appropriate for reducing loss in beans stored in the most common on-farm structure in Rwanda, the open, dung-lined basket. The essential oil, after efficacy and non-target testing, may be appropriate for use in long-term storage at the national warehouses.

CONCLUSIONS

- Essential oil of *Tetradenia riparia* has potential for use against *A. obtectus*, *Z. subfasciatus*, and *R. dominica*.
- Mammalian toxicity studies of the essential oil need to be completed before further insect studies are undertaken.
- Crushed dried leaves of *T. riparia* provide some protection against bruchids when relatively large quantities are added to stored beans. Sensory properties of these beans are acceptable to Rwandan consumers.
- For Rwanda, and other areas where the plant is cultivated in homesteads for medicinal purposes, crushed leaves could be used in combination with another medicinal plant, *Ocimum canum* which provides a fumigative effect.
- For Rwanda, the essential oil, product with medicinal properties, has possibilities for use as a protectant in government storages.

Table II. Mean oviposition, hatch, and percentage of beans oviposited upon (\pm standard deviation) by *Zabrotes subfasciatus* Bohem. with dried leaves of *Tetradenia riparia* (Hochst.) Codd incorporated among the beans (10 reps; 20g beans; 5 males + 5 females; $28 \pm 1^\circ\text{C}$; $65 \pm 5\%$ relative humidity; 12L:12D; oviposition allowed until death of parents).

Dosage % wt/wt	Eggs Laid	Proportion hatched	Percentage of beans oviposited upon
0.0	135.8 \pm 28.6*	0.945 \pm 0.041*	84.4 \pm 7.9*
0.1M	160.0 \pm 27.8*	0.960 \pm 0.014*	89.6 \pm 7.3*
0.5M	145.8 \pm 26.9*	0.932 \pm 0.040*	85.8 \pm 6.1*
1.0M	156.6 \pm 13.3*	0.932 \pm 0.053*	87.1 \pm 7.3*
10.0M	66.6 \pm 36.8 ^{a,1}	0.790 \pm 0.122 ^{b,1}	18.5 \pm 17.2 ^{b,1}
0.1C	147.2 \pm 38.8*	0.943 \pm 0.020*	87.1 \pm 7.3*
0.3C	147.0 \pm 22.6*	0.948 \pm 0.022*	86.8 \pm 5.4*
1.0C	156.4 \pm 17.5*	0.909 \pm 0.034 ^{a,1}	85.8 \pm 4.2*
10.0C	93.8 \pm 23.5 ^{a,2}	0.820 \pm 0.086 ^{b,1}	13.3 \pm 3.7 ^{b,1}

C = crushed leaves; M = milled leaves

Plant material supplied in February, 1990 (= Lot A) was used in this experiment. Insects placed in test chambers at 0-3 da post-adult emergence from bean.

* $P \leq 0.05$; Tukey-Kramer test. ¹ $P \leq 0.05$, pairwise t-test.
² $P \leq 0.05$; pairwise t-tests. ³ $P \leq 0.05$, pairwise t-test.

Dosage % wt/wt	Eggs Laid	Proportion hatched	Percentage of beans oviposited upon
0	121.7 \pm 18.6*	0.970 \pm 0.011*	78.86 \pm 8.81*
1	109.3 \pm 24.2 ^a	0.953 \pm 0.017*	71.66 \pm 8.33 ^{a,b,1}
2	119.4 \pm 12.4*	0.953 \pm 0.020 ^a	59.80 \pm 15.34 ^{b,1}
3	125.1 \pm 18.1*	0.956 \pm 0.021*	58.68 \pm 19.72 ^{b,1}
4	120.3 \pm 28.1*	0.903 \pm 0.077 ^{a,1}	40.01 \pm 19.25 ^{b,1}
10	90.2 \pm 15.6 ^{a,2}	0.860 \pm 0.029 ^{a,1}	7.32 \pm 3.00 ^{b,1}

Plant material was used in a crushed condition and supplied in June, 1990 (= Lot B). Insects placed in test chambers at 0-2 da post-adult emergence from bean.

*Means followed by same letter not significantly different; $P \leq 0.05$; Tukey's HSD test.
¹Means significantly less than those for untreated control; $P \leq 0.05$; pairwise t-tests.

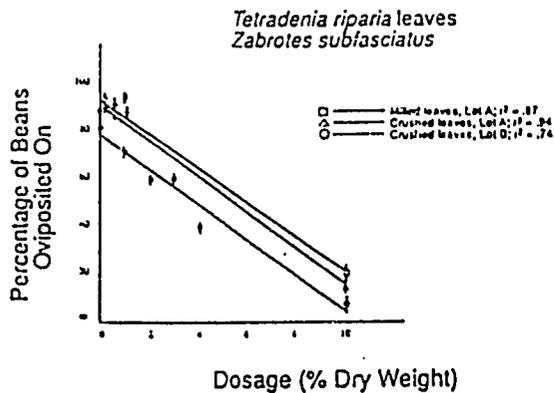


Figure 4. Percentage of beans oviposited (mean \pm standard deviation) on by *Zabrotes subfasciatus* Bohem. when exposed to dosages of dry leaves of *Tetradenia riparia* (Hochst.) Codd ($n=10$; 5 males + 5 females 0-3 da post-adult emergence). Comparisons made between leaf preparations (crushed vs. milled) and plant supply (Lot A vs. Lot B).

