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ANNUAL REPORT

INTER-AMERICAN INSTITUTE OF AGRICULTURAL SCIENCES OF THE OAS 1 JULY 1970

Training and Research Center

Turrialba, Costa Rica

Page 1 of 1

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THE APPLICATION OF NUCLEAR ENERGY TO AGRICULTURE

Annual Report
to the
United States Atomic Energy Commission
Contract AT(30-1)-2043

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Prepared by
Carl C. Moh

Inter-American Institute of Agricultural Sciences of the O.A.S.
Training and Research Center
Turrialba, Costa Rica

July 1, 1970

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-
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1-18
~~RESEARCH~~

~~A.~~ RADIATION BOTANY AND PLANT GENETICS

1. ~~Radiosensitivity of Tropical Plant Species~~
(C. C. Moh and J. J. Alan)

Purpose and methods

The objective of this investigation is to study the radiosensitivity range of tropical plants in a plant family. A more detailed description of the objective has been presented in previous reports. Plant species from American tropical areas are collected and the young plants are exposed to chronic irradiation in the gamma field to study the maximum daily dose that a species can tolerate. The criterion of tolerance is indicated either by the asexually propagated species that can survive a lengthy period (a year or more) under continuous daily irradiation or by the sexually propagated species that can complete their life cycle by producing viable seeds.

Progress report

This year, we have studied the radiosensitivity of two plant families. A total of 44 species from 16 genera in Leguminosae (Pea Family) and a total of 18 species from 6 genera in Convolvulaceae (Morning-glory Family) were investigated. The radiosensitivity results of some species have been reported in previous years. The results on those species completed this year or which will require more time to complete are represented in Fig. 1.

So far the data obtained are suggestive and permit us to draw a tentative conclusion. In Leguminosae, a wide range of radiosensitivity is found among the species. Vicia faba probably can complete its life cycle at a daily dose of less than 25 r, while Dolichos lablab, Mimosa pudica, and Vigna sesquipedalis are seven or more times more resistant than the Vicia. In Convolvulaceae, almost all the species tested are radioreistant, especially Ipomea batatas (sweet potatoes) and Operculina tuberosa (wood rose) which can continuously produce vegetative growth at 300 r per day for a year of exposure. The species of Convolvulaceae are a group of radioresistant plants.

Table 1 summarizes the results on the range of radiosensitivity in the plant families so far obtained.

2. Mutagenic Effects of Cycasin on Higher Plants (C. C. Moh)

Toxicity of Cycads and Cycasin

Cycasin is a chemical compound, methylazoxymethanol- β -D-glucoside, occurring naturally in the cycad plants. The cycads belong to the family Cycadaceae of the Gymnosperms and are widely distributed in the tropical and subtropical regions. The plants are used as ornamentals, as animal feed, as medicine, and as human food. The toxicity of cycads has long been known as in some cases the induction of illnesses or death in animals and humans can be traced to the ingestion of the cycad

CONVOLVULACEAE

FIG. 1. GROWTH RESPONSE OF THE TROPICAL SPECIES IN CONVULVULACEAE AND LEGUMINOSAE TO CHRONIC GAMMA RADIATION. DISTANCE FROM THE CESIUM-137 SOURCE AND THE DOSIMETRY IN THE GAMMA FIELD ARE IN LOG SCALE.

FIG. 1. (CONT.) GROWTH RESPONSE OF THE TROPICAL SPECIES IN CONVULVULACEAE AND LEGUMINOSAE TO CHRONIC GAMMA RADIATION.
 DISTANCE FROM THE CESIUM-137 SOURCE AND THE DOSIMETRY IN THE GAMMA FIELD ARE IN LOG SCALE.

Table 1. Radiosensitivity range of the tropical plant species in Gymnosperms and other plant families

Plant group	No. of genera studied	No. of species studied	Range of maximum doses the species can tolerate or complete the life cycle (r per day)	Radiosensitivity classification
Gymnosperms	5	5	5 (Pinus)- 27 (Cycas)	sensitive
Convolvulaceae	6	18	183 (Quanoelit)- 218 (Ipomea)	resistant
Leguminosae	16	44	25 (Vicia)- 208 (Dolichos)	wide range

or its products (11). In more recent years, because of the high incidence of neurologic diseases occurring on Guam and other islands of the Pacific where the indigenous inhabitants use cycads as a source of food (6, 11), studies on the chemistry of cycads and the biological and pathological effects have proceeded rapidly. It has been demonstrated that the tissues of cycads, or the cycasin, the compound isolated from the tissues, are toxic and carcinogenic in a wide variety of animals (6), and radiomimetic in Allium chromosomes (9).

Further chemical studies and biological tests revealed that the aglycone of cycasin, methylazoxymethanol, is the toxic component (1, 3). Cycasin produces its toxic effects in a biological system only when the cycasin is hydrolyzed by the enzyme,

β -glucosidase, to release its aglycone. Thus, cycasin produced carcinogenic effects in conventional rats but not in germfree rats which lack the glucosidase (2). A similar explanation can be applied to the experimental results that cycasin per se induced no mutations in Salmonella (7) and Drosophila (10), but its aglycone was a potent mutagen.

Finally, it may be pointed out that two events, one historical and the other recent, may have an implication on cycad toxicity.

1) The cycads, in an evolutionary sense, are considered to be ancestral to the flowering plants and predominated over the vegetation during the Mesozoic Era (135 to 200 million years ago or called Age of Cycads). The extinction of dinosaurs was at the end of this era (8), when the cycads thrived. Whether the toxicity of cycads played a role in dinosaur extinction or it was just a coincidence is an interesting speculation.

2) In a recent clinical survey in the El Progreso area on the North Coast of Honduras, Mulholland (5) reported that the prevalence of anemia (hemoglobin 7.5 gm or less) was 24-52% in the villages, and the high recurrence rate among the anemic patients whose treatment was apparently successful for temporary arrest suggested that there are other factors for causing the illness. His survey continued to indicate that: "The residents of the villages with prominent prevalence of anemia believed

that a toxin from a plant called Camotillo (botanically identified as Zamia furfuracea, a member of the Cycadaceae family) contaminates their water supply. This plant grows in the mountains on the banks of the streams from which these villages draw their water directly. The main tributary from the mountains (Guaymas River) flows directly to Guaymites (anemia prevalence: 52%); more distant branches reach Toyos (anemia: 29%) which is 13 km to the north, the Camatote (anemia: 28%) which is 11 km to the south of Guaymitas." The implication of cycad toxicity is worthy of note.

Purpose and Methods

In the present study, the mutagenic effect of cycasin on higher plants is reported. It is known that many plants contain emulsins that are capable of splitting the cycasin into the aglycone (9). Mutagenic effects are expected to be obtained by direct application of the cycasin to the plant materials.

The common bean (Phaseolus vulgaris L.) was used as an experimental material. Besides its agricultural importance in the American tropics, its short life cycle, self-pollinated nature, and diversified genetic characters provide good botanical properties for mutation studies. The β -glucosidase activity in the bean variety (San Fernando) used for the present experiments was tested by Dr. H. J. Teas of the University

of Miami, Florida, U.S.A. Although the activity was not extraordinarily high, its presence was sufficient to warrant the performance of the experiments.

Newly harvested bean seeds, dried in an oven at 34°C for 2 weeks, were treated with cycasin solution of various concentrations at 20°C for 24 hrs. Fifty seeds were used in each treatment and the concentration of cycasin was prepared in a 10-ml aqueous solution with 0.25, 0.50, 0.75 and 1.00 mg of cycasin per seed. At the end of the treatment period, all 10 ml of cycasin solution were absorbed by the 50 seeds. The seeds were then rinsed several times with water and grown in the greenhouse. At maturity, the seeds from each treated plant were harvested separately and were grown in the soil box, one plant per row, for observation of the induced mutations. The observation was carried out for 2 weeks of the seedling period. According to our previous mutagenic experiments, most of the induced mutations, including both chlorophyll and morphological types, can be detected within this period. The pooled data on four identical experiments in which the M_1 plants were grown in the greenhouse are presented in Table 2.

In the experiment in which the M_1 plants were grown in the field, the seeds were treated in the same manner as described above, except that a total of 300 seeds were treated in each concentration of 60 ml cycasin solution. The mutation study was also conducted as described above.

Table 2. Survival rate and mutation frequency induced by cycasin in Phaseolus vulgaris L.

Treatment mg/seed	Number of seeds treated	M ₁ surviving plants		Mutations*	
		number	% of control	number	%
Greenhouse grown					
Control	200	183	100.0	0	0
0.25	200	171	93.4	78	45.6
0.50	200	99	54.1	57	57.6
0.75	200	66	36.1	41	62.1
1.00	200	44	24.0	25	56.1
Field grown					
Control	300	240	100.0	0	0
0.25	300	150	62.5	72	48.0
0.50	300	35	14.6	20	57.1
0.75	300	11	4.6	8	72.7

* The induced bean mutations can generally be classified into two categories: Chlorophyll and morphological. Chlorophyll mutations are those which change the leaf color. For example, yellow-green, yellow, albino, virescent, mottling, shrivel and variegate are included in this category. The morphological mutations are those which change the shape, size, growth pattern, or growth habit of an organ or the whole plant. Examples of these mutations are elongated or wrinkle leaf, dwarf, miniature, vine, etc.

