COO—2505—06

MASTER

THE APPLICATION OF NUCLEAR ENERGY TO AGRICULTURE

FINAL REPORT

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PREPARED BY

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TROPICAL AGRICULTURAL RESEARCH AND TRAINING CENTER

TURRIALBA, COSTA RICA

JUNE 30, 1976

FOR THE
U. S. ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION
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Resume

This project was part of the Atoms-for-Peace Program enunciated by ex-President Eisenhower before the United Nations. The objectives of this project were two-fold: to carry out agricultural research, basic or applied, in the American tropics, using nuclear energy as a tool, and to train students from Latin America in the use and application of nuclear energy techniques to agriculture. The research activities consisted of the following fields: A. radiation botany, plant genetics and crop improvement, B. entomology, C. biochemistry and plant physiology, and D. soil chemistry.

A. Radiation botany, plant genetics and crop improvement.

(1) By using induced mutation technique, it is possible to change the pigmentation (P) gene into its recessive form in the common bean. As a result, the black bean varieties would produce white seeds which are highly acceptable to the consumers. In this manner, many black bean varieties superior in yielding capacity and disease resistance can simply be mutated and can be used as an improved variety. Likewise, the mottling (M) gene in the Pinto beans can also be mutated, and the mutant produces all red seeds. This demonstrated that the mutation breeding method, as compared with the conventional breeding techniques, has its merit when applied to specific cases.

(2) Induced mutation method also can change the protein quantity and quality in beans. A bean mutant (Bayo-2) had higher protein content and a different amino acid spec-

trum when compared with the original parent.

(3) A white bean mutant (NEP-2) with high PER value, less sugars, no tannin content, and good canning quality has been propagated and the seeds have been sent to 34 institutions in 20 countries for their field trials.

(4) An efficient and rapid screening technique for detecting hydrocyanic acid (HCN) released from cyanogenic glucosides in cassava has been developed. By irradiating the stem cuttings of cassava with 4 kR gamma rays, the HCN content in the plants of irradiated population varied to a much larger extent than that of the control population. This result may offer a good possibility for selecting acyanogenic cassava plants.

(5) The coffee seeds and young cassava buds have an unusual biological response to ionizing radiation. When these materials were irradiated, the mutant characters appearing in the R_1 plants were rarely a chimeric change but the whole plant produced a similar mutant characteristic. The evidence indicates that the development of a coffee or cassava plant is from one or two initial cells in the apex.

(6) The radiosensitivity of 84 plant species in the tropics was studied. In general, gymnosperms are a very radiosensitive group of plants. Many species in Compositae, Convolvulaceae, and Cruciferae are resistant to the radiation.

B. Entomology.

(1) The research carried out in entomology was aimed at evaluating the feasibility of controlling the following insects by the male sterilization technique: Mediterranean fruit

fly, Coffee leaf miner, Warble fly (Torsalo) and Meliaceous shootborer. The biology of these insects was studied. The mass rearing technique, and a cheap rearing diet for the insect were developed. The radiation dose for the sterilization, stage of the insect for irradiation, the longevity and mating vigor of the irradiated insects were determined. Flooding ratio of irradiated flies to normal flies was also explored. All this basic information is necessary for the field release of sterilized males for the control.

(2) In a cooperative project with an international institution (OIRSA) supported by the United Nations Special Fund and IAEA, a pilot field trial for testing the effectiveness of the male sterilization technique for the Medfly control was carried out in Nicaragua. From 1968 to 1969, about 40 million irradiated flies were released in an area of 48 km^2 each week. The result showed that the fly infestation was reduced to 90 percent, demonstrating that the Medfly could be controlled by the male sterilization technique.

C. Biochemistry and plant physiology

(1) By use of ^{14}C labeling techniques, it was revealed that the reductive pentose phosphate pathway (C_3 metabolism) is the major photosynthetic pathway in cassava. When $^{14}CO_2$ is assimilated by cassava leaves, sucrose is the major end product of photosynthesis and essentially the only compound translocated. The major portion of translocated sucrose is found in those plant parts which are on the direct route to the root system. The pathway for sucrose conversion in cassava roots is by a reaction catalysed by sucrose-UDP glu-

cosyltransferase (sucrose synthatase) but not by invertase.

(2) Cacao seeds are very sensitive to cold. The seeds chilled at 4ºC for 10 minutes had no germination. However, the vitality could be restored if the chilled seeds were post-heated to 37ºC for 10 minutes immediately. By respiratory test, tissue culture method, and cytological observation, it was proved that the seed death due to cold is caused by the biophysical damage of the cotyledonous cells.

(3) The effect of dehydration on cacao seed viability and on the respiration rate was studied. Normally, cacao seeds contain 40-45 percent water. All germination and respiration ceased at 14-16 percent water. This indicates that water has a much more critical physiological role in cacao seeds than in other seeds. It may also explain why there is a lack of dormancy in cacao seeds.

(4) Two closely related fungi, Gloeosporium musarum, causing severe fruit rots in bananas and Colletotrichum gloesporioides, causing little banana rotting were used in order to study the host-pathogen relationship for disease incidence. In a series of comparative studies on carbohydrate metabolism of these two fungi, no significant metabolic differences could be established which might account for their dissimilar pathogenicities, although these two fungi are not absolutely identical metabolically. A theory for the mechanism of latency, i.e., the inability of G. musarum to develop on unripe banana fruits, was proposed to account for the pathogenicity. The latency is due to the substances in the banana latex inactivating the various enzymes produced by the invading fungus.

(5) By use of ^{32}P as a tracer, the effect of sur-

factants on P absorption was studied. All the commercial surfactants used for the studies increased both absorption and subsequent translocation of ^{32}P in coffee and cacao plants. Ammonium phosphate was absorbed and translocated to a much greater extent than were either sodium or potassium phosphate.

D. Soil chemistry.

(1) The chemical properties of tropical soils were studied. Almost all the soils in Central America are of volcanic origin and are acid. Extractable aluminum was high for the most acid soils. Cation exchange capacity decreased rapidly with the depth of the soils. Cation movement in the soils was slight, even under the effect of 100 inches of water passed through these soils.

(2) In the younger Costa Rican soils, organic phosphates contributed about 50 percent of total phosphates, but in the old, highly weathered Brazilian soils, organic-P contributed only about 20-30 percent of the total P.

(3) Most soils from the Amazon Basin in Brazil were low in phosphates, and had a high fixing capacity of P.

(4) The sulfate movement was studied by means of fagged sulfates. Different cations of the various sulfates had little effect on the movement of this anion. Different soils affected the movement differently. Retention of sulfate was proportional to the combined content of clay and silt.

(5) Mineralization of organic nitrogen was studied in different volcanic soils. The soils showed a negative linear association between allophane content and nitrogen

mineralization. Mineralization in andosols was significantly inferior than in other soils studied.

(6) The chemical properties of volcanic ash from recent volcanic eruption were studied. The ash had a very small cation exchange capacity, was moderately acid, did not contain water-soluble phosphates, and did not fix this element. Fresh ash was also quite a good source of soluble cations and sulfates, but had no nitrogen.

(7) By using radio-tracer ^{86}Rb, it is possible to study the distribution of the roots of a coffee tree which actively absorb the fertilizers. It was found that most of actively absorbing roots are near the surface and close to the free trunk.

In the training aspects, a total of 82 students from 17 countries were trained through the Nuclear Energy Program. Thirty-seven of them received M.S. degrees from our Institute and 17 received Ph.D degrees from the U.S. and other universities.

A total of 224 scientific articles were published during this project period.

In 1953, Ex-President Eisenhower enunciated the Atoms-for-Peace program before the United Nations -- uses of nuclear energy for the benefits of mankind. For Latin America, a committee composed of the scientists from Brookhaven National Laboratory and USDA was set up to study the projects that could be carried out in this area. Since many Latin American countries are agriculturally dependent, it was proposed that a project be granted to the Inter-American Institute of Agricultural Sciences of the Organization of American States. The general objectives of the Nuclear Energy Project were two fold: to carry out agricultural research, basic and applied, in the American tropics, employing nuclear energy as a tool; and to train student from Latin America in the use and application of nuclear energy technique to agriculture.

The research activities of the project consisted of the following fields: 1) radiation botany and plant genetics -- use of induced mutation for crop improvement; 2) entomology -- application of male sterilization method for insect control; 3) biochemistry and plant physiology; and 4) soil chemistry. The research progress and results have been described in detail in the AEC annual reports and triennial reports, and also published in various international journals. The following summaries provide brief descriptions of the research activities in different agricultural fields during the contract period, as well as the training activities.

I. RESEARCH

A. RADIATION BOTANY, PLANT GENETICS AND CROP IMPROVEMENT

1. Mutation Breeding in Beans (Phaseolus vulgaris)

The common bean serves as the main plant protein source in the daily diet of the American Tropics. Many potentially high yield varieties have not been used as a cultivar because there are some other undesirable characters which are not acceptable to the consumers. The use of nuclear energy as a tool for bean improvement should have a great impact in the tropical countries.