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Table III. Summary of results of human sensory preference measured by a trained Rwandan panel for beans cooked after 2 weeks exposure during storage to three plant products: crushed leaves of *Tetradenia riparia* (Hochst)CDD; neem seed extract (Margosan-O); and crude pyrethrin extract. The control was beans treated in the usual manner for storage at national warehouses (1% actellic).

Bean Treatment	% preference for sample	% acceptability of sample
Actellic vs neem	70-30	90-70
Actellic vs <i>T. riparia</i>	45-55	85-75
Actellic vs pyrethrin	55-45	100-79

Bean Treatment	% Appreciation of certain sample properties ²							
	Positive effects				Negative effects			
	Ta	O	Te	A	Ta	O	Te	A
Actellic ¹	54	8	23	15	15	15	23	47
Pyrethrin	41	17	25	17	0	8	42	50
<i>T. riparia</i>	56	11	33	0	11	11	22	56
Neem	56	11	22	11	11	22	11	56

¹ Actellic, in this experiment was considered the control because all beans sold by OPROVIA are treated with actellic.

² Ta = taste; O = odor; Te = texture; A = appearance

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Appendix 8. Manuscript drafted for submission to a refereed journal. "The imboho: a traditional structure for storage of dry pulses in East Africa." for submission to J. Stored Product Res. drafted.

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**The imboho: a traditional structure for storage
of dry pulses in East Africa**

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INTRODUCTION

Frequently farmers in one area develop by trial and error an agricultural technique which works particularly well for the conditions they experience. Because farming techniques are often family secrets or because extension systems are undeveloped and national meetings of e.g. bean or sorghum growers do not exist, such successful techniques frequently do not become widely adopted in a country or even in a similar region of the country. Often it is not until a national survey is conducted by persons interested in indigenous farming practices that such techniques surface and become disseminated.

No previous mention of the Imboho¹ has been located in the literature of any country. There are, however, similar structures that appear in records of this area and in Rwandan oral history. The closest such record is the Burundi storage survey in which an "impeche" is described (Mertens, 1982) for holding beans after harvest. Structures of this type were made of plant materials and held each 50 kg. In the Congo Republic an oblong structure of natural materials is used on-farm for storage (Wittenberger 1985). Staff at OPROVIA^{1/} indicate that structures similar to the imboho were used in the past for transport of beans. There also is a folk tale about a giant imboho. The only historical written or photographic record was found in a text (Paterson, 1907) with no explanation but shown carried on the head of a Ugandan woman.

Of the 9 sectors and 9 collines sampled in our 1984-85 survey only one colline in one sector used the structure. We present the following observations because we consider this pulse storage structure to warrant a careful evaluation. Following this survey of bean and sorghum storage methods, local structures such as the imboho and modified structures were evaluated under somewhat controlled conditions at research laboratories in OPROVIA headquarters in Kigali and some will be evaluated in selected fields or farm sites throughout Rwanda.

INVESTIGATION METHODS

The imboho was first observed during the first visit of our storage survey team to Nyakabanga colline, Murama sector, Rukira commune in the Prefecture of Kibungo, southeast Rwanda (Figure 1). The purpose of the visit was the primary site verification of a sector randomly chosen for the National Grain Storage Survey (see Site Selection Farm Level). The initial observation was made on 22 June 1984 by Ted Wittenberger (Storage Survey Resident Scientist) and his Rwandan counterpart, Joel Kayumba. Three imbohos were observed in one room of a farmer in this area. Two contained beans of the January harvest and one held groundnuts. Arrangements were immediately made to observe the fabrication of a new imboho which would hold dry beans of the new harvest (June 1984). Wittenberger and Kayumba

¹ Imboho is a Kinyarwandan term and it is plural. The singular term does not exist. Because Rwandans which we worked with began calling it imboho, we will use this term in both a singular and plural sense.
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returned to the sector on 28 July 1984 and observed the construction. On 10 July 1985, a return visit was made to the farm to videotape the construction process.

In total, three farmsteads with imbohos were observed within the storage survey which included 47 farms in 3 agroclimatic areas of Rwanda for the June 1984 harvest of beans and 48 farms in the January 1985 harvest. Bean storage practices were followed in 54 different households. A total of 12 visits were made to observe the structure during the course of the survey including a trip by Drs. Harold Cloud and Paul Hanegreefs, agricultural engineers.

On 26 April, 1985 the Rwandan counterpart, Evariste Munyarushoka and Dr. Florence Dunkel, storage entomologist, returned to the colline Nyakabanga to conduct in depth interviews on the cultural history of the imboho. Utilization patterns for the structure obtained during other visits were also confirmed. The following questions were asked:

- 1) Who taught you how to make the imboho? Was it someone from outside your family?
- 2) Do you know of any nearby collines that use the imboho?
- 3) How long have you or your family lived in this colline? Where (what region) did you come from? Where (in what region) did you grow up?
- 4) Do any of your neighbors in the colline use the imboho for storage?
- 5) When do you use the imboho? For June beans? For January beans? For what other crops do you use it?
- 6) When do you open the imboho? Is it a long-term, untouched strategic storage? Do you reuse the imboho or make it new for each crop and each season?