Progress Report

Table 2 summarizes the results on the survivals and mutation frequencies in the bean plants treated with various concentrations of cycasin solution. The number of survivals decreased as the concentration of cycasin increased. The LD₅₀ was about 0.50 mg per seed for the greenhouse experiment and

0.25-0.50 mg per seed for the field experiment. Field grown materials usually exhibit a lower rate of survival because of unfavorable growth conditions. In both experiments, the toxic effect of cycasin on beans, as reflected by the survival rate, is evident.

The mutation frequencies resulting from seed treatments with cycasin were very high (Table 2). As compared with our previous experiments with EMS, the mutation frequency induced by cycasin was at least as high, if not statistically significantly higher, than that induced by EMS. It appears that cycasin is a potent mutagen in higher plants and can be applied directly to the plant materials for inducing mutations providing that the plant has β -glucosidase activity to release the active mutagenic component, the aglycone, from cycasin. Matsumoto and Higa have demonstrated that the aglycone is a strong methylating agent with 7-methylguanine being formed when it reacts with both DNA and RNA (4). The high mutation rate may be attributed to the ease of methylating the genetic materials by the aglycone.

Experiments are being continued to study the mutation spectrum and the genetic behavior of the cycasin induced mutations.

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3. Induced Somatic Mutations in Manihot
(C. C. Moh and J. J. Alan)

Purpose and Methods

Many tropical plant species, some of them serving as most important economic and food crops, are vegetatively propagated. In these species, the induction of somatic mutations is a method of considerable potential value for plant improvement. Several years ago, preliminary work with Manihot was initiated in our program. More than 80 Manihot cultivars were collected from different areas of the American tropics. A study of the botanical characters and cytological observation of these cultivars has been carried out and provided a basis for selecting the cultivars for somatic mutation induction. Because of the separation of the personnel, work on somatic mutation had been temporarily halted. This year, this line of work is being continued.

Manihot serves as an important source of carbohydrates in the tropics and is used as a major food crop in many areas. Most Manihot species are monoecious and can be propagated vegetatively as well as sexually. Because Manihot cuttings can grow easily, the traditional method for cultivation is by asexual means. Usually a foot-long cutting is buried in the ground and a plant will produce an average of 10 lbs. of fresh roots at maturity without much care. A production of 30 tons of fresh roots per acre has been reported by Dutch

agriculturists in Indonesia. Sometimes Manihot is called as "a lazy man's crop".

Several approaches for increasing the somatic mutation rate in Manihot have been proposed in the project proposal of the current year. The results presented below were obtained from the preliminary studies of effects of radiation on Manihot which serve as the basic information for carrying out further experimentation.

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- a) Effect of gamma radiation and EMS treatment on the shoot apex of Manihot cutting.

In this study, effectiveness of the EMS and the radiation treatments was compared. Foot-long cuttings with the lower 5-inch section protected by a 1/8-inch of lead were irradiated with 3 kr of gamma radiation. The buds of similar length cuttings were treated with 0.04 M EMS for 24 hours. Somatic damage of leaf primordium cells was severe in the radiation treatment. However, no somatic damage induced by EMS was noted. Probably the primordium is situated under a thick epidermal layer and the EMS does not penetrate to the primordia to induce the effects. Unless another effective treatment method is found, use of gamma radiation is by far a better method for inducing somatic mutations in Manihot cuttings.

b) Radiosensitivity of the shoot apex.

A preliminary test showed that the buds of Manihot are rather sensitive to gamma radiation. The cuttings were irradiated with a range of radiation doses from 2 to 6 kr. At a dose of 6 kr, no growth of the shoot apex could be recognized after four months of sowing. Apparently this is a complete lethal dose for the Manihot buds. At 4 kr, the sprouting was delayed for a month and the new grown leaves were highly mottled and deformed. Chlorophyll-deficient chimaeras were also induced. At 2 kr, there was no appreciable growth inhibition, but a few chlorophyll-deficient sectors were noted. It appears that 4-5 kr is an optimal dose for inducing somatic mutations study.

c) Propagation of the Manihot cultivars.

Since most Manihot are monoecious and are cross-pollinated, a high degree of heterozygosity among the cultivars is expected. This thought is supported by our previous cytological examinations of the cultivars that not many have a normal meiotic pairing of the chromosomes. On the contrary, many have chromosomal structural changes of various types and degrees. The somatic mutation rate could vary from cultivar to cultivar. Many cultivars from single plant selection are being propagated in the field. The somatic mutation rate of the cultivars will be presented in the next report.

4. Summary of the Mutation Breeding Method in the Seed-Coat Color of Beans.
(C. C. Moh)

a) The development of the breeding method

The success of induced mutation method for plant breeding is largely conditioned by the following two factors. First, it depends upon whether a plant species has the genetic potential to mutate to the desirable traits. Secondly, it depends upon the ease of detecting the desirable mutants. For the first, knowledge on the genetic constitution of the species and information on the mutability of the desirable traits are essential. For the second, an efficient screening technique for isolating the induced desirable mutants is helpful. It is known that the seed-coat of beans has a wide range of color variations in the natural population. During the past years, while working on the induced mutations in beans with various mutagens, it was noted that the genetic factors controlling seed-coat color were mutable. Since the seed color is a major agronomic character to determine the acceptability of a bean variety for consumption, it appears that induced mutation technique provides a promising method for the seed color breeding.

The development of the screening technique for the seed colors was based on the observation of the plant characters of several hundred bean varieties collected in Latin America. A correlation has been established between the seed color and the hypocotyl color of early seedling stage. Essentially, the seed

colors can be divided into four principal groups: black, bayo (from deep brown to light yellow), red, and white. The correlation between the seed color and the hypocotyl color can be seen in Table 3. All the black varieties observed produced red hypocotyl, and all the white varieties produced green hypocotyl. The bayo and red varieties could produce either red or green hypocotyls. This correlation provides a criterion to detect the possible seed color mutants induced from a black variety.

Table 3. Correlation between the seed-coat colors and the hypocotyl colors of 271 bean varieties.*

Seed-coat color	Hypocotyl color		Total varieties
	red	green	
black	93	0	93
bayo	38	21	59
red	54	22	76
white	0	43	43
Total varieties	185	87	271

* A test of independence showed that the total $\chi^2 = 108.56$, P value is highly significant.

b) The screening procedure

In this section, the procedure of the screening method is summarized in steps for breeding practices.

1. The R_1 plants are harvested individually after the mutagenic treatment. The selfed progenies of an R_1 plant are sown in a row in a soil box. A 3 x 0.7 m box is sufficient for 40 rows for the selfed progeny test.
2. When the hypocotyl hooks of the seedlings begin to emerge, normally 4 to 5 days after sowing, the mutants with a change in hypocotyl color from red to green or to light pink are marked for transplanting.
3. When the selected mutants are a week old and the first trifoliate leaf begins to appear, they are transplanted to a soil pot for further growth observation.
4. According to our experimental records, some mutants such as some dwarfs or miniatures are also associated with a hypocotyl color change. Those considered as deleterious ones can be discarded shortly. Only those with normal appearance and growth are maintained to maturity.
5. When the mutant plants mature, the seeds are examined for their color identification.

c) The advantage and weakness of this method

During the last year, we have obtained 6 white and 28 bayo (covering a wide range of brown to yellow color) seed mutants from different black seed varieties. Obviously, it is an

effective breeding technique for seed-coat colors of beans. As compared with the conventional hybridization method, this mutation breeding technique has the following advantages:

- 1- Most of the induced mutant characters are of a recessive nature and are inherited in a simple Mendelian manner. For plant varieties which require one or two genic changes to improve their agronomic standing, such as the present case of seed color improvement, mutation breeding can produce the results with a minimum of expense and effort.
- 2- The bean flowers are a rather delicate structure for emasculation and the seed set from crosses usually is very low. Mutation breeding eliminates the technical difficulties for making hybridization.
- 3- A homozygous line can be established quickly for the field trial.

The weakness of mutation breeding is that a desirable mutant is not always procurable. In the present case, a red seed mutant has not been obtained although mutants covering a wide range of other colors have been isolated with relative ease. Another problem commonly encountered in the mutation breeding is that undesirable pleiotropic effects or semi-sterility are induced in the mutants. Under these conditions, a purification of the mutant line is necessary before it can be used for the field trial.

B. ENTOMOLOGY

1. Sterilization of the Mediterranean Fruit Fly and Its Application to Fly Eradication (K. P. Katiyar and E. Ramírez)

a. Mating competitiveness of Medfly males irradiated during pupal or adult stage

Purpose and Methods

In the pilot test carried out in 48 sq. km. in Carazo, Nicaragua, by the Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA), in cooperation with the International Atomic Energy Agency (IAEA), to evaluate the feasibility of Medfly control or eradication by the sterile male method, the flies were irradiated at pupal stage 48-24 hrs before emergence. In our 1964 annual report, we had reported that the mating vigor (insemination efficiency) of 10 kr irradiated males is affected by the pupal stage at which irradiation is applied. The closer to adult emergence that the pupae are irradiated, the higher the insemination efficiency of the treated males.

Last year, some experiments were carried out to compare the mating competitiveness of Medfly males irradiated 24 hrs before or 24 hrs after emergence. The tests were carried out at a 40:1 ratio (sterile:normal adults), and the results showed no difference in the mating competitiveness of males irradiated as pupae or as adults. Further studies were carried out this year to determine if males irradiated as adults are more competitive than those irradiated at the pupal stage.