At the outset of the bean improvement program, studies on bean radiosensitivity, factors modifying the sensitivity, chemical mutagen effects, and mutation frequency were carried out. Finally, beneficial mutants were selected for the breeding program. The results are summarized below:

a. The LD_{50} of dry bean seed (about 12% moisture content) was 12 kR, when an acute gamma radiation dose was employed.

b. When bean plants were exposed to chronic irradiation in the gamma field, the maximum dose with which the plants could complete their life cycle by producing seeds was 75 r per day.

c. Both acute and chronic gamma irradiation were equally capable of inducing mutations. In analyzing the mutation frequencies in the R_2 and R_3 generation of selfed progenies, a much higher mutation frequency was obtained in the R_3 progeny test. This may be due to the small induced chimeras

in which a number of mutations did not express their mutant characters in the R_2. The mutation frequency induced by chronic plant irradiation was at least as high as the frequency induced by acute seed irradiation.

d. All the induced mutations observed have been of a recessive nature; dominant mutations have not been found. In studying the genetics of the mutations induced by the acute seed irradiation, 74% segregated a simple Mendelian radio (3:1), 19% segregated a low, and 6% segregated a high mutant ratio. For the chronic plant irradiation, only 42% of the induced mutations segregated in a 3:1 ratio, but 56% segregated a low and 1% segregated a high mutant ratio.

e. A treatment of bean seeds with 0.06 M ethyl methanesulfonate (EMS) solution for 6 to 8 hr was very effective for inducing mutations in beans. As compared with gamma irradiations (LD_{50} dose), the mutation frequency induced by EMS was higher, and it appeared that the mutation spectrum from the EMS treatment was also broader.

After a few years of research work on inducing mutations in the common bean, we found that the mutation method is very efficient in inducing certain desirable agronomic characters of this crop, which may be applied directly to the benefit of agriculture. Examples are given below:

a. Mutation breeding in seed-coat colors of bean.

Seed-coat color is an important agronomic character to determine the marketability of a dry bean variety. People in a location have a specific preference for certain colors

of beans. However, seed-coat colors in beans are determined by the interaction of many color factors and modifiers. To improve seed-coat color by the conventional method, it needs many generations of back-crossing. The number of crosses would be increasingly larger as the backcross generation advances. Obviously, the conventional method is more tedious, and time consuming.

It is known that many black bean varieties are superior in disease resistance and yielding capacity, and because of their seed-coat color, the cultivation is restricted in many bean growing areas. By treating the seeds of black bean varieties with gamma rays or EMS, it is possible to induce a range of seed-coat colors from white, yellow, to grey brown. We have used this method to obtain 12 white and 41 yellow or brown mutants from 4 black bean varieties. Genetically, these mutants are due to the changes of pigmentation factor (P) or other color modifiers from dominant to recessive.

One of the white bean mutants, NEP-2, has been propagated and the seed have been sent to 34 institutions in 20 countries for field trials or laboratory tests. The results sent back showed that this white mutant has other good characteristics.

1) The field trial carried out in Uganda indicated that the NEP-2 mutant has exceeded in yield all other small white beans from several hundreds in their bean collection and remained entirely free from rust.

2) The field trial in Ecuador showed that the NEP-2 mutant was one of the top producers in a location of

high altitude (5740 ft.) which is about the maximum that beans can grow.

3) In a field trial in Michigan State, the NEP-2 mutant appeared to be a very promising variety, and in the laboratory test, NEP-2 has a low percentage of stachyose, raffinose, and sucrose. These sugars are believed to be implicated in the flatulence problem in beans.

4) A nutritional study carried out by INCAP (a nutrition research center of Central America) showed that the NEP-2 has a higher PER value in rat feeding tests. (1.72 for NEP-2 and 1.16 for its black parent).

5) Analysis carried out in Purdue University showed that the NEP-2 has no detectable quantity of tannin.

6) NEP-2 has a good canning quality according to the H.J. Heinz Co. of England.

The NEP-2 mutant is being used as a new bean variety for field trial (in Uganda) or as a new genetic material for hybridization (in Michigan).

b. Change of the pinto character. Pinto bean is characterized by the mosaic pattern of red and white color on the seed-coat. Genetically, it is due to a dominat mottling factor (M). Because of the mottling seed-coat color, pinto beans in general are not accepted by the consumers in many areas. By treating the soaked seeds of a vigorous variety of pinto bean (Brasileno) with EMS solution, a pure red bean mutant was isolated in the M_2 generation. This mutant was obtained in an 8-month period (2 bean generations). By using hybridization method, it may need many more generations

for producing a pure color line. This, again, demonstrates the efficiency of induced mutation method to improve some plant characters in agriculture.

c. High protein content mutant. In analyzing the nutritional value of the seed-coat color mutants of beans, a brown seed mutant, Bayo-2, had a considerably higher protein level than its parent (30-34% for the mutant vs. 22-22% for the parent). This mutant was also sent to the Protein Laboratory of IAEA, Vienna, for further analysis. The results confirmed the findings of our laboratory. Moreover, there was a noticeably different amino acid composition in the Bayo-2 mutant than the parent. Since the seeds of this mutant are small, it can be used as a genetic material for hybridization. The result well demonstrates the possibility of using induced mutation method for protein improvement in agricultural crops.

2. Mutation Breeding in Cassava (Manihot esculenta)

The roots of cassava are a staple food crop in the tropics and provide a major source of carbohydrates in the daily diets of more than 200 million people. Because of its great potential in root production (10-30 tons of fresh root per acre), cassava is probably one of the few food crops which can make a prominent contribution to the world food supply. However, it is known that the cassava is a cyanogenic plant. The cyanogenic glucosides, when hydrolysed, release hydrocyanic acid (HCN) which is poisonous. While a great part of the glucosides can be eliminated during food processing or preparation, concern has arisen in recent years

as to the probable toxic effects on humans in high cassava consumption areas. Medical evidence has shown that tropical ataxic neuropathy in West Africa may be a manifestation of chronic cassava poisoning. Research on this crop was carried out to explore the feasibility of whether the glucoside level could be lowered or eliminated by induced mutation method.

a. Cytogenetics and methods for inducing mutations in cassava

Cassava is a cross-pollinated crop and has a marked protogynous flowering habit. Cytogenetically, it is a polyploid and different cultivars have various degrees of chromosomal aberration. We found that seed irradiation is not an efficient means for inducing mutations in cassava. Pollen irradiation and stem irradiation are better methods.

By irradiating the cassava pollen with 5-6 kR of gamma rays, a mutation rate as high as 50% in the R_1 generation could be obtained. Most of the mutations were morphological, and were permanent when propagated vegetatively. Some mutants with vigorous erect growth were isolated as an improved cultivar.

b. Screening for acyanogenic mutations.

1) Screening method. For mutation breeding, an efficient method for screening the desirable mutants is essential. We found that the sodium picrate method is most practicable for the screening purpose. Because this method is extremely simple and sensitive, we can screen 100-200 samples a day. It is also more efficient and less time consuming to use the leaves instead of the roots as screen-

ing materials. In a recent study of the cassava cultivars in our collection, we found that the HCN levels in the leaf and in the root are correlated.

2) Screening results. By irradiating the young stem cuttings (6-8 months old wood) of a cassava cultivar with an acute gamma dose of 4 kR, it was found that in a number of cases, the induced mutant characters appeared in the whole R_1 plants, or in large chimeric sectors. This result suggested that a cassava plant could develop from one or two initial cells in the shoot apex, and gave us a guide line to sample the leaves for screening.

Several thousand leaves from irradiated plants were screened. As compared with the control, some leaves had higher and some had lower HCN level. This indicated that the irradiation had increased the variability and offered a possibility for selection. The selected plants with low HCN level were propagated for further testing.

3. Biological Response of Coffee (Coffea arabiaca)

Coffee is one of the most important economic crops in the tropical belt. The export of many Latin American countries largely depends upon this one crop. Research was conducted to study the genetic effects of radiation on coffee. The information obtained would serve as a basis for further mutation breeding.

The coffee seeds had an unusual response to radiation. When the seeds were irradiated, the mutant character appearing in the R_1 plants was not a chimeric change as in

many higher plants, but rather the whole plant produced a similar mutant characteristic. This evidence indicates that the development of a young coffee shoots is from a single initial cell of the apex. Most of the mutations induced in the R_1 generation were morphological, especially the angustifolia (ag) type. That the ag frequency dose relationship with thermal neutrons was linear and the relationship with gamma or X-rays was exponential suggests that the ag mutations were the results of two-hit chromosomal aberrations. Because of lack of chimera productions, seed irradiation should be an efficient method for coffee mutation breeding.

4. Radiosensitivity of Tropical Plant Species.

The objective of this study was to obtain information on the range of radiosensitivity in tropical plant species. The information obtained not only served as a guideline in the use of radiation doses for inducing mutations in plant breeding, but also allowed us to predict the effects of radioactive fallout.