RESULTS AND DISCUSSION

Construction Procedure (based on observation of construction)

The imboho is made primarily from the dried primary sheaths of banana plants. These dry sheaths are collected the evening before construction and moistened with water just prior to construction (Figure 2). They are then shredded into strips longitudinally. These strips are about 1.5m long. Strip length depends, however, on the overall size of the imboho to be built. The strips are shredded into two widths 3 to 5 cm and 10 to 20 cm. At this point a frame or building guide¹ consisting of 2 parallel logs (8cm diameter) was placed on ground. The narrower strips are twisted and are laid parallel on the ground 10 cm apart (Figure 3a). If the frame is used,

¹ We were told this is not usually used, but was used at this time to facilitate the construction. It was not used in the construction observed in 1984.

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the twisted strips are laid over the log building frame and perpendicular to it. Then, the wider strips are laid flat, perpendicularly over the twisted pieces (and parallel to log frame) until an area of about 75cm x 150cm is covered.

A bed of dry grass (cut 2 days prior to construction) is then laid on top of the flat strip (Figure 3b). The species of dry grass is apparently not important but the length is (0.5m). At the ends of the imboho under construction they are placed in a v-shape.

At this point, the beans to be stored are emptied onto the grass bed. During one construction the farmer indicated Malathion had been previously applied to the stock which they were presently encasing. Ash and kaolin were also sometimes used. The leaves of a local plant ("UMWISHEKE") (Ocimum kilimunjarum, related to sweet basil, Ocimum bascillicum) were also mixed into the beans during both of the imboho constructions observed. These leaves had a strong pungent odor. Their function was insect repellency and/or insecticidal. Bunches of the small leaves were plucked from the flowering green plant and dropped onto the beans.

After the beans were emptied onto the grass bed, a stout stick 1.5 meters long, 5 to 7 cm thick with a tied "ball" of banana leaf strips at one end was laid on the beans and half buried in the beans with the ball in the beans about one-fourth of the distance from the end of the bed (Figure 3b). The other end of the stick extended out past the bed and underlying mat. The main purpose of this pole is support, but it may serve to be a rodent barrier and provide a passive aeration effect around the entire bulk of beans or other legume.

Additional grass was then laid over the bed and stick. Subsequently, another layer of wide flat sheath material was laid to cover the grass. When this was completed, the ends of the twisted strips were tied up, starting with the apical end. This formed a cocoon like structure (Figure 3c). The loose ends were trimmed off with a knife. A final twisted strand was tied to each encircling strand and it ran the length of the entire structure. The purpose of this final strand is apparently to keep the other tied off strands from slipping. Tying of the strings requires the help of 3 to 4 persons. While the tying occurred, the farmer continually pounded the side of the imboho with his fist to reposition the beans more compactly.

The entire construction process took about 1.5 hours not including the time it took to collect materials. This particular imboho held about 60 kg. but the builder commented that he was capable of fabricating one with a 200 kg capacity.

Umushenga Variation of Imboho

Another variation on the imboho is the umushenga. The umushenga has a similar construction but the wooden shaft running up the center is absent. It is also suspended from a rafter or overhead beam inside the house and no part of it touches the walls or floor. The imboho on the other hand, leans up against the wall on its wooden foot or a hole is dug for the foot to be inserted and the container stands upright. One farmer mentioned that the

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umushenga was used for soybeans or peanuts but not beans. Beans are stored in the imboho.

Location within the House

In 100% of the visits to 47 farms after the June 1984 harvest and to 48 farms after the January 1985 bean harvest (for a total of 52 different farms), beans were stored inside the house. Within the house, the most frequently encountered location was an actual storage area or store room. Other areas used were the sleeping area, foyer and cooking area. All imbohos observed were stored in a storage area in the house. Two farmers interviewed indicated their family has in the past placed the imboho outside the house and constructed it much larger when yield was greater and theft was not as much a problem.

Utilization

The imboho and umushenga storages are not built immediately after the harvest and post-harvest treatment of the crop. The crop, instead, is placed in temporary storage first, usually a basket lined with cow dung. The duration of this temporary storage is apparently 4 to 8 weeks and during this period some insecticidal application may be performed either with ash or a synthetic insecticide such as malathion. The beans in imbohos we observed were used up 4-5 months after harvest. The farmers indicated that when possible they keep beans in these containers up to 2 years.

Although only beans (Phaseolus vulgaris) were sampled in the imboho, the imboho may be used for other legumes. The umushenga is used for soybeans and peanuts but not beans. Neither structure is used for storage of peas, sorghum, or maize.

Once the imboho or umushenga is constructed, access to the stock is accomplished by simply spreading the wider sheaths apart at the lower 1/3 of the container and letting the stock fall into a waiting receptacle. After the stock has been reduced, the beans or other legumes are removed by hand from the upper portion of the structure. When a sufficient quantity has been removed, the hole is closed by shifting or gently pushing the sheaths back into position. It is a 'disposable' structure, used only as long as the stock that was put in it when it was made, lasts.