Tests were carried out in the laboratory at $25 \pm 3^{\circ}\text{C}$ temperature and $75 \pm 5\%$ relative humidity in 1 ft^3 wooden frame cages covered with plastic screens. The sterile males used in the experiment were irradiated with 10 kr. The adults were sexed out within 12 hrs after emergence. The sterile and normal flies were put together in the cages 48 hrs after adult emergence.

Mating competitiveness of sterile males irradiated at three different stages (as pupae 24 hrs prior to emergence and as adults 24 hrs or 48 hrs after emergence) was tested by caging them with normal males and normal females. The mating competitiveness of each type of male was tested at three different ratios: 1:1:1, 2:1:1 and 3:1:1 (sterile male:normal male:normal female). The test included two controls to check the fertility of irradiated and non-irradiated males. Each cage had 50 normal females per ratio.

The egg collection started when adults were 5 days old and it was continued until the flies were 4 weeks old. Eggs were collected 5 times a week. Each sample consisted of 200-400 eggs. The experiment was repeated 5 times, each time with different batches of pupae.

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The percent egg viability of the mixed fly populations of sterile males (irradiated as pupae, 24 hrs before emergence, or as adults 24 or 48 hrs after emergence), normal males and normal females, is summarized in Table 4. The results, in general, do

Table 4. Mating competitiveness of Medfly males irradiated with 10 kr as pupae (24 hrs before emergence) or as adults (24 or 48 hrs after emergence).

Sex ratios ^{a/}	Female fertility (%) ^{b/} when caged with males treated as		
	24 hrs pre-emergent	24 hrs post-emergent	48 hrs post-emergent
0 : 1 : 1	92.9 (25,063) ^{c/}	90.7 (26,795)	93.1 (24,760)
1 : 0 : 1	0.7 (27,348)	0.4 (23,788)	0.2 (26,641)
1 : 1 : 1	61.5 (27,512)	66.0 (25,678)	59.2 (26,603)
2 : 1 : 1	49.9 (26,208)	45.4 (27,246)	43.3 (26,615)
3 : 1 : 1	46.1 (26,157)	41.2 (27,488)	42.2 (27,242)

a/ Treated ♂ : untreated ♂ : untreated ♀ (50 ♀/ratio).

b/ Average for 6 replicates.

c/ Figures in parenthesis are total number of eggs examined on which percent egg-hatch is based.

not show any marked mating competitiveness superiority of males irradiated as adults (24 or 48 hrs after emergence) over those irradiated as pupae (24 hrs prior to emergence). A slightly better mating competitiveness of males irradiated as adults over those irradiated as pupae is found when the tests were carried out at 2:1:1 and 3:1:1 ratios of sterile male:normal male:normal female. At 2:1:1 ratio of treated male:normal male:normal female, the mean egg hatch were 49.9%, 45.4% and 43.3%, respectively, when males were irradiated 24 hrs prior to eclosion and 24 or 48 hrs post-eclosion. Similarly at 3:1:1 ratio, the mean egg viability of normal females was slightly lower when caged with sterile males irradiated as adults than with those irradiated as pupae. At 3:1:1 ratio, the percent egg-hatch of 46.1%, 41.2% and 42.2%, respectively, were found in cages having sterile males irradiated as 24 hrs pre-emergent, 24 hrs post-emergent and 48 hrs post-emergent.

b. Insemination frequency of Medfly males irradiated during pupal or adult stage

Purpose and Methods

One of the basic requirements for the successful application of the sterile male technique to control an insect is that irradiated males should reasonably compete in mating with normal males. In a test designed to measure the insemination capacity of irradiated males, we found that the mating vigor of males irradiated with 10 kr at pupal stage was affected by the stage of development

at which irradiation was applied. The closer to the adult emergence that the pupae were irradiated, the higher the insemination frequency of the treated males. The sterilization dose of 10 kr seems to kill all germ cells except spermatids and sperm, which are later utilized by the treated adults for inseminating the females. The advanced stage male pupae are expected to have greater portion of their testes full of spermatids and sperm. Consequently, males irradiated at a later pupal stage would have greater insemination capacity than those treated at an early stage. Based upon the above information, it seems that the mating vigor of the treated males can be increased further by irradiating the adult stage. The present experiment was carried out to see if mating vigor (insemination efficiency) of the irradiated males can be increased by applying radiation to the adult stage (24 and 48 hrs after emergence).

The insemination capacity of males irradiated at three stages of development (24 hrs before eclosion, 24 hrs after eclosion and 48 hrs after eclosion) was compared with untreated males. Sterilization dose of 10 kr was chosen as this dose induces more than 99.9% dominant lethal mutations in the sperm of treated males. The adults used in the experiment were sexed within 12 hrs after emergence and kept in separate cages until used.

Insemination capacity of males of each treatment were measured by confining an individual male with six virgin females in a small cage for 48 hrs. Then, females from surviving males were removed

and their spermathecae were dissected out and examined for the presence of sperm under a compound microscope. The females which carried at least a trace of sperm in spermathecae were scored as inseminated. In the Medfly female, motile sperm was found in the spermathecae 24 hrs after adult's death. Each surviving male was given six virgin females for another 48 hr period of insemination. This mating procedure was repeated until males were 3 weeks old. At this time, most of the males had died in all the treatments. Each treatment had 15 males at the beginning of the test. The age of the females used in the experiment varied from 3-8 days.

The experiment was carried out in the laboratory at $25 \pm 3^{\circ}\text{C}$ temperature and $75 \pm 5\%$ relative humidity. The males were given females for insemination for the first time at the age of 3 days.

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Table 5 presents the average number of females inseminated per male during nine mating series by different types of sterile males (irradiated as pupae or as adults) and untreated males. The results indicate that 1) irradiation reduces the insemination vigor of the treated males; 2) males irradiated at adult stage (24 or 48 hrs after eclosion) have slightly greater insemination capacity than those irradiated as pupae (24 hrs before eclosion). During 3 weeks of adult life a normal male inseminated an average of 21.6 females compared to 9.9, 13.8 and 14.8 females inseminated by males irradiated as 24 hrs

Table 5. Consecutive insemination of Medfly females caged for 2 days with 10 kr irradiated males at a ratio of 6:1

Mating series	Male age (days)	Av. number of females inseminated (per male) in different treatments			
		Normal	24 hrs pre-emergant	24 hrs post-emergant	48 hrs post-emergant
1	3 - 4	3.5 (12) ^{a/}	2.7 (15)	3.4 (15)	3.1 (15)
2	5 - 6	2.4 (10)	2.1 (14)	3.5 (13)	2.8 (13)
3	7 - 8	2.7 (9)	1.5 (12)	2.2 (13)	2.1 (11)
4	9 - 10	3.1 (8)	0.6 (11)	1.4 (9)	2.8 (8)
5	11 - 12	3.4 (8)	1.7 (9)	1.4 (7)	2.6 (7)
6	13 - 14	2.6 (5)	1.1 (8)	0.4 (5)	0.7 (6)
7	15 - 16	2.2 (5)	0.2 (6)	1.5 (2)	0.5 (4)
8	17 - 18	1.0 (4)	0.0 (4)	0.0 (2)	0.2 (4)
9	19 - 21	0.7 (3)	0.0 (3)	0.0 (1)	0.0 (4)
Total for 21 days		21.6	9.9	13.8	14.8

^{a/} Figures in parenthesis are number of males used for matings.

pre-emergent, 24 hrs post-emergent and 48 hrs post-emergent, respectively.

The maximum number of females inseminated by an individual male during 3 weeks adult life were 30, 19, 19, and 20, respectively, for untreated males and males irradiated (as pupae 24 hrs before emergence or as adults 24 or 48 hrs after emergence).

- c. Laboratory studies to evaluate sexual mating competitiveness of Medfly males irradiated at various dosages

Purpose and Methods

Sterilization of the Medfly at pupal stage (1-2 days before adult emergence) with 10 kr induces more than 99.9% dominant lethal mutations in sperm of treated males. But males so treated do not compete in mating with normal males. It is believed that perhaps lowering the sterilization dose from 10 kr (inducing almost 100% sterility in males) to sub-sterilization doses (leaving up to 10.0% fertility in treated males), the sexual vigor of the males can be increased. Last year some experiments were done with 5 kr, 7 kr, 9 kr and 11 kr dosages at a 5:1 ratio (treated:untreated adults). The results did not show any increase in the mating vigor (based on egg viability) of the males treated with low sterilization doses. This year, in cooperation with Dr. C. H. Hooper of Siebersdorf Laboratory of the IAEA, further experiments were carried out to compare the mating competitiveness of Medfly males irradiated with gamma irradiation with various dosages. Results of the experiments carried out at

Turrialba laboratory are presented here.

Mating competitiveness of sterile males (irradiated at four different sterilization dosages: 5 kr, 7 kr, 9 kr, and 11 kr) with untreated males was tested at a ratio of 9:1:1 (432 sterile males:48 normal males:48 normal females). Further evaluation on sexual vigor of males treated with 5 kr and 11 kr was carried out at 19:1:1 ratio (475 sterile males:25 normal males:25 normal females). The experiment included one control of untreated flies at 10:1 ratio (480 normal males:48 normal females).

Males were irradiated with OIRSA's ^{60}Co source at dose rate or 10,500 r/min at pupal stage (24-48 hrs before emergence). All the flies used in the experiment were sexed (under the influence of CO_2) within 12 hrs after emergence.