A total of 84 plant species in the tropics have been tested for their radiosensitivity to chronic radiation in the gamma field. As one compares the two classes of plants, Gymnospermae and Angiospermae, the former class in general is more sensitive to the radiation. Among the 5 species in gymnosperms tested, none could survive at a daily dose of 27 r after more than two years of chronic exposure. In angiosperms, the radiosensitivity of 79 species in 18 plant families was tested. Many species in Compositae, Convolvulaceae, and Cruciferae are resistant to the radiation.

The species in Laguminosae and Solanaceae showed a wide range of radiosensitivity. Some species in Euphorbiaceae are also radioresistant.

B. ENTOMOLOGY

The research carried out in entomology was aimed at evaluating the feasibility of controlling or eradicating some economically important agricultural insect pests in Latin America. Investigation utilizing the male sterilization technique was carried out on the following insect pests:

Mediterranean fruit fly, Ceratitis capitata Wiedemann (Diptera: Tephiritdae)

Coffee leaf miner, Leucontera coffeella (Guérin-Méneville) (Diptera: Lyonetiidae)

Torsalo, Dermatobia hominis (Linnaeus, Jr.) (Diptera: Cuterebridae)

Meliaceous shootborer, Hypsipyla grandella (Zeller) (Lepidoptera: Pyralidae)

1. The Mediterranean Fruit Fly

The Mediterranean fruit fly is one of the most destructive pests of tropical and subtropical fruits. Besides citrus, medfly attacks more then 200 other fruit and vegetable crops. The history of this insect indicates that it has been constantly spreading from its original home in Africa. The Medfly was first reported in Central America from Costa Rica in early 1955. Within 7 years it had spread to the neighboring countries of Nicaragua and Panama.

A cooperative project was started in 1961 between the Inter-American Institute of Agricultural Sciences (The Institute is an USAEC contrator) and the Organismo Internacional

Regional de Sanidad Agropecuaria (OIRSA) to evaluate the feasibility of eradicating the medfly in Central America using gamma sterile males. In this cooperative project we supplied all basic research needed for field trials and OIRSA did the pilot field tests to determine the effectiveness of the technique.

Towards the end of 1965, OIRSA in cooperation with the International Atomic Energy Agency (IAEA), initiated a program (Funded by UNDP/SF and operated by IAEA) in Nicaragua to determine the effectiveness of sterile male technique for medfly. From 1968 to 1969, each week about 40 million irradiated flies were released in a test area of 48 km^2 (6 x 8 km). The Nicaraguan test was carried out in the center of a wide medfly infested area. In the test area the fly infestation was reduced about 90%. The results of this experiment supported the conclusion that the medfly could be eradicated by the sterile male technique in Central America.

a. Rearing and biology. The use of the sterile male technique requires large scale rearings of adults in the laboratory for field releases. OIRSA laboratory in San Jose, during pilot tests in Nicaragua in 1968-1969, reared about 55 million flies weekly. Larval food was the most expensive item besides labor and equipment in the rearing program. We tested several types of less expensive yeasts commercially available. It appeared that medfly larvae do not have very specific yeast requirements, as the use of any yeast by us did not adversely affect the weight of individual pupa, per-

cent pupal recovery from larval medium, adult emergence from pupae, fertility and fecundity of females and longevity of adult flies. Based on our findings the Joint IAEA/OIRSA project in San Jose used torula type B yeast ($0.18/lb. plus freight) in mass rearing of medflies which saved several thousand dollars in rearing cost of medflies.

We also worked out the optimum larval densities for bagasse and carrot substrate diets which greatly contributed in medfly production costs with optimum utilization of larval diet. Improvements were also made in lowering adults food cost by substituting cheaper protein hydrolysate S-150 and omitting orange juice powder.

In the beginning we experienced erratic egg-hatch in several experiments from normal medfly adults. Careful studies carried out later revealed that medfly eggs desiccate rapidly at relative humidities lower than 85%.

When used for sterile fly releases, pupae were shipped long distances in shipping containers where pupal temperature rose considerably due to metabolic heat. The experiments carried out indicated that heat treatment of normal or irradiated pupae higher than 40°C for 2 hr was lethal. A A temperature of 40°C or lower had no latent effect on reduced adult emergence or longevity.

Studies were carried out to find the dispersal habit and longevity of adult medflies in nature by releasing P^{32} labelled adults. It was found that about 88% flies remained within 240 meters of the release point. Maximum distance travelled by a labelled adult was 1300 meters. Released

flies were recaptured for up to 26 days.

b. Gamma sterilization. Most of the effort in this investigation was directed toward determining the proper sterilization dose, insect stage for sterilization and irradiation effects on sexual vigour, mating competitiveness and longevity of treated insects. Basic information on all the above mentioned points, necessary for field releases, were worked out in the laboratory.

Sterility in medfly adults can be achieved by irradiating either the pupal stage (1-3 days before adult eclosion) or newly emerged adults (1-2 days post emergent). When egg viability was used to measure the radiosensitivity, males were more radiation resistant than females. A dose of 5 kR induced complete sterility in females, compared to a dose of 10 kR needed for producing completely sterile males.

Our studies clearly indicated that sterilization reduced mating competitiveness of sterile males (based on egg-hatch data of mixed sterile and normal adult populations). In all tests, at every ratio of sterile: normal flies studied, the observed egg-hatch was higher than the expected egg-hatch. However, sterilization did not reduce longevity of treated adults.

An attempt was made to determine the cause of reduction in mating competitiveness of males. It was determined that when irradiation was applied to the advanced pupal stage (1-2 days short of emergence) at several levels 5-11 kR, sexual competitiveness decreased as the dose increased.

Since successful sterility can be induced in males

over a wide range of development (1-3 days before adult emergence to 2 days after adult emergence), a series of experiments was undertaken to determine any relation between the degree of insect development and the effect of sterilization on the male sexual vigor. The tests were carried out to measure insemination capacity and mating competitiveness (using egg viability data of mixed sterile and normal flies) or irradiated males. The results indicated that insemination frequency of sterile males increased with advancement in insect development stage at which irradiation was applied. This seemed to be quite logical as a dose of complete sterilization (10 kR) destroyed all germ cells except spermatids and sperm, which were later utilized by treated adults for inseminating females. The advanced stage males in pupal or adult stage were expected to have a greater portion of their testes full of spermatids and sperm.

Mating competitivenes tests carried out in the field with caged fruiting coffee trees indicated that laboratory strains of sterile males were very inferior in competing for mates with wild males. A ratio of 120:1 (Sterile: wild) males was needed to reduce egg viability in coffee berries to 1%. This indicates that for actual field trials high overflooding ratios are needed to suppress medfly reproduction.

Since medfly females are multimating in habit, effects of sterile and normal male matings (in sequential tests) were studied to determine the females mating behavior. It was shown that females, initially mated to sterile males, have a

higher tendency to remate with normal males than in a reverse situation. Results of alternate mating tests showed that in twice mated females (one mating with normal and the other with sterile males) egg fertility increased greatly when the second mating was normal, but egg-hatch was only slightly reduced when the second mating was sterile. Sterile males seemed to transfer a sufficient quantity of sperm. Thus it appeared that somehow irradiation lowered the mating competitiveness of irradiated sperm.

We have also determined that sterilization does not have any significant adverse effect on the rate of the sexual maturity, mating cycle, and mating duration of treated males. Also, remating in female medflies was not dependent on the quantity of sperm present in the spermathecae.

By no means has all the research related to application of sterile male technique for medfly been completed. However, we have obtained most of the basic information necessary for large scale field releases. The research to be done for the successful application of this technique is mostly ecological studies to be carried out in the field. These include host survey, density of fly and seasonal movements of flies, etc.

2. Biology and Sterilization of the Coffee Leaf Miner

This is a very destructive pest of coffee in all the coffee growing countries of the Western Hemisphere. The only satisfactory control of this insect so far is the use of highly toxic and hazardous systemic insecticides. In an effort

to find some other non-chemical alternate control method for this insect, studies were carried out to evaluate the applicability of gamma sterile male technique for controlling or eradicating the coffee leaf miner. The research done under this study is briefly summarized below.

a. Biology and rearing technique

Although some information on the life cycle of this insect was available it was not sufficient for the application of the sterile male technique. We studied further in the laboratory the biology of this insect. These studies included optimum temperature and humidity ranges for development of eggs, larval and pupal stages. Information on fecundity and ovipositon patterns of the female was gathered. Technique was devised for obtaining mating and oviposition of fertile eggs in the laboratory. This enabled us to develop successful mass rearing of this insect for the first time in the laboratory. The rearing was done on potted coffee plants. No attempt was made to develop artifical rearing medium. We mass reared about 2000 adults per day over a period of 1 year continuously using this technique.

b. Radiation sterilization

Like most Lepidopterous species the coffee leaf miner was found highly radio-resistant (when egg-hatch was used as the criterion to measure the radiation sensitivity).