Farmers claimed this structure was superior to the open baskets in maintaining bean quality during storage. The open basket was used by 77% of the farmers in the national survey. The specific types of bean quality mentioned as being improved by the imboho were insect infestation, lack of mold problems, and bean cookability. Bean cookability, after a long storage period, was particularly superior in imboho beans. It is possible that the imboho is particularly used after small harvests due to e.g., low rainfall. In that case, the imboho would create conditions which will maintain bean quality longer, possibly over another dry season.

Of the beans harvested, 59.1% are consumed directly on the farm. The next most frequent mode of utilization is to sell them in the market. Approximately 39 markets were visited during the varietal survey (Lamb and

Hardman, 1986) and 4 markets were separately visited in the Storage Survey (Dunkei et al., 1986) at no time was an imboho observed being carried to market. It is possible that the imboho is either not widely used or the beans placed in the imboho are consumed on-farm and not sold. It is also possible that small quantities from the imboho are placed in another container. The imboho is not presently a typical transport structure for beans. It was never observed in transit in Rwanda during the project January 1983 to October 1985. Rwandan coworkers, however, upon seeing video tapes of the construction process or seeing them at our experiment station site indicate that these were once very common transport containers in Rwanda. When used for transport, however, they were much smaller.

Hypotheses Regarding Origin

Three hypotheses have arisen from this preliminary investigation of the imboho. First, one can hypothesize that the colline Nyakabanga or that commune in which it is located is the evolutionary center of origin of this storage structure. The evidence for this is the lack of observation of this structure in any of the 51 other households surveyed for storage practices in Rwanda during 2 bean harvests (June 1984 and January 1985) and during a preliminary survey in a commune in Butare (DeJaegher 1980). Additional support of this hypothesis is from the people we interviewed who use the imboho. They all indicated the structure was always made by their ancestors and all their family use it.

A second hypothesis is that the structure is an ancient one once used throughout Rwanda, but it was used for transporting food rather than for food storage. The evidence for this is that there are folk tales in the oral tradition of Rwandans from at least the Rutobwe Commune in the Gitarama Prefecture (180km from the Nyakabanga colline - 3 hours on present roads). Actually, the first and second hypotheses are not mutually exclusive. A combined hypothesis could be made as follows: the imboho was an ancient structure used for carrying food on long journey. Someone in the colline Nyarabakura evolved a similar, but bigger structure that could be propped up or hung inside the home for stationary storage of pulse crops. Due to lack of surveys of this type and an absence effective extension system in Rwanda, the success of this method never spread. Meanwhile, the use of the structure for general transport of food disappeared. Perhaps it was replaced by the locally manufactured plastic bags or buckets now seen throughout Rwanda.

A third hypothesis is that it is an indigenous storage or food transportation structure in a neighboring country and someone from the colline in Kibungo visited this country and brought it back to Rwanda. The evidence for this is a photograph of Ugandan women carrying on their heads a similar but smaller structure (Patterson 1907, Figure 4).

Other evidence is the similar structure (impeche) used for storage of beans in Burundi (Mertens 1982).

Further surveys in other geographical regions of Rwanda and research in the cultural anthropological literature of the area is warranted to test these 3 hypotheses.

CONCLUSIONS

At this point in our investigation the imboho/umushenga can be described as a form of traditional storage unique to Rwanda and indeed found only in one region of this country. Farmers who employ this method of storage have claimed that they encounter fewer insect problems than with other forms of containers. They say that they have no mold problems at all. Further, and perhaps most important, individuals who use these storages have claimed that the hardness problem encountered in dry beans stored over 6 months is less pronounced than in beans stored by other traditional methods.

These statements coupled with the fact that the imboho and umushenga are made completely out of indigenous material and cost nothing to make except for a minor labor commitment indicate that these structures might be a viable item for extension to other parts of Rwanda and other areas where beans and other dry pulses are stored adjacent to banana groves. Bananas are grown virtually everywhere in Rwanda yet these containers are apparently found only in this one region.

Not only is the banana sheath package a previously undocumented kind of storage container, but leaves themselves and leaf wrap are unusual storage containers. Leaves, thought to be banana, were found used in a Badeku village for kola nut storage (Williams 1973). In this case, the wrapping was replaced with fresh leaves every three weeks. A suspended structure made of leaves and tied with fiber ropes was also observed in use for on-farm storage in the Congo (Wittenberger 1985).