Irradiated and normal flies were caged together immediately after sexing. Perforated quart-sized polyethylene round freezer jars were used for oviposition. First egg collection was made when flies were 5 days old and it was continued until adults were 3 weeks old. Eggs were collected 5 days per week. Individual egg sample consisted of 200-400 eggs.

Adults were confined in 1 ft³ wooden frame cages covered with plastic screens. The experiment was replicated seven times. Each replicate was set on a different day with a different batch of pupae. All the tests were performed in the laboratory at $25 \pm 3^\circ\text{C}$ temperature and $75 \pm 5\%$ relative humidity. The summarized results of this experiment are presented in Table 6.

Table 6. Mating competitiveness of Medfly males irradiated at various dosages with gamma radiation at 24 hrs before adult emergence.

Treat- ment	Adult population ^{a/}	% egg-hatch in different replications						Average % egg-hatch	
		1	2	3	4	5	6		7
11 kr	432: 48: 48	32.7 (3518) ^{b/}	22.1 (3358)	27.3 (3001)	51.1 (2174)	21.4 (2792)	38.8 (2749)	16.7 (2819)	30.0
9 kr	432: 48: 48	24.6 (3472)	17.5 (3132)	31.3 (3376)	26.2 (2924)	26.7 (2724)	29.8 (2753)	26.6 (3446)	25.8
7 kr	432: 48: 48	26.5 (3435)	32.1 (2966)	23.7 (3203)	25.5 (2592)	35.2 (3174)	14.2 (2758)	18.0 (3351)	25.0
5 kr	432: 48: 48	36.5 (3327)	27.6 (2852)	26.0 (3059)	16.9 (1492)	22.2 (3158)	34.9 (2364)	26.0 (3247)	27.2
11 kr	475: 25: 25	17.6 (3310)	25.3 (2883)	16.6 (1897)	27.8 (1610)	5.8 (1530)	22.6 (2393)	23.3 (2663)	19.9
5 kr	475: 25: 25	17.7 (2924)	14.6 (2078)	13.9 (2192)	20.6 (1650)	14.6 (2406)	20.7 (2354)	22.2 (2314)	17.7
Check	0:480: 48	92.9 (3343)	93.7 (2459)	93.5 (3039)	95.0 (2343)	84.9 (2209)	92.7 (3122)	91.7 (2922)	92.0

^{a/} Treated males : Normal males : Normal females.

^{b/} Figures in parenthesis are total eggs examined on which the percentages are based.

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Table 6 presents the percent egg-hatch of normal females caged with sterile males (irradiated with 5 kr, 7 kr, 9 kr and 11 kr) and normal males in a ratio of 9:1:1 (sterile male:normal male:normal female). The results indicate that low sterilization dosages (tested at 9:1 and 19:1 ratios of sterile males:normal males) do not increase the mating competitiveness (based on % egg-hatch) of irradiated males. Ratio test of 9:1 (sterile males:normal males) gave an average of 30.0%, 25.8%, 25.0% and 27.2% egg viability when sterile males were irradiated with 11 kr, 9 kr, 7 kr and 5 kr, respectively. Similarly ratio test of 19:1 (sterile males:normal males) did not show any marked difference in mating competitiveness of males irradiated with 11 kr or 5 kr. The mean egg-hatch of the females caged with sterile and normal males (at 19:1 ratio) were 19.9% and 17.7%, respectively, when sterile males were treated with 11 kr and 5 kr dosages, respectively.

- d. Suppression of the reproductive potential of the Medfly by gamma irradiated males in caged coffee trees

Purpose and Methods

At OIRSA laboratory in San Jose it has been noticed that irradiated laboratory strain Medfly males do not equally compete in mating with untreated wild flies. Last year an experiment was carried out in large outdoor cages to measure the mating competitiveness of irradiated laboratory strain adults with normal wild flies. The results of this experiment were inconsistent. Further experiments were, therefore, carried out this year to measure the mating

competitiveness of sterile laboratory strain flies with wild flies in large cages under outdoor conditions.

The mating competitiveness of sterile flies was evaluated at ratios of 40:1, 80:1 and 120:1 (irradiated:untreated). The test included one control with only normal flies. The experiment was carried out in the field in large screen cages (12 ft x 12 ft x 8 ft) erected over coffee trees in fruiting. The cages were erected over coffee trees (4 to 5 trees per cage) with only green berries several weeks before the fly releases in order to avoid the presence of natural infestation on the coffee trees under test.

The first fly release (affected on Sept. 5, 1969) consisted of 40 normal wild flies (20 males and 20 females) per ratio in each cage. The wild flies were obtained from infested tropical almond (Terminalia catapa) fruits collected from the Puntarenas area. In subsequent releases, the number of adults was reduced to half. The flies were released once every week. The number of sterile flies to be released in each treatment was calculated volumetrically (1000 pupae per 17 ml, and 90% adult emergence from the pupae).

The adults were released 12 hrs to 30 hrs after emergence. All the normal flies used for releases were sexed out within 12 hrs after emergence and males and females were kept in separate cages until released. This precaution was taken to avoid any matings of normal females with normal males before release. An

attempt was made to release same age normal and sterile flies.

First 8 releases of sterile flies consisted of adults irradiated with 10 kr and the following 9 releases were affected with 9 kr irradiated adults. Daily protein hydrolysate type-M plus sugar solution in water was sprayed over the coffee foliage to provide food for the adults.

Mature coffee berries were picked up once or twice every week from each cage and were examined for the presence of larvae and unhatched eggs. The unhatched eggs were incubated in the laboratory for 72 hrs on moist filter paper in order to determine egg viability. Maximum of 250 berries were examined at one time from any cage. Unexamined coffee berries were stored for 10-12 days in order to allow the development of hatched larvae to pupae.

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Weekly egg viability data from different treatments are presented in Table 7. The percent egg-hatch in Table 7 is based upon the number of larvae and viable eggs found in the sample. Like previous year, in the present experiment the egg deposition is very poor and erratic. The reason for this oviposition pattern of Medfly females in coffee berries is not known. However, based on mean egg-hatch data over a 17-week period, it seems that sterile laboratory strain flies do not compete equally with normal wild flies. Releases of sterile (both males and females) and normal wild flies (males and females) at a ratio of 120:1

Table 7. Suppression of reproductive potential of Medfly (based on egg-hatchability) by weekly releases^{a/} of gamma sterilized adults (males and females) in various ratios.

Adult ratios	Percent egg-hatch during 17 successive weeks ^{b/}																	Average %
Sterile:Normal	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	egg-hatch
0: 1 ^{c/}	-	83.3	97.0	98.5	-	98.0	100.0	99.6	99.5	97.1	100.0	-	97.9	97.8	100.0	100.0	100.0	98.7
	(0)	(18)	(34)	(66)	(d)	(50)	(117)	(275)	(222)	(138)	(102)	(d)	(49)	(90)	(16)	(8)	(19)	(1204)
40: 1	0.0	0.0	0.0	3.3	0.0	0.0	36.0	45.4	57.4	15.4	4.8	-	-	2.6	0.0	100.0	0.0	19.0
	(5)	(14)	(34)	(30)	(19)	(8)	(25)	(22)	(61)	(78)	(21)	(d)	(0)	(39)	(12)	(2)	(3)	(373)
80: 1	100.0	8.3	10.5	0.0	100.0	4.8	29.4	50.0	50.0	2.7	4.3	100.0	-	0.0	20.0	0.0	-	13.2
	(1)	(12)	(19)	(2)	(2)	(21)	(17)	(16)	(4)	(73)	(23)	(1)	(0)	(4)	(10)	(7)	(0)	(212)
120: 1	0.0	0.0	0.0	0.0	-	0.0	0.0	1.2	0.0	-	-	-	-	14.3	0.0	-	-	1.0
	(11)	(14)	(22)	(8)	(0)	(4)	(40)	(83)	(5)	(0)	(0)	(d)	(0)	(7)	(6)	(0)	(0)	(200)

a/ In each treatment adults were released in cage erected over 4 coffee trees bearing fruits. For the first 8 releases of sterile flies were irradiated with 10 kr and the rest with 9 kr.

b/ Figures in parenthesis indicate total no. eggs examined.

c/ Initial release consisted of 40 flies (20 of each sex) per ratio. In subsequent releases number of flies was reduced by 50%.

d/ Records were not taken.

was most effective in suppressing the reproductive potential of normal flies (based on percent egg-hatch). Such a mixed fly population laid over a 17-week period an average of 1.0% viable eggs. Releases of sterile flies with normal flies at ratios of 40:1 and 80:1 lowered the fertility of normal flies to 19.0% and 13.2%, respectively.

Table 8 presents weekly pupal yield data from different treatments throughout the experimental period of 17 weeks. The pupal yield came from the coffee crop left over in each treatment after the weekly samples of 300-500 berries were taken out for the percent egg-hatch records. The data indicates that maximum suppression in reproductive potential (based on pupal yield) of wild flies by irradiated flies is observed when normal population is over-flooded with sterile flies at a ratio of 1:120. At this over-flooding ratio wild normal flies produced 3 pupae (1.0%) during the 17-week period compared to 288 pupae (100.0%) produced by the same number of normal flies during the same period, but in absence of sterile flies.

Since results of these experiments have been inconsistent during the past two years, next year we plan to repeat this experiment with some modifications.