Studies carried out to determine the suitable insect stage and dose level for inducing complete sterility indicated that pupal stage of this insect was not appropriate for irradiation. A dose of 60 kR applied to advance age pupae

(a few hours prior to adult emergence) was lethal. Complete sterility in the males was achieved by irradiating 24 hr old moths with 90 kR. However, moths so sterilized were not sexually competitive and their longevity was drastically reduced. But we found that completely sterile males were able to transfer sperm to famale spermathecae during mating. Also males receiving a high radiation dose (90 kR) elicited a normal ovipositional response in the normal females. Complete sterility in the female coffee leaf miner was induced by a low radiation dose of 20 kR.

c. F_1 inherited sterility

When it was confirmed that the use of completely sterile males had little value in population suppression by sterile male technique, research was directed to discover the presence of so-called F_1 inherited sterility phenomenon. It was interesting to observe that in coffee leaf miner, highly sterile F_1 progeny (both sexes) could be produced by applying relatively low irradiation doses (20-30 kR) to the parent males. We have carried out studies on the mating competitiveness and longevity of highly sterile F_1 progenies and found that although F_1 adult longevity was only slightly reduced the mating competitiveness of these moths was significantly reduced (specially when in the mating competitiveness tests of F_1 males, F_1 females were not included). Some preliminary tests carried out towards the end of the study where mating competitiveness of F_1 males were studied, using F_1 males and females and normal males and females, showed encouraging results. Egg-hatch of such a mixed moth population was greatly reduced.

All our studies were conducted in the laboratory. Is is very important that the laboratory findings should be tested under field conditions. Perhaps some studies in outdoor field cages would give a more correct picture concerning the utility of F_1 partial inherited sterility in controlling this insect.

3. Biology and Sterilization of the Torsalo

This insect is of great economic importance to the cattle industry of many Latin American countries. With the exception of Chile, it occurs in all Latin American countries from Mexico to Argentina. It has not been recorded in other parts of the world outside Latin America. The damage is inflicted by the parasitic larvae which causes cutaneous myiasis in cattle.

The control or eradication of the torsalo by sterile male technique offers promising aspects. Adults of torsalo have a very short life, since they do not feed as their mouth parts are vestigeal. The low mating frequency of sterile males (as found in most cases) would not be so vital a factor as in long lived insects. The torsalo population in nature is very low in certain seasons of the year. The available chemical methods can reduce the population density to a low level in areas of heavy infestation. Eradication can be achieved in areas where torsalo population is maintained by reservoirs of wild animal hosts which are inaccessible for insecticidal treatment.

A study was made to rear torsalo larvae in the

laboratory on an artificial diet prepared from fresh meat and blood. The life cycle of this insect was also studied in the laboratory by rearing torsalo larvae on guinea pigs. The number of larval instars and the duration of each stadium was worked out. Morphological characters of each larval instar were studied and identifying characters were established which enabled us to separate the various instars. Work was also done on the effect of some physical factors, such as temperature and humidity on the developmental rate of eggs and pupae of this insect. We found that eggs and pupae of torsalo were very susceptible to desiccation at a relative humidity lower than 80%. The biology of torsalo is rather unusual. The females lay eggs on active blood sucking or day flying insects which visit cattle frequently. In nature, the Mediterranean fruit fly has not been reported as a vector for the torsalo eggs. In the laboratory, we have used the Mediterranean fuit fly adults extensively as oviposition material for torsalo females. In the presence of the common house fly (a natural vector for torsalo eggs) and the Meditarranean fruit fly in the same cage female torsalo preferred Mediterranean fruit fly for egg-laying.

Some gamma sterilization studies were carried out by using field collected pupae. Sterilization dose for males and females were worked out. A dose of 10 kR induced complete sterility in males as well as females. The females were somewhat less radiation resistant (measured in terms of egg viability) as compared with the males.

4. Biology and sterilization of the Meliaceous shootborer.

This shootborer is a serious pest of valuable Meliaceous trees such as the mahogany (Swietenia sp.) and the Spanish cedar (Cedrela sp.), both native species of Latin America. All attempts to establish large scale plantations with these tree species have failed mainly due to severe attacks by the larvae of this insect.

Due to growing concern about the damage of this shootborer in natural Meliaceae plantations, cooperative studies with the Inter-American Working Group on Hypsipyla problems were conducted to develop an artificial rearing diet, study the mating and oviposition behavior of the moths and finally to evaluate the feasibility of controlling this insect by gamma sterile male technique.

Suitable artificial diet for larval rearing was worked out, and satisfactory mating and oviposition techniques under outdoor conditions were developed. The insect stage and sterilization dose to induce complete sterility in the moths of both sexes were also studied. Results indicated that sterilization doses did not drastically reduce the longevity of treated males.

C. BIOCHEMISTRY AND PLANT PHYSIOLOGY

1. Physiology of Cassava (Manihot esculenta)

a. Photosynthesis of cassava leaves: Metabolic pathway and net rate of CO_2 fixation.

By use of rapid ^{14}C labeling techniques, the first photosynthetic products from CO_2 were establised. It

was concluded from this work that the reductive pentose phosphate pathway (C_3 metabolism) is the major photosynthetic pathway in cassava. Also it was shown that cassava has an active glycolate metabolism, which suggests that a high rate of photorespiration occurs.

The net rate of photosynthesis was determined as 45 ± 5 mg CO_2 per hour per square decimeter of leaf area and was shown not to change significantly with the age of the plant. The net rate also did not differ when various cultivars were compared. The net photosynthetic rate of cassava is 20% greater than the common bean and 20% less than sugarcane when measured under the same experimental conditions.

b. Translocation of assimilated carbon from cassava leaves: Distribution and rates of flow.

When $^{14}CO_2$ is assimilated by cassava leaves, sucrose is the major end product of photosynthesis and essentially the only compound translocated. Small amounts of the related oligasaccharides, raffinose and stachyose were the only other products found in the translocate. The major portion of translocated sucrose is found only in those plant parts which are on the direct route to the root system.

The net rate of translocation from cassava leaves was determined and compared to the rates in bean and sugarcane. Sugarcane had the fastest rate and bean the slowest, with cassava between these two plants. The rate of translocation appears to be directly proportional to the rate of photosynthesis. When the translocation rate was measured in different cultivars of cassava, no significant difference was

observed.

c. Sucrose metabolism in cassava roots.

The major pathway for sucrose conversion in cassava roots was found to occur by a reaction catalysed by sucrose - UDP glucosyltransferase (sucrose synthatase) and not by invertase. This enzymatic reaction appears to be regulated by the nucleotide balance and the presence of metal ions. UDP - glucose, a product of this degradation of sucrose, was shown to be the major precursor for starch synthesis.

The specific activity of cassava root sucrose synthatase was measured at monthly intervals and shown to decrease in activity with increasing maturity. This same pattern of activity was shown to occur when a range of root sizes was selected from one plant rather than on the basis of plant age. The concentration of sucrose and reducing sugar also decreased with increasing maturity of the root. Both the activity of sucrose synthatase as well as the concentrations of sugars varied widely between different cultivars.

d. Synthesis of cyanoglucosides in cassava

^{14}C - L - valine, a known precursor of linamarin, was administered to cassava leaves and its metabolism traced by analysis of the plant parts by paper chromatographic techniques. After 4 hours of ^{14}C - valine metabolism, a radioactive compound was detected on the chromatogram of the leaf extract which showed all the characteristics of authentic linamarin. Glutamate, glucose, fructose, sucrose, and five other unknown compounds were also present in the leaf extract. The extract of the source leaf petiole, however, contained only

sucrose with traces of glucose and fructose. There was no detectable linamarin present in the translocate. These results show, at least in young cassava leaves undergoing active photosynthesis, that linamarin is synthesized but not translocated.

When ^{14}C-L-valine was applied on the inner layer of bark of the root, or injected directly under the peel and left for up to 8 hours no metabolism of valine was observed.

2. Effect of Temperature on the Germination of cacao seed (Theobroma cacao)

a. Sensitivity of cacao seed to cold

Cacao seeds were soaked in a water bath at temperatures from 0ºC to 60ºC and for 2 to 128 min. The seeds were then planted and those which had expanded colyledon 3 weeks later were recorded. Between 14º and 44ºC, no effects were noted. Decreased growth occurred at higher or lower temperatures, and damage was greater, the longer the exposure. Cacao seeds are unusually sensitive to above freezing temperatures. The seeds chilled at 4ºC for 10 min died.

b. Reversibility of seed death caused by cold

1) Conditions leading to irreversibility of seed death

It was demonstrated that cacao seeds chilled at 4ºC for 10 minutes could be restored to viability if post-treated to 37ºC for 10 minutes. This suggested the function of a biophysical phenomenon in the cold death of cacao seeds. If a lipid in the cellular membranes changed state during cold

treatment, this could lead to death by cellular destruction. Heating re-converted the lipid to its original state and the integrity of the membrane as a result, was not destroyed. It was noted, however, that after 15 minutes at 4ºC, no amount of heat treatment at any reasonable temperature (60ºC also kills the seed) could restore viability. These data suggest that in combination whth a biophysical effect there is some physiological effect with time.