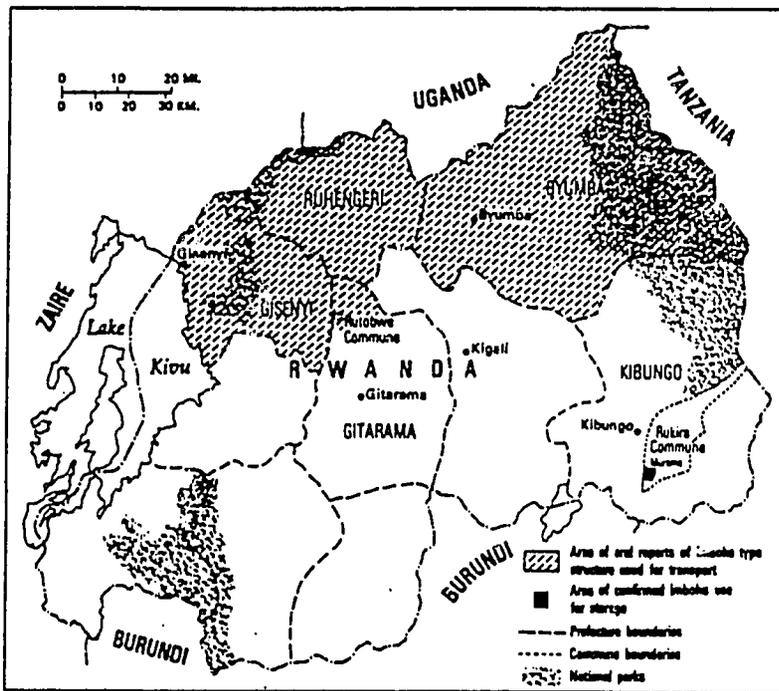
Additional research in the following areas is necessary prior to an extension commitment: a) a broader survey of indigenous storage practices which would determine if the imboho is used in other areas of Rwanda or if different methods of on-farm storage, which also would be worth investigating, have evolved in Rwanda, b) controlled comparative experiments conducted by the OPROVIA Grain Quality Laboratories, using the imboho intact and testing the properties of its components, e.g., the banana leaf, c) on-farm trials to test the claims that this is indeed a better form of storage than the other indigenous storage structures, such as open baskets lined with cow dung, d) an etymological analysis of the term imboho and a historical study, and e) a planned series of interviews with farmers who use the imboho to determine why certain portions of the construction are included and what the 'safe storage' parameters of it are.

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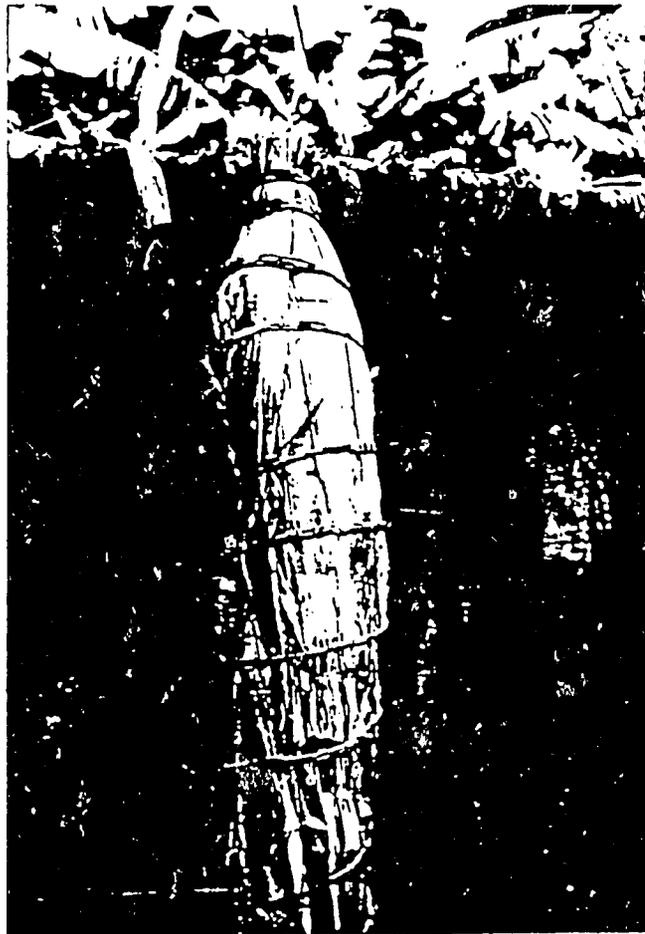
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Figure 1. Location of oral reports and observations of the imboho in use, either as a storage or as a transport structure.



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Figure 2. Photos of imboho from previous storage period, after beans contained in it had been used and just prior to discarding structure. (Photos by F.V. Dunkel).



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Figure 3. Imboho during construction process a) top view before placement of grass or beans, b) side view after placement of support stick, half of the beans and half of the banana sheaths, c) after completion of tying.