- e. Effect of gamma sterilization on the mating duration of the Medfly males

Purpose and Methods

Sterile Medfly males do not compete equally in mating with

Table 8. Suppression of reproductive potential of the Medfly, measured in terms of pupal production^{a/}, by weekly releases^{b/} of gamma sterilized adults (males and females) in various ratios

Adult ratio Sterile:	No. pupae produced weekly																	Total pupae obtained	% pupal production
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		
Normal																			
0 : 1 ^{c/}	0	0	0	8	82	1	0	7	18	86	6	44	7	22	1	5	1	288	100.0
40 : 1	0	0	0	0	0	0	1	5	5	17	2	0	1	2	1	0	0	34	11.8
80 : 1	0	1	0	9	2	2	0	3	1	1	2	1	1	2	0	0	0	25	8.7
120 : 1	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	1	0	3	1.0

a/ From the coffee crop left over, after taking out the samples for the egg fertility data.

b/ In each treatment adults were released in cage erected over 4 coffee trees bearing fruits. For first 8 releases of sterile flies were irradiated with 10 kr and the rest with 9 kr.

c/ Initial release consisted of 40 flies (20 of each sex) per ratio. In subsequent releases number of flies was reduced by 50%.

normal males (based on egg viability data of normal females caged together with normal males and sterile males). It is suspected that perhaps during matings sterile males transfer fewer sperm than normal males. The low volume of sperm transfer affected by sterile males may be the results of relatively shorter mating duration of sterile males as compared to that of normal males. The present experiment was designed to find out if irradiation of the Medfly males at various sterilization levels affects the mating durations of the treated males.

Effects of three sterilization dosages (6kr, 8kr, and 10kr) on the mating durations of the males was compared with untreated males. The adults used in the experiment were irradiated at pupal stage (24 hrs before emergence). The flies used for matings were sexed out within 12 hrs after emergence.

The individual pair mating was affected in 4 oz. glass baby food jars. In order to facilitate the ventilation inside the jars, the central portion of the metal lid had a hole of approximately 2 cm in diameter covered with 52-mesh plastic screen. In each jar one virgin normal female was confined with one same age unmated normal or irradiated male. The mating was observed one day from 7 am. to 5 pm. Records were made on the mating durations of all the couples.

Number of fly pairs set for mating varied 40-60 per treatment in different tests. The experiment was repeated on four

different days, each day with a different batch of adults. The age of the flies used in all the tests varied from 3-6 days. Adults were not provided food or water while confined in mating jars. All the tests were carried out in the laboratory at temperatures ranging from 23-27°C and relative humidity varying from 70-80%.

Results

Table 9 presents the average mating durations of the untreated and treated (6 kr, 8 kr or 10 kr) males. The data indicate that sterilization of the Medfly males up to 10 kr did not reduce significantly the mating duration of the treated male. Average mating duration of males irradiated with 10 kr was 140 minutes compared to 153 minutes of average mating duration of untreated males. Maximum mating duration found was 277 min (4 hrs 37 min) in normal male while 10 kr males had maximum mating duration of 230 min (3 hrs 50 min). It appears, therefore, that under the present experimental conditions sterilization does not adversely affect the mating duration of the treated males up to 10 kr.

f. Effect of gamma sterilization on the sexual maturity of the Medfly males.

Purpose and Methods

Irradiation with 10 kr induces more than 99% sterility in the treated males. However, the males irradiated with 10 kr do not compete equally with normal males. Experiments were carried out last year in the field in cages (10'x10'x8') to determine

Table 9. Mating durations of Medfly males irradiated with different doses as pupae (24 hrs prior to emergence)

Treat- ments	Average mating duration (minutes) per male ^{a/}					
	Rep. I	Rep. II	Rep. III	Rep. IV	Four reps.	
					Range	Average
Normal	132 (26) ^{b/}	154 (30)	153 (21)	173 (37)	3-277	153
6 kr	129 (22)	125 (24)	148 (19)	165 (37)	4-242	142
8 kr	134 (20)	148 (26)	137 (15)	150 (33)	10-230	142
10 kr	131 (23)	151 (30)	135 (23)	142 (36)	17-230	140

^{a/} Age of males used in the experiment varied 3-6 days.

^{b/} Figures in parenthesis are number of males on which mating durations are based.

the mating competitiveness of males irradiated with sterile (10 kr) and sub-sterile (8 and 6 kr) doses. In these experiments irradiation was found to reduce slightly the mating competitiveness of treated males. As pointed out before, irradiated males do not compete equally with normal males (based on the egg viability data of normal females). It is suspected that perhaps irradiation delays the sexual maturity of the treated males. The present experiment was carried out with two objectives: 1) to determine in the laboratory the mating competitiveness of males sterilized with different dosages; 2) to find out if irradiation at sterilization and sub-sterilization levels has any effect on

the rate of sexual maturity of the treated males.

Effects of three sterilization levels (6 kr, 8 kr and 10 kr) on mating competitiveness and sexual maturity were studied. The males were irradiated at pupal stage 24 hrs before emergence. All the adults used in the experiment were emerged between 5-9 am. and the sexes were separated within 12 hrs after emergence. One hundred twenty-five young unmated males of each treatment (6 kr, 8 kr, 10 kr and normal) were simultaneously caged with 250 young virgin normal females.

Mating pairs were collected individually in small shell vials. Time of initiation of each mating was noted on the vials. The males of different treatments were marked with different fluorescent color powder dyes by mixing the dye powder with the pupae. Later on the males were identified under dissecting microscope in ultraviolet light by means of the dye color mark present on the ptelenum.

In order to eliminate the influence of any particular dye on the mating behavior of the flies, the colors were rotated in different experiments so that males of each treatment had been marked at least once with each dye color.

On the day of emergence, mating pairs were not collected as on this day as soon as males and females were put together (between 3-4 pm.) the cages were removed for overnight storage in dark at 25°C in temperature controlled cabinets. During the following three consecutive days, after the day of emergence,

each day the mating pairs were collected from 7 am. to 4 pm. Every day, during the rest of the period when flies were not under mating observations (4 pm.-7 am.) cages were stored in the dark (to prevent mating) at 25°C.

The experiment was carried out on three different times, each time using a different batch of pupae. Each test had three replicates. All the tests were carried out in the laboratory at a temperature range of 23-27°C with relative humidity varying from 70-80%.

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Table 10 presents the total number of males of each treatment mated during the first three consecutive days after emergence. The results indicate that the sterilization of the Medfly males at pupal stage (24 hrs prior to emergence) adversely affects the mating ability of the treated males. Mating competitiveness of the sterile males decreased with the increase of the radiation dose. Males irradiated with dosages of 6 kr, 8 kr and 10 kr mated 84.9%, 66.9% and 57.6%, respectively, as frequently as normal males.

Table 11 presents the mating frequency data of males of each treatment (6 kr, 8 kr, 10 kr and normal) for the first four days of the adult life. No mating took place on the day of emergence and very few one-day old males (maximum of 4 in normal and 6 kr) mated through out the whole first day after the day of emergence. On the third day of adult life, i.e. 2nd day

Table 10. Effect of different sterilization doses on the mating competitiveness of the Medfly males ^{a/}.

Treat- ment	Number ^{b/} of males mated on different days ^{c/} after emergence ^{d/}				% matings
	1st day	2nd day	3rd day	Total	
Normal	4	254	358	616	100.0
6 kr	4	253	276	523	84.9
8 kr	2	218	192	412	66.9
10 kr	2	174	179	355	57.6

a/ Irradiated at pupal stage 24 hrs prior to emergence.

b/ Based on total of 9 repetitions from 3 tests (each test with 3 repetitions). In each repetition, 250 virgin normal females were caged with 500 unmated males (125 males of each treatment).

c/ Matings were allowed 7:00 am to 4:00 pm each day. During rest of the time (4:00 pm to 7:00 am) adults were kept in the dark in incubators at 25°C. No mating occurred on day of emergence as males and females were put together between 3:00-4:00 pm.

d/ Adults emerged between 5-10 am and no mating occurred on the day of emergence.

after emergence when males were 2 days old, in all the treatments, mating started since early morning and continued until 4 pm. with the peak mating frequency occurring between 9 am. and 1 pm. On the fourth day of the adult life, males started mating immediately at 7 am. when they were brought in the light from the temperature controlled cabinets where they were stored in the dark to prevent unobserved matings. In all the treatments by this time all the males were fully sexually matured as the highest mating frequency

Table 11. Mating competitiveness data of normal and irradiated (6 kr, 8 kr and 10 kr) Medfly males simultaneously confined with normal females during 3-day period after emergence.