2) The pause effect in cacao seed death caused by cold.

It was found that, if, after treating cacao seed at 4ºC for 10 minutes, one did not immediately place the seeds in water at 37ºC, but rather, paused 5, 10, 15, or 20 minutes before so doing, only a very few seeds of the 5 minute pause treatment were re-vitalized, all other seed were dead. This phenomenon argues in favour of some physiological event, brought on by cold, which is the ultimate cause of death. It could possibly be a lysis of the cells of the seed cotylédon caused by biophysical changes induced by cold, followed by autolysis caused by the seed itself.

3) Resistance of various clones of cacao to cold

A number of clones of cacao were studied for cold resistance at 4ºC for 5 and 10 minutes. The most resistant seeds at 5 minutes were UF-613 and ICS-29 (60% survival), ICS-39 and ICS-1 (50% survival) and UF-667 (34% survival), R-9 and R-10 had 25% survival, while UF-667 and UF-676 showed a 20% survival rate at 4ºC for 5 minutes. None of the other clones studied could survive even the 5 minutes

treatment at 4ºC. These were: UF-618, UF-296, UF-650, UF-668, R-2, IMC-67, SPA-9, and IAL-407. A normal control (no cold treatment) of these various clones showed between 96-100% survival in all cases.

c. Role of the cotyledon in cacao seed death caused by cold

1) Respiratory changes in cacao seed cotyledon coincident with seed death

Warburg studies of normal vs. cold treated cotyledon demonstrated that in cold treated tissues, endogenous respiration rises precipitantly compared to normal material. This is accompanied by the production of a strongly coloured brown pigment which is due to the action of polyphenol oxidase. Cacao seed radicle tissue shows no respiratory change between normal and cold treated samples, In addition, cold treated radicle tissue free of the cotyledon grew just as well as normal tissue in sterile culture. These data support the idea that the main site of cold germination inhibition action lies in the cotyledon and not in the radicle. Respiratory studies with the cotyledon chilled 0.5, 10, 15 and 20 minutes, demostrated a gradual increase in endogenous respiration as exposure to cold increased. If, after exposure to cold at the various times, the seeds were then treated at 37ºC (the restorative heat treatment) it was noted that the 5 minute cold respiration rate had dropped down to normal levels while the other rates were approximately the same. It is apparent that there is some relationship between the increase of respiration following chilling of the cotyledon and the irreversible death point of the seed after cold treatment.

This is supported by the fact that restoration of viability by post-heat treatment results in a decrease in respiration of the seeds which have been chilled in water for 5 minutes at 4ºC. This lower rate of respiration is practically indistinguishable from that of the normal system. The major effect of irreversible cold-induced death occurs in the cotyledonary tissue during the period 10-15 min in water at 4ºC. The increase in respiration with chilling time suggests a progressive effect of cold treatment on the cotyledon. Such an effect could be attributed to a gradual change in the physiological properties of the cotyledon, possibly a solid state change in the membrane lipids. Restoration of viability following heat treatment could then be due, at least in part, to a reversal of such physiological change.

2) Cytological changes induced by cold in cacao seed cotyledon

After cold treatment, portions of cacao seed cotyledon were fixed and prepared for microtoming. Analysis of tissue sections showed that the cold-treated tissues differed significantly from those of normal cotyledons. The tannin cells present in the normal tissue was absent in the cold treated tissue, being replaced by tannin cell 'ghosts'. In general, the cold-treated tissues seemed to be in worse shape than those of normal cells.

3) The effect of cold on mitosis in root tips of cacao radicle tissue grown in sterile culture

In order to prove that cold did not effect mitosis in cacao radicle tissue and to demonstrate that the growth in sterile culture of these tissues was not due to

elongation, cacao radicle tissue grown in sterile culture was fixed and prepared for microtoming. Analysis of normal and cold treated tissues showed no significant difference in mitosis between the two.

3. Physiology of Cacao Seed

a. Effect of sugars, alcohols and Krebs' cycle intermediates on respiration of cacao seed radicle tissue.

Warburg respirometer studies of respiration in cacao radicle tissue showed that dextrose and sorbose are metabolized at a rapid rate by these tissues while sucrose and galactose do not show a significant increase in respiration over the endogenous. These data were used as the basis to modify a tissue culture medium which used sucrose so that cacao radicle tissues could grow on it. A number of sugars had an inhibitory effect on endogenous respiration, e.g., fucose, lactose, maltose and sedoheptulose. Phenol, butanol, cyclohexanol, and ethanol all increased the respiration rate of radicle tissue, while ethylene, glycol, isoamyl alcohol, and isopropanol had no effect or were inhibitory. Of the Krebs' cycle intermediates studied, fumaric and oxalacetic acids stimulated respiration while citric acid inhibited endogenous respiration.

b. Effect of Krebs' cycle intermediates and phenol on respiration of cacao seed cotyledon

In the cacao seed cotyledon citric and fumaric acid were stimulatory to cacao cotyledon, doubling the rate of the endogenous system. Oxalacetic acid had no effect. Phenol almost tripled the rate of endogenous respiration in the coty-

ledon. Phenol is possibly a substrate of some phenol oxidase in the cotyledonary tissue, in addition, it may stimulate release of other enzymes due to its lytic properties.

c. The relationship between respiration, germination and moisture content in cacao seeds

The mature cacao seed contains 40-45% water. This high content is possibly the cause or perhaps the result of a lack of dormancy in cacao seeds. The effect of dehydration on cacao seed viability and the respiration rates in these tissues were studied. All germinative ability and respiration ceased at 14-16% water, based on wet weight. Germination ability remained 100% until approximately 20% of the water remained and then began to drop precipitously. Respiration fell in a straight line at about 35-20% at which time it too fell precipitously.

The loss of respiration as water loss occurred is logical, since all metabolic processes require water. The loss of respiration and germinative ability at 15% moisture content indicates that in cacao seeds, water has a much more critical role than in other seed systems.

The majority of seeds are viable and respire at values lower than 12% moisture content. In cacao, it may be that water serves a structual function as well as a metabolic one, as for instance, in certain critical unions in cellular membranes. Cacao seeds represent a metabolic system with no period of dormancy and this suggests a study on the use of metabolic inhibitors as a means to store cacao seeds for later planting.

4. Sulphur Metabolism Employing S^{35}

a. Sulphur metabolism in photosynthetic bacteria, Rhodospirillum rubrum

Our investigation has demonstrated that sulpholipid was produced in photosynthetically grown R. rubrum. This organism is capable of a dual existence, that is, under anaerobic conditions, in the light, it can exist as a photosynthetic microorganism, while under aerobic conditions, light or dark, it exists as a heterotroph. One hypothesis involved the possibility that the S-lipid was important in the photosynthetic process and therefore should not be found in heterotrophs. Since R. rubrum could be grown both as a photosynthetic organism and a heterotroph, it lent itself well to testing of this hypothesis.

It was found that dark grown R. rubrum (Heterotrophic) possessed no S-lipid. If heterotrophically grown R. rubrum were placed under light, anaerobic (photosynthetic) conditions, a simultaneous return of S-lipid and chlorophyll could be noted. Analysis of cellular fractions employing a Spinco ultracentrifuge demonstrated that most of the S^{35} activity as sulfolipid was found in the bacterial chromatophore, the analog to the chloroplast of plant cells. These results demonstrate that the S-lipid is associated with the organ of photosynthesis in some intimate fashion and does not exist, in the case of R. rubrum, unless photosynthetic metabolism is evident.

b. Mobilization of S^{35} in cacao seeds.

Normal and chilled (4ºC, 20 minutes) cacao seeds

were used for the experiment. S^{35} was placed at the apex of the cotyledon or on the radicle in both normal and cold treated seeds. Sulphur migration and absorbtion was measured in radicle, middle of cotyledon, and top of cotyledon in all cases. Sulphur was transported from cotyledon to radicle and vice versa in normal and in cold treated seeds. When S^{35} was applied to the normal cotyledon, a higher amount of S^{35} was transferred to the radicle than in the cold treated seed. On the other hand, when S^{35} was applied to the radicle, a larger amount of S^{35} was found in the cotyledon in the case of cold treated seeds. This could be explained in the following way: in a normal seed, nutrient transport occurs from the cotyledon towards the embryo. In chilled seeds, this phenomenon does not occur as efficiently as in normal seeds, possibly due to a breakdown of vascular or cellular continuity between embryo and cotyledon. Thus, we see a higher concentration of S^{35} in radicles of normal seeds than in radicles of chilled seeds when S^{35} is applied to the cotyledon. Applying S^{35} to the radicle in chilled systems leads to a higher count in the cotyledon than in normal seeds because the natural activity of the radicle absorbs S^{35} and transfers it without competition from the 'flow' of material from the cotyledon.