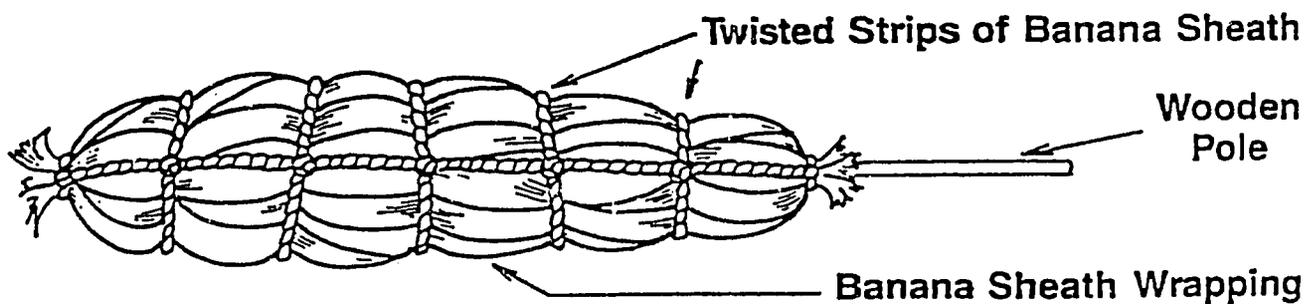
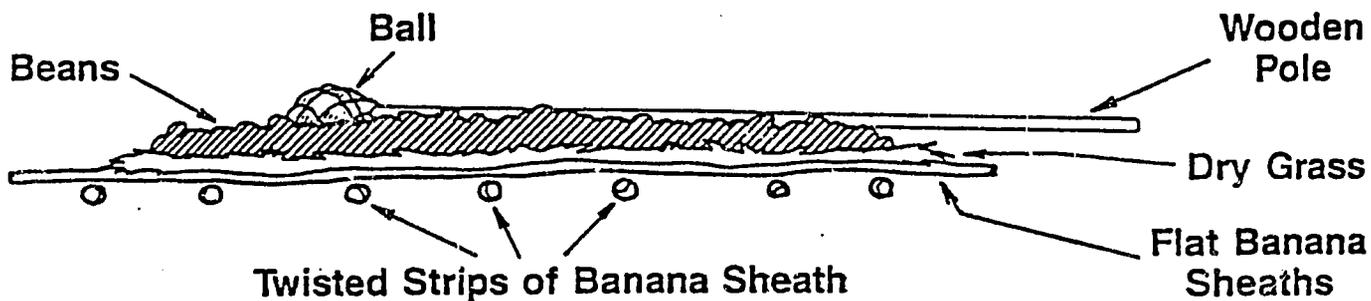
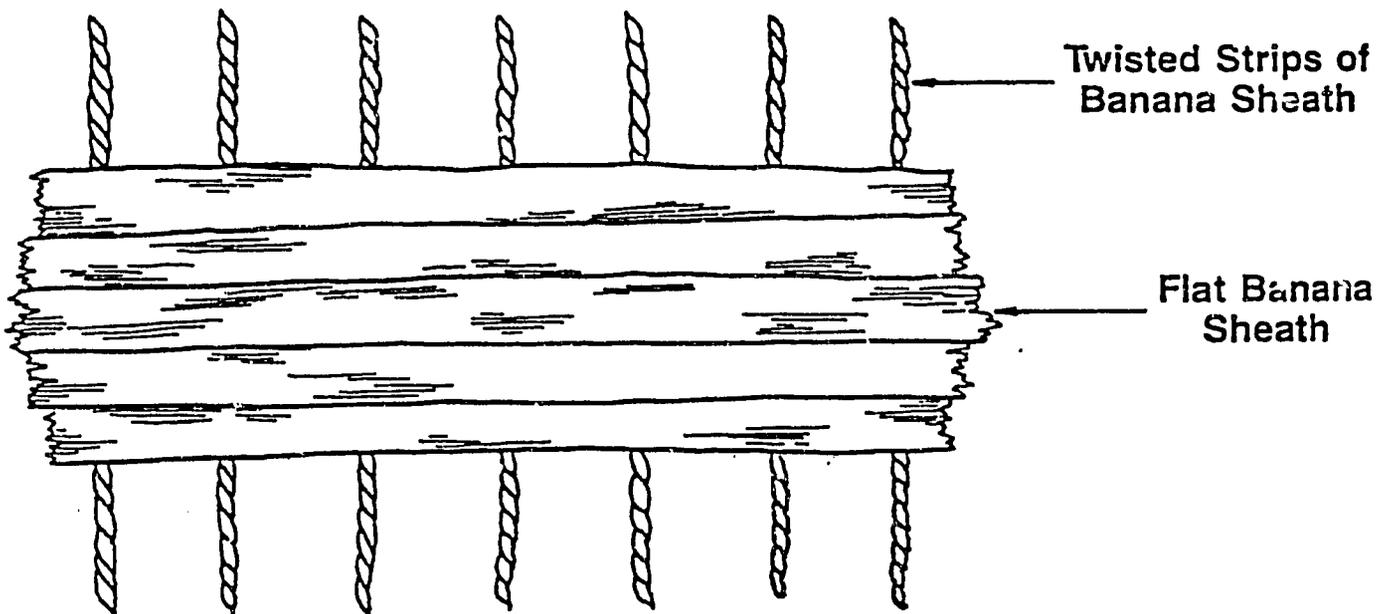


Figure 4. Illustration from Patterson (1907) of Ugandan women carrying supplies of food in structure similarly shaped to imboho.



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Appendix 9. Published refereed journal article. "Evaluation de la toxicite et de l'effet repulsif de certaines plantes du Rwanda contre les bruches du haricot: Acanthoscelides oblectus Say et Zabrotes subfasciatus Boheman" Insect Sci. Applic. 12:695-697.