Day of adult life	Observation time	Percent male matings ^{a/} observed during first four days of adult life in various types of males			
		Normal	6 kr	8 kr	10 kr
Day of emergence ^{b/}	c/	0.0 (0) ^{d/}	0.0 (0)	0.0 (0)	0.0 (0)
1st day after emergence	7:00 am. to 4:00 pm. ^{e/}	0.6 (4)	0.8 (4)	0.5 (2)	0.6 (2)
2nd day after emergence	7-8 am.	0.2 (1)	0.4 (2)	0.2 (1)	0.6 (2)
	8-9 am.	0.6 (4)	1.0 (5)	1.2 (5)	1.4 (5)
	9-10 am.	6.2 (38)	8.0 (42)	7.3 (30)	10.1 (36)
	10-11 am.	9.7 (60)	10.7 (56)	13.8 (57)	11.8 (42)
	11-12 am.	9.7 (60)	13.2 (69)	13.3 (55)	10.1 (36)
	12-1 pm.	6.7 (41)	6.5 (34)	7.0 (29)	6.5 (23)
	1-2 pm.	4.4 (27)	3.4 (18)	4.4 (18)	3.9 (14)
	2-3 pm.	1.8 (11)	1.5 (8)	3.4 (14)	3.4 (12)
	3-4 pm.	1.9 (12)	1.7 (9)	2.2 (9)	1.1 (4)
	Total	41.2 (254)	46.4 (243)	52.8 (218)	48.9 (174)
3rd day after emergence	7-8 am.	16.2 (100)	13.4 (70)	10.0 (41)	13.5 (48)
	8-9 am.	15.6 (96)	19.9 (104)	16.5 (68)	14.1 (50)
	9-10 am.	9.1 (56)	8.2 (43)	7.3 (30)	12.1 (43)
	10-11 am.	10.4 (54)	7.1 (37)	5.1 (21)	5.6 (20)
	11-12 am.	2.4 (15)	1.7 (9)	2.7 (11)	2.5 (9)
	12-1 pm.	1.6 (10)	1.5 (8)	3.2 (13)	1.7 (6)
	1-2 pm.	1.5 (9)	0.6 (3)	1.2 (5)	0.6 (2)
	2-3 pm.	0.8 (5)	0.4 (2)	0.2 (1)	0.0 (0)
	3-4 pm.	0.5 (3)	0.0 (0)	0.5 (2)	0.3 (1)
	Total	58.1 (358)	52.8 (276)	46.7 (192)	50.4 (179)

a/ Based on total of 9 repetitions from 3 tests (each test having 3 repetitions). In each repetition, 250 virgin normal females were caged with 500 unmated males (125 males of each treatment). In each column total mating for 4 days = 100%.

b/ Flies emerged between 5:00 and 10:00 am.

c/ No mating pairs observed since females were added to males between 3:00 to 4:00 pm.

d/ Figures in parenthesis are total no. matings on which percentages are based.

e/ Each day between 4:00 pm. and 7:00 am. flies were kept in dark in incubators at 25°C.

was recorded during the first four hrs of the observations, i.e. 7-11 am.

The results indicate, therefore, that sterilization up to 10 kr does not delay the maturity rate of treated males. The mating frequency pattern of irradiated (6 kr, 8 kr and 10 kr) males is similar to that of normal males. On the third day of adult life, when, for the first time, the majority of males had reached sexual maturity, 41.2% normal male matings took place compared to 48.9% 10 kr sterile male matings on the same day. On this very day, during the peak mating period, i.e. 9 am. to 1 pm., the hourly mating frequency of 10 kr sterile males varied 6.5% to 11.8% compared to 6.2 to 9.7% mating frequency of normal males recorded during the same period.

g. Effect of gamma sterilization on the sperm transfer ability of the treated males.

Purpose and Methods

Medfly females have multimating habits. In an alternate mating experiment, we found that more females mated a second time with normal males when initial mating was with 10 kr irradiated males and somewhat fewer females mated a second time with sterile males when the original mating was with normal males. This mating behavior of the females may be caused by a diminished amount of sperm transferred by sterile matings. The present experiments were carried out to see if sterile male matings transfer fewer sperm compared to normal male

matings and whether receptivity of females is controlled by the amount of sperm received by the females from the first mating.

Three to six day old young virgin females were allowed to mate individually with same age unmated males. The mating was carried out in 4 oz. glass baby food jars. One male and one female were placed in each jar. The metal lid of the jar had a window of about 2 cm. in diameter in the center covered with fine plastic screen in order to facilitate ventilation inside when flies were confined. The mating was observed constantly from 7 am. to 4 pm. The females which accepted matings were observed for a second mating. As soon as a mated female accepted the second mating, the couples were forcibly separated and the male was removed. Such females were considered twice mated.

Later on, the spermathecae of all the females that accepted two matings and an equal number of females which mated once, but did not remate, were examined under microscope for the sperm contents. The sperm quantities of the spermathecae were given five arbitrary ratings based on sperm volume calculated by visual observations.

First category 'abundant' was given maximum rating of 4. Both spermathecae in this category were full with sperm. Second category 'many' with rating of 3, had both spermathecae more than half full, third category 'few' with rating of 2, had small

number of sperm in either one or both spermathecae. Fourth category 'very few' with rating of 1 had only traces of sperm. The fifth category consisted of no sperm and was given zero rating.

All the experiments were carried out in the laboratory at temperatures ranging between 22-27°C and relative humidity fluctuating between 70-80%. The mating experiments were started at 7 am. and were concluded at 4 pm. During the mating period the adults were not given food or water. In each experiment 200 pairs were set (100 pairs for each treatment, i. e. normal and 10 kr). The experiment was repeated on five different occasions.

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Table 12 presents the summarized data from five experiments on the amount of sperm present in the spermathecae of the females re-mated or refused second mating by normal or sterile male during a 9-hr test period (7 am. - 4 pm.). The results indicate that sterilization with 10 kr does not reduce the sperm transfer capacity of treated males, at least in the first mating. The sterile males, like the normal males, transfer a good amount of sperm in most of the matings. An equivalent amount of sperm is transferred by normal as well as sterile male. Sperm content ratings of females mated once to normal and irradiated males were 3.4 and 3.2, respectively.

The sperm content ratings of the females which accepted second matings were very similar to those which refused second mating.

Table 12. Sperm contents in the spermathecae of the Medfly females remained receptive or non-receptive immediately after first mating with irradiated^{a/} (10 kr) or untreated males.

Mating history of females	% females with different degrees of sperm contents in their spermathecae ^{b/}					Av. rating for the group
	Abundant (Rating 4)	Many (Rating 3)	Few (Rating 2)	Very few (Rating 1)	None (Rating 0)	
Twice ^{c/} with normal male	38.6 (22)	36.8 (21)	10.5 (6)	3.5 (2)	10.5 (6)	2.9 (57)
Twice ^{c/} with sterile male	48.3 (14)	44.8 (13)	3.4 (1)	0.0 (0)	3.4 (1)	3.3 (29)
Once with normal male	53.6 (30)	35.7 (20)	7.1 (4)	0.0 (0)	3.6 (2)	3.4 (56)
Once with sterile male	49.0 (25)	31.4 (16)	15.7 (8)	0.0 (0)	3.9 (2)	3.2 (51)

a/ Treated with 10 kr as pupae, 24 hrs before emergence.

b/ Figures in parenthesis are number of females observed.

c/ Include females which after first mating re-mated but in second mating the couples were forcibly separated soon after initiation of mating to prevent sperm transfer from second mating.

This indicates that receptivity of females was not controlled by the amount of sperm present in the spermathecae of the females. The sperm content ratings for the females remated were 2.9 (normal mating) and 3.3 (sterile mating) compared to ratings of

3.4 and 3.2 of those females which refused second mating with normal and sterile males, respectively. More experiments will be carried out to determine the cause of receptivity in Medfly females.

h. Induction of visible Medfly mutants

Purpose and Methods

Work from last years is continued to breed some visible Medfly mutants which can be used in sterile male release programs. Three-day old males are fed on 0.025 M ethyl methanesulfonate (EMS) in sugar water solution through saturated cotton wicks for 24 hrs. Adults are starved 24 hrs, before offering EMS in solution. Two days after feeding on chemical, treated males (100-150) are allowed to mate (in mass) with same age virgin females (150-200). Twenty-five to thirty mating pairs are removed and placed individually into cages to obtain F_1 flies. All F_1 individuals arising from a single pair are mated in mass. F_2 flies are allowed individual sib matings in isolation. F_3 adults are examined for visible mutants.

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We are unable to give sufficient time for this experiment. Three batches of F_3 flies were examined this year and so far we did not obtain any mutant. We are considering to discontinue this project.

2. Studies on the Biology and Sterilization of the Coffee Leaf Miner, Leucoptera coffeella Guer.
(K. P. Katiyar and J. A. Reyes)

Purpose and Methods

Work has been continued to evaluate the application of gamma sterile male technique to control or eradicate the coffee leaf miner. This year experiments were carried out to determine the dose-response curve of male and female moths. Effects of sterilization on the longevity of treated moths (males as well as females) have been studied.

Newly emerged moths (less than 24 hrs old) were irradiated with several dosages between 1 kr and 90 kr. The treated moths (male or female) were confined in cages with untreated moths of the opposite sex. Each cage had 10 pairs of moths. Daily egg collection was made for 8-day period. Data on the adult mortality were taken in all the experiments.

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The results in tabulated form are not yet available as experiments are not yet completed. However, general results indicate that male moths are more radioresistant (based on egg-hatchability data) than females. Sterilization dose of 30 kr induces 99.9% sterility in female while 90 kr is needed to induce 99.8% sterility in males. Irradiation reduces the longevity of treated males. Average longevity of untreated and 90 kr treated males were 16 and 8 days, respectively. In females, sterilization slightly increases

the adult's life. Average longevity of normal and 30 kr treated females were 12 and 14 days, respectively.

Unlike tobacco budworm, Heliothes virescens (F) and the cabbage looper, Trichoplusia ni (Hübner), the fecundity of normal coffee leaf miner females mated to sterile males was not adversely affected. Average number of eggs deposited per female, during 8-day oviposition period, were 63 and 56, respectively, by females mated to untreated and 90 kr treated males. Detailed results of these experiments will be reported in next year's annual report.