5. Plant Disease Physiology: Host-parasite Interractions on banana fruits.

In the consideration of disease physiology, two entities, the host and the potentical parasite, are recognized, and each of these has its own unique chemical make-up and pattern of metabolism. The ways in which these entities interact

determine whether or not disease will occur, and what the cause of the disease will be. In nature, any given plant during the course of its lifetime comes in contact with many and diverse potential pathogens; only a few of these are actually capable of inducing a disease. What is remarkable is that there is so little disease, considering the possibilities which exist.

The present study proposed to delve into this problem of disease by utilizing a comparison of a virulent banana fruit rotting organism, Gloeosporium musarum, and a closely related fungus, Colletotrichum gloeosporioides, which causes severe rotting on other tropical fruits such as papaya and avocado, but which is saprophytic or weakly parasitic on banana. The fruit rot was chosen since it has a disease cycle which may conveniently be carried through in the laboratory. In addition, the host fruit undergoes a period in which it is completely resistant to the G. musarum, but as ripening progresses becomes susceptible. This is of obvious survival value in the case of wild diploid seed-bearing bananas, where seed release is brought about by autolysis and microorganism breakdown of the fruit, a process which does not commence until the seeds and the fruit have ripened.

When considering the reasons why one organism is pathogenic and another is innocuous, one is faced with several possibilities. The non-pathogen may be incapable of attacking the potential host because it lacks certain necessary extracellular enzymes which are required to turn host into utilizable food. Or, because of certain internal metabolic differ-

ences, the non-pathogen does not derive required energy or essential nutrients from the host. A third possibility is that within the host tissue there exists compounds which prevent establishment of the fungus as a parasite.

These hree alternatives do not exhaust all the possible mechanisms to explain resistance, but were the ones considered most pertinent for the present investigation.

Prior studies established that, as far as fungal extracellular enzymes such as those controlling pectolytic and amylytic processes were concerned, no difference between the two fungi under study could be detected. It was observed that the C. gloeosporioides was capable of utilizing the metabolic blocking agent 2-deoxy-D-glucose as its sole carbon source, whereas G. musarum could not grow on this sugar.

In carrying out the study, two questions were asked: a) Are there significant differences in carbohydrate metabolism between the two fungi which might account for their dissimilar pathogenicities? b) Does the banana fruit contain substance which inhibit both organisms while it is green, and C. gloeosporioides after becoming ripe?

After having carried out a series of studies, no significant metabolic differences could be established which would serve to answer the first question, but the routes and controls of carbohydrate were worked out, adding to the knowledge of these fungi for future reference. A theory for the mechanism of latency, i.e., the inability of G. musarum to develop on unripe fruits, was proposed to partially answer the second question; however, no materials which could selectively

inhibit the non-pathogen were obtained from the ripe fruits. The basic question of why one fungus is pathogenic and the other not thus remains unanswered at the present time.

a. Comparative studies on carbohydrate metabolism of Gloeosporium musarum and Colletotrichum gloeosporioides

1) Growth on starch and other carbohydrates

To observe whether any gross differences existed in the utilization of various carbohydrates, both fungi were grown in liquid media containing a number of sugars, starches, and so on. It was noted that C. gloeosporioides grew much better on galactose than did G. musarum, but apart from that the reactions on the other substrates were similar. The same was true for amylase activity.

2) Warburg respirometry using metabolic blocking agents

In these studies the oxygen uptake of washed mycelial fragments was studied in the presence of a series of blocking agents. G. musarum and C. gloeosporioides, both grown on glucose, and C. gloeosporioides grown on 2-deoxy-D-glucose, were all compared with respect to their reactions to the various agents. Based on the results obtained, it was concluded that there was a good chance that the two fungi had different pathways of carbohydrate catabolism.

3) Radiorespirometry with ^{14}C sugars

For these tests the two fungi were grown in shake culture, the mycelium removed and washed, and then placed in reaction flasks containing the various glucose-^{14}C substrate. The respiratory CO_2 was collected from the CO_2-free air passed through the flasks, and the alcohol formed in the reaction

medium was distilled off and degraded to CO_2. These respiratory products were then analyzed for radioactivity. Again, the two fungi gave differing results, suggesting that they had unlike catabolic pathways.

4) Enzymes of the glucose catabolism pathway

In this series, the fungi were extracted to obtain the enzymes intact, either by grinding and ultracentrifugation, or by preparing acetone extracts of the mycelium. The extracts were then reacted with various substrates of the catabolic pathways and with the necessary co-factors, and the results read in a spectrophotometer.

In this case, no major difference could be detected between the two organisms. Both apparently lacked phosphofructokinase, and it was concluded that under the conditions of the experiment glucose breakdown was via the pentose phosphate pathway.

An overall examination of the results obtained from these experiments shows that the two fungi under study are not identical metabolically, but are quite similar as far as the parameters tested have revealed.

b. Host control over fungi growth patterns

It was stated earlier that the differences in pathogenicity noted between G. musarum and C. gloeosporioides on banana fruits might also be due to controls exerted on the parasites by the host tissue, and the phenomenon of latency in the case of the pathogen was mentioned. Several series of experiments have been carried out to attempt to explain the observations made in relation to host control of the invading

organism.

1) A theory for the mechanism of latency

A number of postulates have been put forth to explain latency in anthracnose infections of various fruits. None of these have been completely satisfactory, so this phenomenon was studied in the hope that the mechanisms involved might serve to illuminate the basic nature of the disease.

To study this, tannic acid and banana tannin were mixed with a banana enzyme, β-amylase, and the reduction in activity measured. It was found that the banana tannin was very active against this enzyme system, and it was postulated from these results that latency is due to the inactivation by materials in the banana latex of the various extracellular enzymes produced by the invading fungus.

2) Effects of banana phenolics on fungus growth

From the foregoing results it would be expected that banana latex, since it contains tannins which are capable of inactivating extracellular enzyme systems, would be active in vitro and inhibit growth in petri dishes. Unfortunately such is not the case. The latex is a fine medium for fungal growth, which means that either the food materials from the medium can diffuse directly into the mycelium without being transformed by the extracellular enzymes of the organism, or that only certain enzymes are inactivated, and those which remain provide enough food materials so that there is mininal interference with growth. The latter would seem unlikely since the tannin-protein complex is unspecific. What remains then is to determine whether the complexing of the tan-

nin with the enzyme actually renders the latter incapable of acting as a catalyst, and whether all of the enzyme present is inactivated.

Since it is known that the principal tannin of banana latex is leucodelphinidin, a series of tests were performed in which the effects of this material, in its leuco and oxidized forms, and of other similar substances, on fungus growth, was studied. No leads which would resolve the problem of pathogenicity were uncovered, nor were there any indications that differences existed between the two fungi.

The nature of host-parasite relationship, being a very intimate one, is not adapted to being studied by observing the separate entities apart from one another, and then trying to draw conclusions as to what occurs when they are conbined. However, such a study as this can yield more basic information which may allow us to understand more fully the nature of pathogenicity, and thereby allow a more rational means of controlling diseases of plants.

6. The Mechanism Controlling Dwarfism in a Radiation Induced, Single Gene Bean Mutant.

During studies on the genetics of radiation-induced mutants of beans, it was found that the Dwarf-1 mutant of the Mexico 80-R line was controlled at a single locus. By establishing the biochemical basis underlying this form of dwarfism and comparing it with the parent lines, it was thought that some of the mechanisms which affect growth control might be elucidated.

Experiments with applied growth regulators showed

that dwarfism could be reversed by application of gibberellic acid (GA_3). It was ogical to suppose that a deficiency in the endogenous gibberellins might account for the phenonemon. The first series of experiments established that when the mature seeds were extracted and fractionated, the only fraction from the normal line which could reverse dwarfism in the mutant was that called the 'ethyl acetate neutral fraction'. The other fractions contained active materials as evidenced with several bioassays, but only the neutral fraction contained the control factors.

A later series of tests then attempted to resolve the dwarfism problem by chromatographing the neutral fraction and testing the various zones for activity in a dwarf bean leaf bioassay. From this, it was found the both the normal and dwarf lines were possessed of identical gibberellins, rather than the dwarf being deficient as was first thought. At the same time, it was seen that the Dwarf-1 line contained in addition a powerful inhibitory material which was specifically inhibitory to the neutral fraction gibberellins, but not to GA_3. It was concluded that the single gene mutation affected not the gibberellins, but the inhibitors which control them.

7. The use of Wetting Agents in Foliar Nutrition

By using P^{32} as a tracer, it is possible to study the effectiveness of wetting agents in foliar nutrition. Three commercial surfactants (Anionic: Fenopon-Ac-78; Cationic: Hyanime-2389; Nonionic: Tween-80) were used. All the surfactants increased both absorption and subsequent translocation of P^{32},

as compared with the control in coffee and cacao plants. Ammonium phosphate was absorbed and translocated to a much greater extent than were either sodium or potassium phosphate.