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J. KAYITARE
20-3-1992

EVALUATION DE LA TOXICITE ET DE L'EFFET REPULSIF DE CERTAINES PLANTES DU RWANDA CONTRE LES BRUCHES DU HARICOT: *ACANTHOSCELIDES OBTECTUS* SAY ET *ZABROTES SUBFASCIATUS* BOHEMAN

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Résumé—L'effet toxique et répulsif de quatre plantes *Chenopodium procerum*, *Ocimum canum sinus*, *Tetradentia riparia*, et *Capsicum frutescens* a été testé sur *Acanthoscelides obtectus* Say et *Zabrotes subfasciatus* Boheman. L'effet toxique a été observé sur feuilles d'*Ocimum canum sinus* et de *Chenopodium procerum* Hochst. Les quatre plantes évaluées n'ont montré aucun effet répulsif sur les bruches.

Mots Clés: Toxicité, répulsion, *Acanthoscelides obtectus*, *Zabrotes subfasciatus*, *Chenopodium procerum*, *Ocimum canum sinus*, *Tetradentia riparia*, *Capsicum frutescens*

Abstract—The toxic and repellency effects of four plants *Chenopodium procerum*, *Ocimum canum sinus*, *Tetradentia riparia* and *Capsicum frutescens* were tested on *Acanthoscelides obtectus* Say and *Zabrotes subfasciatus* Boheman. The toxic effect was found on leaves of *Ocimum canum sinus* and *Chenopodium procerum* Hochst. The four plants tested did not show any repellency effect on the bruchids.

Key Words: Toxicity, repellency, *Acanthoscelides obtectus*, *Zabrotes subfasciatus*, *Chenopodium procerum*, *Ocimum canum sinus*, *Tetradentia riparia*, *Capsicum frutescens*

INTRODUCTION

Certaines plantes peuvent servir à plusieurs fins. C'est le cas des plantes du genre *Chenopodium*. Considérées comme nocives pour l'agriculture en tant qu'adventices, elles ont servi à des fins alimentaires pendant la disette dans plusieurs pays comme le Mexique, la Suisse et l'Afrique du Sud (Coon, 1960; Holm, 1977). La poudre et l'extrait de la même plante furent utilisés comme insecticide contre les larves de la moustique anophèle (Jacobson, 1953). Au Rwanda le

Chenopodium est utilisé en médecine traditionnelle. Le Centre Universitaire Rwandais de Pharmacopée et la Médecine Traditionnelle se sert du *Chenopodium* pour faire des médicaments (Ntezurubanza, 1988). Enfin, cette plante est utilisée par les agriculteurs de Kibungo (Rwanda) pour préserver les récoltes de haricot contre l'attaque de bruches pendant le stockage (Munyemana, 1986). D'autres plantes utilisées dans cette étude appartenant aux genres *Tetradentia*, *Ocimum*, *Capsicum* servent également en médecine traditionnelle au Rwanda (Van Puyvelde, 1988; Ntezurubanza, 1988). Par ailleurs, Munyemana (1986) a montré l'efficacité des plantes citées dans la lutte contre la bruche du haricot, *A. obtectus* Say. Ainsi nos recherches

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s'inscrivent dans le cadre de la valorisation des plantes du Rwanda pour mieux exploiter leurs potentialités insecticides pour la conservation des récoltes par les agriculteurs.

MATERIEL ET METHODES

L'évaluation de l'effet toxique et répulsif de *Chenopodium*, *Ocimum*, *Tetradentia*, et *Capsicum* a été réalisée sur les bruches du haricot *A. obiectus* et *Z. subfasciatus*. Les insectes ont été multipliés en masse au laboratoire de l'Université de Wisconsin, Madison. Les feuilles, les fleurs et les fruits ont été utilisés séparément dans cette étude. Le matériel végétal a été séché au soleil puis broyé au mixeur pour en obtenir une poudre qui a été utilisée par la suite. Pour évaluer l'effet toxique des plantes, les essais ont été conduits dans des boîtes de pétri tapissées d'un papier filtre sur lequel était déposé 1 g de poudre de matériel végétal. Dix adultes de *Z. subfasciatus* âgés de 3 jours ont été ensuite placés dans des boîtes de pétri à raison de 4 répétitions. Les témoins n'ont pas subi de traitement. Les essais ont été conduits dans des conditions de température et d'humidité relative ambiantes. Le contrôle a été effectué deux fois par jour (matin et soir) pendant 15 jours pour déterminer le nombre d'insectes morts.

L'effet répulsif des plantes a été testé sur le *Z. subfasciatus*. L'expérience a consisté à mettre 400 gr de haricot dans des bocalux spéciaux traversés par des tubes en plastique perforés et munis d'orifice sur le fond pour recevoir les insectes fuyant le produit. Le haricot contenu dans les bocalux a été traité à la dose de 0,4 g de poudre de plantes. Quatre lots de 25 bruches de *Z. subfasciatus* ont été ensuite placés dans des bocalux.