C. SOIL CHEMISTRY

1. Soil Acidity and Cation Retention Properties of Tropical Soil

a) Exchangeable and titratable acidity (K. Igue and R. Fuentes)

Purpose and Methods

Aluminum and iron play a very important role in tropical soils. They are associated with high acidity of the soil, high adsorption of phosphates (4) and sulfates (3) and in many instances cause Al toxicity to the plants (1). Determination of these elements as occurring in different forms is very important. Although free Al and Fe sesquioxides have been determined (2), little has been done to characterize exchangeable, non-exchangeable and organic matter bounded Al. A common technique used to determine exchangeable acidity consists in extracting soil samples with unbuffered salt

solutions (5,9) and buffered salts such as NH_4OAc at pH 4.8 (6,7). In order to further characterize Al in some volcanic soils, studies were initiated using salt displacing methods.

Ammonium acetate has been a very common extractant for the exchangeable cations in soils. This method has been used also for the extraction of aluminum.

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Figure 2 shows the amount of Al extracted with NH_4OAc at different pHs. Higher amounts of Al were extracted at low pH. This is in accordance with the data presented by others (6,7). Ammonium acetate is ineffective at neutral pH. Higher amount was observed for Coronado soil (Ultisol) followed by Cervantes (Andosol) and Colorado (Inceptisol). From the graph it is difficult to separate the exchangeable and non-exchangeable Al. At low pH a significant amount must come from the dissolution of $\text{Al}(\text{OH})_3$, aluminum phosphate and Al bounded by organic matter (9).

Table 13 presents the exchangeable acidity displaced by unbuffered N KCl pH 7.0 by successive leaching of the soil and collecting 25 ml increments. The total Al extracted from Colorado is higher than Birrisito. The data is plotted in Figure 3. A continuous rise is observed until it reaches a total volume of 150 ml and then starts to level off. The flat portion of the curve resembles that of dissolution described by Skeen & Sumner

Fig. 2. Aluminum extracted with $\underline{N}$ NH_4OAc and $\underline{N}$ KCl as a function of pH.

Al me/100 g

Fig. 3. Amount of Al extracted with N KCl pH 7.0 by successive leaching.

Table 13. The amount of exchangeable Al^{+++} and H^+ displaced from 5 g soil by successive increments of N KCl at pH 7.0

Leaching Fractions ml	Colorado A			Birrisito A		
	Al^{+++}	H^+	Total	Al^{+++}	H^+	Total
	me/100 g			me/100 g		
0 - 25	1.90	0.52	2.42	0.43	0.23	0.66
25 - 50	0.78	0.24	1.02	0.41	0.07	0.48
50 - 75	0.48	0.24	0.72	0.34	0.08	0.43
75 - 100	0.39	0.19	0.58	0.34	0.04	0.38
100 - 125	0.24	0.10	0.34	0.24	0.10	0.34
125 - 150	0.19	0.14	0.33	0.18	0.14	0.32
150 - 175	0.15	0.14	0.29	0.16	0.14	0.30
175 - 200	0.12	0.13	0.25	0.13	0.17	0.30
200 - 225	0.10	0.09	0.19	0.12	0.12	0.24
225 - 250	0.08	0.11	0.19	0.10	0.16	0.26
Total	4.42	1.90	6.33	2.45	1.25	3.71

(8) for South African Soil. According to these authors, the exchangeable Al can be obtained by extrapolating the flat upper portion of the curve back to the zero extraction (Y axis). Following this procedure the exchangeable Al will be 3.4 me/100 g and 1.4 me/100 g for Colorado and Birrisito soils respectively.

As the pH of N KCl solution was increased, higher amounts of Al were displaced due, probably, to the dissolution of $Al(OH)_n$ or desorption of Al-organic matter complex. Figure 2 presents the data for N KCl at different pH for Colorado soil only. More Al is extracted with N KCl at neutral pH although less at lower pH. This seems to indicate that 'true' exchangeable Al is close to what is obtained by unbuffered salts, the pH of which tends to be close to that of the soil.

Pratt and Bair (7) suggested an extraction with N KCl using as little time as possible, which minimizes the non-exchangeable form.

When different unbuffered salts were used, CaCl_2 seems to be the most efficient. The preliminary data is presented in Table 14. Skeen and Sumner (8) indicated that NH_4Cl was the most suitable extractant for exchangeable Al. They also indicated that, when acetate was the anion, a higher value was always obtained. In our preliminary data N NaCl was the least effective, but the values obtained are close to the values obtained from Figure 3 by extrapolating the curve back to zero extraction as suggested by Skeen and Sumner.

Further studies are needed in order to characterize exchangeable and non-exchangeable Al in soils of wet tropics and to check for the validity of the Nernst type curve for the interpretation of exchangeable Al.

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Table 14. Exchangeable acidity displaced with different salt solution

Extracting solution	Colorado			Birrisito		
	Al ⁺⁺⁺	H ⁺	Total	Al ⁺⁺⁺	H ⁺	Total
	me/100 g			me/100 g		
N KCl pH 7.0	4.43	1.90	6.33	2.45	1.25	3.71
N NaCl pH 7.0	3.65	0.82	4.45	1.01	0.78	1.80
N CaCl ₂ pH 7.0	n.d.	n.d.	6.67	3.49	1.62	5.11
N BaCl ₂ * pH 7.0	3.22	-	-	1.40	-	-
NH ₄ OAc* pH 4.8	10.8	-	-	4.05	-	-

* Determined by Aluminon procedure (Bornemisza 1965).

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b). Cation exchange properties of volcanic soils
(K. Igue and R. Fuentes)

Purpose and Methods.

The presence of amorphous materials associated with high organic matter content in volcanic soil indicates quite variable values in the CEC of these soils. Variations in CEC as a function of dehydration-rehydration, pH of the solution, have been observed in Hawaiian and similar soils (3,6). Dehydration decreased markedly the CEC of latosols studied. The pH-dependency characteristics of these soils is quite pronounced for buffered NH_4OAc and less noticeable with N CaCl_2 method (2). The retention of acetate anion have been indicated as the cause of high CEC in soils high in amorphous material (7). To further understand the effect of allophane and organic matter in volcanic soils the study has been initiated.

Ammonium acetate and CaCl_2 method were tested at different pHs. Isotopic exchange procedure was tested using ^{45}Ca as acetate and chloride forms, following basically the procedure described by Blume and Smith (1). The soils were initially saturated with N calcium salts and then washed with known concentration of Ca solution, followed by isotopic equilibration.

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The values obtained for CEC as a function of pH are presented in Table 15. Marked effect of pH can be observed under $\text{N NH}_4\text{OAc}$ for the soils studied. This is not so for N CaCl_2 solutions,

Table 15. Cation exchange capacity of Costa Rican soils as determined by NH_4OAc and CaCl_2 at different pHs.

Soil		pH	CEC		me/100 g			pH H ₂ O	M O	Total cations me/100
			<u>N</u> NH ₄ OAc	<u>N</u> CaCl ₂	Ca	Mg	K			
Colorado	A	7	32	14	2.60	0.26	0.30	3.6	10.0	3.16
		6	30	13	1.00	0.24	0.32			1.56
		5	24	13	0.25	0.23	0.34			0.82
		4	22	-	0.25	0.24	0.33			0.82
	B	7	22	8	1.21	0.20	0.10	4.6	1.0	1.50
		6	21	7	0.50	0.13	0.20			0.83
		5	17	6	0.20	0.16	0.30			0.66
		4	16	-	0.10	0.16	0.40			0.66
Birrisito	A	7	52	16	1.10	0.29	0.30	5.8	15.6	1.69
		6	48	14	0.02	0.28	0.30			0.60
		5	44	12	0.02	0.28	0.34			0.64
		4	35	12	0.18	0.30	0.40			0.78
	B	7	42	8	0.75	0.10	0.10	5.8	9.1	0.95
		6	39	7	0.02	0.10	0.10			0.22
		5	36	7	tr	0.10	0.10			0.20
		4	23	4	0.02	0.11	0.11			0.24
Cervantes	A	7	58	21	6.00	2.90	0.92	4.5	13.8	9.21
		6	55	20	4.80	2.60	0.96			8.36
		5	45	19	1.20	2.20	1.12			4.52
		4	38	19	1.00	2.20	1.29			4.49
	B	7	39	16	6.00	0.99	3.00	5.8	3.0	9.99
		6	36	15	4.80	0.97	3.10			8.87
		5	31	9	1.20	0.81	3.30			5.31
		4	27	-	1.20	0.82	3.70			5.72
A. Poasito	A	7	40	19	2.00	1.10	0.70	6.1	20.0	3.80
		6	39	19	1.85	1.25	0.78			3.88
		5	34	18	0.32	1.25	0.90			2.47
		4	26	17	0.46	1.41	1.05			2.92
	B	7	42	14	3.25	0.41	0.46	6.2	15.0	4.12
		6	39	13	0.75	0.54	0.49			1.78
		5	33	12	0.06	0.49	0.56			1.11
		4	24	11	0.17	0.54	0.68			1.39
Paraiso	A	7	34	15	2.70	1.10	0.35	5.7	12.0	4.15
		6	32	14	1.20	1.10	0.38			2.68
		5	30	14	0.32	1.20	0.45			1.97
		4	26	13	0.37	1.40	0.50			2.27
	B	7	23	11	0.60	0.28	0.13	5.7	4.3	1.01
		6	22	9	0.30	0.30	0.14			0.74
		5	21	9	0.04	0.30	0.17			0.51
		4	18	7	0.06	0.37	0.20			0.63

which show only slight increase with pH increase. This was explained by Rich (7) as due in part to the salt retention when the anion is acetate. When $\underline{N}$ $\text{Ca}(\text{OAc})_2$ was used the result was similar to that of NH_4OAc as shown in Figure 4. Higher values are obtained for NH_4OAc at lower pH as compared to $\text{Ca}(\text{OAc})_2$, whereas the difference decreases as the pH increases. To certain degree this can be explained by Ca preference developed by organic matter as the negative charge is developed with an increase in pH (5).