D. SOIL CHEMISTRY

Little is known about the chemical and fertility properties of soils in the humind tropics. However, the rapidly increasing population makes it necessary to use these soils more and more intensively, which requires a thorough knowledge of them. A better understanding of these particular soils will also contribute to a better understanding of soils in general. Volcanic ash soils are very important in the tropics because their present as well as potential productivity is high. To understand them better their parent material, fresh ash, was studied when eruptions of Irazu and Arenal volcanoes supplied the needed raw material.

1. Acidity, Cation Saturation, and Cation Movement in Tropical Soils

The work was initiated with a brief exploration of the acidity status of the soils of the region. All soils around Turrialba are acid, some strongly acid. Appreciable exchangeable (KCL extractable) Al was noted only for soils with a pH below 5.0.

Extractable Al (with NH_4OAc at pH 4.8) was rather high, with up to 11 meq/100 g for the most acid soils.

Cation exchange capacity determinations were made to evaluate the base saturation status of these soils. However, these determinations gave high values when soils were satu-

rated at pH 7.0 with $\underline{N}$ NH_4OAc as is the general practice. These high values did not agree with the low total exchangeable bases and the moderately acid pH found. To understand this phenomenon better, a study of the cation exchange capacity determination was undertaken. Determining the exchange capacity at different pH values and with NH_4OAc or $CaCl_2$ as saturating solutions, it was observed, as could be expected for soils high in amorphous components, that there was a considerable difference between the capacities measured at high and at low pH. It was concluded that, as these soils are acid, determinations at pH 7 overestimate considerably the exchange capacity. It was also noted that at the same pH, the use of NH_4OAc results in higher exchange capacity data, which agree less with the overall soil properties, than the use of $CaCl_2$. Based on the literature it was considered that this is due to acetate adsorption on the large surface of the amorphous materials present.

As a consequence it was decided to use the $CaCl_2$ method as routine determination on soils where high amorphous mineral or sesquioxide content makes acetate adsorption a likely disturbing factor in cation exchange capacity determinations.

It was also concluded, based on exchange capacity determinations in the presence and after the destruction of organic matter, that while organic matter is an important component of the exchange complex, its contribution to cation exchange capacity decreases rapidly with depth, not only due to a decrease of organic matter content but also due to a

lower exchange capacity per gram of the organic matter deeper in the soil.

The movement of soil cations has received attention also. In an experiment with small lysimeters, four latosols were studied using radioactive tracers of Ca, Sr, Mn, and W. Ca and Mn were chosen due to their biological importance while Sr and W are important fall-out products. In addition to untreated samples, the effect of liming, of NH_4NO_3 application, and of the doubling of the organic matter content was studied. Generally, cation movement was slight. Sr was found to be the most mobile and W the least mobile cation. Addition of organic matter enhanced W and Mn mobilization, reduced that of Sr and had a variable effect on Ca. The movement of Ca and Sr was favored by nitrogen application in three of the soils studied and that of Mn and W in all of them.

As a general conclusion, cation movemnt was found to be rather slight (of the order of a few centimeters) even under the effect of a 100 inches of water passed through these soils.

2. Phosphate Studied in Soils of the Humid Tropics

The phosphate studies included three fields: a. Organic phosphates and the methodology for their determination was investigated followed by mineralization studies, b. experiments were realized on the uptake of P from applied fertilizer in case of three crops, nine soils (alluvial soils, andosols and latosols), and three dates of planting after P application, c. the P status of several important soil types common in the Amazon Basin of Brazil was investigated.

a. Organic phosphate studies

This series of experiments was initiated with a methodological study on the determination of total organic P, in soils of the humid tropics. It was found that the ignition techniques of Legg and Black and of Saunders and Williams do not give a satisfactory recovery of organic P due to the fixation as Al phosphates of a considerable part of the P which is mineralized during the ignition. The Mehta et al. acid-base extraction technique was found to be the most efficient extractor. However, this method also extracts an appreciable amount of Fe from some samples and this of course interferes with the subsequent colorimetric determination of P. This was solved by passing the solution through an exchange resin column to eliminate the interfering Fe without introducing any undesirable component.

With an adequate technique, a number of soils from Costa Rica and Brazil were studied to establish the contribution of organic P to total P content. It was observed that in the younger Costa Rican soils, organic P contributed slightly over 50% of total P. The organic C/organic P ratios were lower than those usually found in the literature for the tropics. It is believed that these discrepancies, indicating a higher organic P content, are mainly due to the improved extraction technique.

The old, highly weathered Brazilian soils were very low in total P. For these, the contribution of organic P was only of the order of 20 to 30% of the total P and the organic C/organic P rations were high, of the order of 200-300

and similar to values reported in the literature.

Another experiment was initiated to study the mineralization of organic P in controlled conditions. The effect of inorganic P addition, of liming, of temperature, and of the addition of inorganic nutrients excluding P was investigated for 3 soils, including one high, one medium, and one low in organic P, respectively. Preliminary data have shown little mineralization in the initial phase of the experiment. On the contrary, some of the treatments resulted in an increase of the organic P present. An appreciable increase in Al- and Fe- phosphates was observed on P application. Different temperatures and liming had no observable effect on changes in P.

b. Phosphate uptake experiments with ^{32}P.

In this experiment, corn was grown in 9 soils treated with complete fertilizer tagged with ^{32}P. Fertilizer uptake was generally very low. It was highest for the alluvial soils with the latosols second. Corn planted 15 and 29 days after fertilizer application showed little effect of the time the fertilizer spent in the soil previous to planting. Comparing tomatoes, corn, and Phaseolus calcaratus (a leguminous test plant), it was noticed that tomatoes were on the average the most and corn the least efficient P extractors. It was also concluded, that the use of L or A values is impossible in these conditions as fixation of this element makes a true equilibrium impossible between fertilizer and soil. This observation explains the high A values reported for P deficient tropical soils by many authors.

c. P status of soils from the Amazon Basin in Brazil

The Amazon Basin is one of the least known parts of the Americas. As indirect evidence indicated a very low P content in the area and as carefully selected samples from two profiles were available, the P status of these soils was studied by several different methods. As expected, most soils were low in P in all categories studied. P content decreased with depth and the relationships among the P measurements was typical for highly weathered soils. In addition to the low concentration of P, many of the studied soils fix it intensively. This implies that intensive agriculture in the area would require considerable additions of fertilizers.

3. Movement, Adsorption, and Desorption of Sulfates

To cover a wide range of soil properties, three rather different soils were studied, namely a latosol, an andosol, and an alluvial soil.

The sulfate movement was studied in small lysimeters using tagged sulfates. Different cations of the various sulfates applied had little effect on the movement of this anion. The different soils, however, behaved quite differently. Retention was proportional to the combined content of clay and silt.

The adsorption curves indicated a physical adsorption on the soil surface. The data fitted Freundlich type equations well and no maxima of adsorption were found in the range studied, up to 1000 ppm. Sulfate was rapidly displaced by phosphate solutions but only slowly by water.

The soils lost hydroxyl ions proportional to their

sulfate adsorption.

Sulfate adsorption was much higher than that observed for sulfate-adsorbing soils of the temperate region. This strong sulfate retention explains the appreciable sulfate accumulation reported for latosols in Costa Rica.

4. Free Iron and Aluminum Oxides in Tropical Soils

The content of free Fe and Al oxides in soils is important as these components influence many processes. However, little information is published on this characteristic of tropical soils.

Methods were studied and the conclusion reached that Kilmer's method, slightly modified to accomodate the large amounts of free iron oxides present in tropical soils, can be used satisfactorily.

For the determinations of free Al, Hashimoto and Jackson's extraction was followed by a colorimetric determination.

Soils from Brazil, Colombia, Costa Rica, Panama, and Peru were tested. On the average, alluvial soils had about half the Fe_2O_3 content of latosols, and about one third less Al_2O_3. However, the ranges in R_2O_3 contents of alluvial soils was very large and extended into the range of latosols. Recent volcanic soils were high in free sesquioxides also.

It was concluded that the free sesquioxides can be adequately determined in a great variety of tropical soils with the methods recommended and they form a large percentage of most latosols, andosols and some of the alluvial soils.

5. Mineralization of Organic Nitrogen in Soils on Volcanic Materials.

Many soils on recent volcanic ash contain large amounts of organic N. However, crops growing on these soils often demand a heavy N fertilization. To explore this contradictory behavior the mineralization of organic nitrogen was studied together with the allophane content in surface and subsurface samples of eight soils. The soils included four on recent volcanic ash (andosols), two on old strongly-weathered volcanic materials, and two alluvial soils.

The effect of liming, organic matter addition and of drying and wetting cycles was also explored.

The surface soils (0-20 cm) showed a negative lineal association between allophane content and nitrogen mineralization, indicating the depressing effect of allophane. On the average, mineralization in andosols, was significantly inferior than in the other soils studied. Problems can be expected in soils with over approximately 15% crude allophane.