RESULTATS ET DISCUSSIONS

Les résultats consignés dans le Tableau 1 montrent que les plantes du genre *Chenopodium* suivies d'*Ocimum* ont plus d'effet toxique sur le

Tableau 1. Mortalité due à l'effet toxique des différents végétaux sur les adultes de *Z. subfasciatus* au 12ème jour après le traitement

Traitements	Moyenne
<i>Capsicum frutescens</i> var. à fruits longs	5.75 cde
<i>Capsicum frutescens</i> var. à fruits ronds	6.00 cde
<i>Chenopodium procerum</i> feuilles	8.50 ab
<i>Chenopodium procerum</i> fleurs	7.00 bcd
<i>Chenopodium</i> spp. fleurs	9.25 a
<i>Ocimum americanum</i> fleurs	7.25 abc
<i>Ocimum canum sinus</i>	7.25 abc
<i>Tetradentia riparia</i> feuilles	4.75 e
<i>Tetradentia riparia</i> fleurs	6.00 cde
Témoin	4.50 e

Tableau 2. Mortalité due à l'effet toxique de différents végétaux sur les adultes d'*A. obiectus* au 12ème jour après le traitement

Traitements	Moyenne
<i>Capsicum frutescens</i> var. à fruits longs	0.75 cd
<i>Capsicum frutescens</i> var. à fruits ronds	2.25 bcd
<i>Chenopodium procerum</i> feuilles	5.50 a
<i>Chenopodium procerum</i> fleurs	3.00 bcd
<i>Chenopodium</i> spp. fleurs	3.25 abc
<i>Ocimum americanum</i> fleurs	4.00 ab
<i>Ocimum canum sinus</i>	2.50 bcd
<i>Tetradentia riparia</i> feuilles	3.00 bcd
<i>Tetradentia riparia</i> fleurs	0.50 d
Témoin	1.00 cd

Tableau 3. Effet répulsif des plantes contre *Z. subfasciatus*: % de répulsion d'insectes ($\bar{X} \pm S.E.$) ($n = 4$)

Traitement	Jour 1	Jour 2	Jour 3	Jour 4	Jour 5
Test 1					
<i>O. canum sinus</i>	6.0 ± 3.0	20.0 ± 7.04	38.0 ± 10.8	53.0 ± 15.5	59.0 ± 15.5
Témoin	11.0 ± 2.5	33.0 ± 5.1	52.0 ± 2.4	72.0 ± 2.2	85.0 ± 5.3
Test 2					
<i>O. canum sinus</i>	8.0 ± 2.0	25.0 ± 2.2	40.0 ± 1.4	59.0 ± 2.16	67.0 ± 22.0
<i>C. procerum</i>	22.0 ± 1.7	40.0 ± 4.24	48.0 ± 9.5	60.0 ± 9.5	67.0 ± 11.5
Témoin	21.0 ± 7.3	41.0 ± 9.0	54.0 ± 12.3	66.0 ± 16.0	71.0 ± 17.2
Test 3					
<i>T. riparia</i>	17.0 ± 5.16	37.0 ± 3.2	54.0 ± 55.0	70.0 ± 5.3	78.0 ± 5.7
<i>C. frutescens</i>	17.0 ± 7.2	48.0 ± 5.1	63.0 ± 6.2	70.0 ± 4.1	79.0 ± 3.8
Témoin	4.0 ± 2.0	48.0 ± 5.1	62.0 ± 5.36	72.0 ± 6.3	82.0 ± 1.7

Z. subfasciatus. Les feuilles de *C. procerum* apparaissent être plus toxiques que les fleurs. Les fleurs de *T. riparia* se montrent plus efficaces que les feuilles de la même plante.

L'*O. canum sinus* rend immobile les bruches et provoque une haute mortalité dès les premiers jours qui suivent le traitement. Le *C. frutescens* met les bruches en mouvement continue semblable à celui de témoin. Les insectes tentent de fouir le produit et se mettent sur les parois et les couvercles des boîtes de pétri. La différence de mortalité des adultes pour les différents traitements s'observe avant le 10^{ème} jour. Après cette période la mort naturelle commence à apparaître aussi bien dans les traitements que chez le témoin.

Les bruches *A. obiectus* se montrent plus résistantes aux produits végétaux (Tableau 2). Le *C. procerum* et l'*O. canum sinus* sont les plus toxiques aussi bien pour les adultes d'*A. obiectus* que ceux de *Z. subfasciatus*. En seconde position vient le *T. riparia* et enfin en dernier lieu se classe le *C. frutescens*.

Les résultats sur l'effet répulsif montrent l'absence de signification entre les témoins et les lots traités (Tableau 3). En effet dans les trois évaluations de l'effet répulsif des plantes à pouvoir insecticide contre le *Z. subfasciatus* il a été observé que les bruches des témoins étaient plus mobiles que celles des traitements. La toxicité des produits végétaux aurait diminué la mobilité des insectes qui se déplacent difficilement pour être retenu dans les récipients destinés à leur récupération.

Il faudrait continuer l'étude en milieu réel pour mieux confirmer l'effet toxique trouvé dans certaines plantes (*Chenopodium*, *Ocimum*) et mettre au point la technologie d'utilisation qui serait facile à réaliser et peu coûteux aux agriculteurs en vue de lutter contre les insectes de stockage.

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