The drying-wetting treatment generally increased N mineralization particularity for fine-textured soils.

The organic matter addition had no clear effect while liming, using either $CaCO_3$ or $Ca(OH)_2$, reduced nitrogen mineralization effectively.

One andosol behaved differently from the other three, showing a higher mineralization than could be expected.

It was concluded that nitrogen will have to be included in the fertilizers on these soils, as the very low

nitrogen mineralization in many of them does not put the ample reserves of this element at the disposal of the plants. This same phenomenon, probably caused by adsorption on the large amount of surface, is suggested as an explanation for the frequently observed intensive organic matter accumulation in volcanic ash soils in the tropics.

6. Soil Chemical Properties of Recent Volcanic Ash

Two recent volcanic eruptions in Costa Rica (Irazu between 1963 and 1965 and Arenal in 1968) have supplied raw material for studies on fresh volcanic ash.

As ash such as this was the initial material for many soils in the area and as it affected large, agriculturally important areas, its chemical properties were determined.

It was observed that fresh ash has only a very small cation exchange capacity, it is moderately acid, it does not contain water-soluble P, and does not fix this element. However, there are acid and alkali-soluble phosphates in the ash which probably improve the soils in the long range. Fresh ash is also a quite good source of soluble cations. Arenal ash is a better source than Irazu ash. Apparently, soluble cation content decreases with the distance of sampling from site of emission.

The content of both soluble and exchangeable Mn is very low in all samples.

Both deposits are good sources of sulfates, but Arenal ash has a higher content than Irazu ash. Arenal ash retains its sulfate also somewhat more efficiently than Irazu ash.

Both ashes are sources of small amounts of soluble silicates. The solubilization of silicate increases when the other anions get washed out. This indicates that after a moderate period, silicates are a very important anion in the leachates of recent ash. They might be responsible also for the intensive hardening of the top ash layer observed in both cases. This layer, which can be as hard as 2 mg/cm^2, and is little permeable to water, caused the intensive runoff which resulted in considerable damage at the foot of Mt. Irazu.

Recent ash has no nitrogen. This is the main limitation of its fertility. However, if nitrogen is added, ash contributes appreciably to soil fertility as shown by the excellent crops on farms where N was applied during the years following the ash deposition.

7. Study of the Spatial Distribution of the Absorbing Roots in Coffee

Because of the economic importance of coffee in many Latin American countries, a large quantity of fertilizers is usually applied annually to the coffee trees to insure a good production of this export crop. By using radio-tracers (e.g. Rb-86), it is possible to study the distribution of the roots which actively absorb the nutrients. The result obtained many prove to be useful for fertilizer application. It was found that most of the actively absorbing roots are near the surface and relatively close to the tree trunk.

E. INSTALLATION OF RADIATION FACILITIES

In 1957, the first gamma field was installed in Latin America (at Turrialba, Costa Rica). The original radiation source was 200 curies of cobalt-60. Because of the relatively short half life of cobolt-60 (5.3 years), the source was replaced by 1,300 curies of cesium-137 (half life, 30 years).in 1961. The dosimentry of this new source in the gamma field was also determined.

In 1962, a water pool irradiator was built in Turrialba for seed and insect irradiation. This gamma pool contained 1,800 curies of cobalt-60 and permitted simultaneous irradiation of five samples. This facility was mechanically operated and has given no problems during the last fourteen years of operation. Because of the fluctuation of electrical voltage and current supply, and the difficulties of obtaining technical service, X-ray machines are not a practical radiation set-up in many developing countries. The gamma pool is superior by far.

F. COLLECTION OR RAINFALL FOR FALLOUT ANALYSIS

Since August, 1959, in cooperation with the Health and Safety Laboratory (HASL) of the U.S. Atomic Energy Commission, monthly fallout collections by using ion-exchange columns have been carried out at Turrialba. The resin columns and the monthly rainfall records were sent to HASL for radiochemical analysis.

II. TRAINING

In the training aspects of this project, the staff members gave seminars, or lectures as to the use of nuclear energy in agriculture. In addition, the staff members directed the research of students for their thesis work at the M.S. level. It has been considered that the Research and Training Center at Turrialba is a good stepping-stone to further higher learning in the universities of the U.S. for the Latin American students. A total of 82 students from 17 different countries have received their training during this project period by means of attending short courses in the use of radioisotopes, or conducting research as a research assistant under the senior scientists. Thirty-seven of them (45%) received M.S. degrees from our Institute, and 17 of them (21%) have received Ph.D. degrees from the universities in the U.S. (15), Canada (1), and England (1).

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IV EVALUATION OF THE PROJECT

As has been pointed out previously, this project was part of the Atoms-for-Peace Program proposed by ex-President Eisenhower before the United Nations in 1953. The objectives of the project were two-fold: to carry out agricultural research in the tropics, using nuclear energy as a tool, and to train students from Latin America in the use and application of radiation technique to agriculture. If we look at the tropical belt of the world, -- the geographical region situated between the tropics of Cancer and Capricorn -- almost all the nations located in this area are developing countries and are agriculturally dependent. Very little biological research has been done in the tropics, and the knowledge obtained from the temperate areas usually cannot apply to the tropical phenomena. A research and training project of this nature would have a great impact on the developing nations.

In investigating the effects of ionizing radiation on tropical plant species, a few species (Coffea and Manihot) had an unusual morphological response. The mutant character appearing in the R_1 plant was not a chimeric change, but rather the whole plant produced a similar mutant characteristic. As a rule, the shoot apex of higher plants is a multicellular system, chimera is expected to be induced. The lack of chimeric production indicates that in these tropical plant species, the whole plant could develop from one or two initial cells in the apex. This unusual characteristic would greatly facilitate the mutation breeding in these tropical plants.

The common bean is a basic food in the American tropics.

It provides a major protein source in the diet of the people and in some areas, it is almost the only source of protein intake. The mutation work in this project has demonstrated the feasibility of converting an undesirable character into a desirable one (e.g. seed-coat color mutants) and also the feasibility of inducing high protein mutants (e.g. Bayo-2 mutant). In some aspects, we have also demonstrated that mutation breeding is by far a more efficient method than the conventional breeding methods (e.g. to eliminate the mottling gene in the pinto bean or to induce white bean mutants). A white bean mutant has thus been developed by mutation breeding technique. This mutant has a high value of protein efficiency ratio (PER) as well as other good chemical properties. Seeds of this mutant have been sent to 34 institutions in 20 countries for field trials. This is an example of the use of nuclear energy for agriculture improvement which certainly has a great impact on the developing countries. Students from Brazil and Peru have come to our program to learn the mutation breeding method for their plant improvement.

In the tropics, insect pests and plant diseases usually cause severe damage in agriculture. One of the most destructive insects is Mediterranean fruit fly. This insect was first reported in Costa Rica in 1955 and has gradually moved northward. The research on the feasibility of using male sterilization technique for the fly eradication in our laboratory has catalyzed the interest of several international organizations, and led to the development of an international cooperative project for the Medfly control research. In 1965, a Central

American institute (OIRSA) received support of about one million dollars from the United Nations special funds and the IAEA to further study the feasibility of using the sterilization technique for medfly control in a field trial. A pilot test in Nicaragua showed that a 90 percent reduction in fly infestation in the sterile fly release area. The result demonstrated the effectiveness of controlling the medfies by the sterilization method. However, a team sponsoved by the USAID made an evaluation and indicated that a total eradication of the medflies in Central America would cost about 40 million dollars. At present, no sponsor is available to support this total eradication project, but the methods for the control and the research results are always available once they are needed.

Plant diseases are also destructive to the agricultre of the American tropics. Studies on banana fruit rot gave us an understanding of the host-pathogen relationship in this disease of this importan export crop, and allowed a more rational means for the disease control.

Several tropical crops have a phenomenal yield. We have studied the photosynthetic efficency and the translocation of photosynthetic products in cassava, in an effort to understand the reason for high production of carbohydrates in this root crop. Due to the energy crisis at the present time, it is of significance to discover the highly productive plants which may be used as a substitute for part of the energy demand. Sugar cane is another tropical crop which should be looked into, because of its high sugar production (alcohol source)

and the usefuness of its residue as a fuel.

Many soils in the American tropics have a very high phosphate-fixing capacity. Improper application of fertilizers usually causes large losses on a farm. Our studies on foliar nutrition, ion movements in the soils, element uptakes by plants, and the distribution of absorbing roots in soil have offered a more efficient method for the application of fertilizers.

In the training aspects, 82 students from 17 Latin American Countries were trained through our Nuclear Energy Program. Thirty-Seven received M.S. degrees from the Institute and 17 received Ph.D. degrees from universities in the U.S. and other countries. When they returned to their native countries, they either continued their academic work in the universities or occupied significant positions in their government offices. Thus, this project has established an excellent academic relationship with the people of Latin America.