In Table 16 the values of CEC calculated according to Blume and Smith (1) are presented. The values calculated for isotopic dilution factor (IDF) and Ca apparently mobilized calculated according to McConaghy et al. (4).

As the pH decreases the IDF factor increases for acetate, whereas it maintains constant for CaCl_2 . This means that at low pH more Ca is present in solution. The constancy in IDF when CaCl_2 is used seems to indicate the inability of unbuffered salt to keep the pH at desired values. Under this condition the pH tends to be that close to the soil pH.

Apparently, mobilized Ca also varies with the method and when acetate is used it is lower than CEC at high pH and higher at lower pH. For the CaCl_2 system the values are always higher than CEC, indicating release of Ca to the solution possibly due to lower pH attained.

A more detailed study will be necessary in order to determine the effect of organic matter upon calcium and CEC properties

Fig. 4. Cation exchange capacity of Birrisito (Andosol) soil as measured by radiometric method.

Table 16. Values obtained for CEC, IDF (Isotopic dilution Factor) and apparently mobilized Ca using radiometric procedure for two different accompanying anions

Soil	pH	Ca(OAc) ₂ N			CaCl ₂ N		
		CEC	IDF	Ca*	CEC	IDF	Ca*
		me/100 g	ppm	me/100 g	me/100 g	ppm	me/100 g
Birrisito	8.0	55	86	42	12	127	15
	7.0	49	81	35	11	127	15
	6.0	37	86	30	11	127	15
	5.0	25	130	32	11	127	15
	4.0	15	144	23	10	125	14
Coronado	8.0	63	76	42	15	125	18
	7.0	57	78	39	15	127	18
	6.0	56	70	34	15	127	18
	5.0	37	96	32	14	127	17
	4.0	14	145	22	15	125	18

CEC = calculated by method of Blume and Smith

IDF = according to McConaghy et al. 1967.

Ca* = apparently mobilized Ca.

$$IDF = \frac{Ca_{total} - Ca_{initial}}{\frac{Sf}{Si}}$$

$$Ca* = IDF \times \frac{Si}{Sf}$$

Si, Sf = initial and final specific activity.

of these soils.

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2. Mineralization of Organic-Phosphate in Three Costa Rican Soils.
(K. Igue, R. Fuentes)

Purpose and Methods

Organic phosphorus constitutes up to 77% of the total P in some representative volcanic and alluvial soils of Costa Rica (5). In the past, experiments showed that crops grown on these soils did not respond to P application. This was due to a high fixation of P by amorphous materials present in these soils (7). No information is available concerning the availability of organic phosphorus in Costa Rican soils. Acquaye (1) and Awan (2) indicated the significance of organic fraction to the nutrition of cocoa and corn respectively in tropical conditions.

The present study was undertaken in order to provide information about the rate of transformation of organic-P fraction in selected volcanic soils (Table 17). The treatments imposed to these soils were described in the previous annual report

(NYO-2043-194). The methods of organic-P extraction were carried out according to the modified Mehta et al. (6). The fractionation of organic P followed that of Chang and Jackson (7).

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The mayor effect of different treatments upon P transformations during the incubation period are shown in Tables 18, 19 and 20. The effect of incubation time is summarized in Figures 5 and 6 for organic and inorganic fraction respectively. The results indicate that mineralization did not occur in the case of Colorado series. The corresponding inorganic fraction increased for both Juray and Birrisito but decreased for Colorado soil. During the initial periods of incubation immobilization was observed in the soils studied. This indicates that upon treatment with nutrients an energy source was provided which allowed the buildup of microbial population in the soil. This is similar to that observed by Blasco et al. (4) in Colombian soil. They demonstrated that incubation of volcanic soils for 4-8 weeks decreased available P in the soil. They observed a high increase in bacteria and fungi population following the incubation period.

In the present case a prolonged incubation promoted mineralization for Juray and Birrisito whereas immobilization continued to occur up to 10 months incubation period for Colorado soil. This soil is considered very low in available nutrients. The total P content is higher for Juray and Birrisito. McCall et al. (9) indicated that P addition increased mineralization which

Table 17. Characteristics of the soils used to study mineralization of organic phosphorus.

Soil	pH	OM (%)	Fe ₂ O ₃ %	Al ₂ O ₃ %	C/P	C/N
Juray sandy clay	5.5	7.35	6.4	15.5	85	12
Colorado sandy clay	4.8	9.70	4.6	19.1	224	15
Birrisito sandy clay	5.4	6.04	5.5	20.6	54	12

Table 18. Phosphate transformation in Colorado Series during 10 months incubation at room and 35°C.

Treatment	P _{total}	P _{inorg}	P _{Al}	P _{Fe}	P _{Ca}	Org P
-----ppm-----						
Lime without	1290	770	112	215	38	537
with	1297	741	107	197	33	551
Temp. room	1293	765	113	229	36	541
35°C	1294	746	106	183	34	546
Nutri- check	1138	640	51	164	32	500
ents others	1168	660	49	164	32	521
P	1428	861	170	248	39	572
P+other	1440	862	167	247	39	582
Time initial	1248	775	120	183	24	474
1 month	1337	818	92	186	36	544
3 months	1287	745	102	204	26	528
10 months	1306	684	123	252	57	629

Others = NK and micronutrients.

Table 19. Phosphate transformation in Birrisito Series after 10 months incubation at room (23°C) and 35°C.

Treatment		P _{total}	P _{inorg}	P _{Al}	P _{Fe}	P _{Ca}	Org P
----- ppm -----							
Lime	without	1560	620	166	73	91	940
	with	1493	593	162	73	87	899
Temp.	room	1513	607	166	70	87	907
	35°C	1540	607	162	76	91	932
Nutri- ents	check	1349	456	99	41	64	896
	others	1373	456	92	42	64	916
	P	1695	752	232	104	113	941
	P+other	1689	763	233	103	115	926
Time	initial	1453	504	229	84	110	948
	1 month	1523	528	150	37	102	994
	3 months	nd*	nd	nd	nd	nd	nd
	10 months	1604	789	114	96	56	817

* nd = not determined

Table 20. Phosphate transformation in Juray Series after 10 months incubation at room (23°C) and at 35°C.

Treatment		P _{total}	P _{inorg}	P _{Al}	P _{Fe}	P _{Ca}	Org P
----- ppm -----							
Lime	without	2137	1162	240	394	67	975
	with	2174	1166	234	398	74	1003
Temp.	room	2153	1169	234	370	69	987
	35°C	2157	1159	239	421	73	991
Nutri- ent	none	2070	1054	189	334	65	1012
	others	2053	1051	189	347	62	993
	P	2265	1272	285	454	77	988
	P+others	2234	1279	285	449	79	962
Time	initial	2188	1152	215	374	63	1039
	1 month	2246	1162	293	605	71	1078
	3 months	2196	1101	257	317	99	1104
	10 months	1991	1241	182	288	50	736

Fig. 5. Organic phosphorus and inorganic P determined after different incubation time.

Fig. 6. Fractions of inorganic P after different periods of incubation.

increases available P. Van Diest (10) showed that organic P increased up to 30% in cultivated and fertilized soils. He assumed this increase to be due to an incorporation of plant material and some microbial residues.

The data seems to show that mineralization after 10 months occurred according to the C/P ratio as indicated (3). But this was not true when the incubation time was short in accordance with that observed by Blasco et al. (4).

It is more reasonable to think that mineralization occurs when available P is present for microbial activity. On the other hand, the C/P ratio can be also a function of total P of the soil.

Figure 6 shows the fractions of inorganic P according to Chang and Jackson. Birrisito shows a decrease in Al-P and Ca-P, but the Fe-P decreases and then increases. A slight increase was observed in the case of Colorado soil, whereas in the case of Juray there was initial increase and then decrease with prolonged incubation. The increase in Fe-P for Juray is quite significant.

The interpretation of the inorganic fraction is somewhat difficult in these high phosphate fixing soils. When P was added the Al-P, Fe-P and Ca-P were significantly increased for the three soils. Nevertheless the values of the 3 fractions are somewhat low compared with total inorganic P extracted by Mehta et al. procedure (9). This seems to indicate that part of

mineralized or added P can become fixed in a form not extracted by the procedure used. It is possible that some of these fractions can be occluded forms. Liming and temperature had little effect.

This experiment was terminated and from the results presented we may conclude that:

- a) Mineralization of organic-P occurred after prolonged incubation in those soils high in total P. Immobilization occurred in soils with low total P content and high organic matter content.
- b) Mineralization rate followed the established C/P ratio after 10 months incubation period.
- c) Nutrient application favored initial immobilization of P.
- d) Application of inorganic P increased organic-P for Colorado soil, but no increase was observed for Birrisito and Juray.
- e) Mineralized P or added P is fixed by the soil in a form that can not be extracted by NH_4F , NaOH and H_2SO_4 . It is assumed to be in occluded form.

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3. Zinc-Phosphorus Interaction in Costa Rican Soils (K. Igue and M. L. Marinho)

Purpose and Methods

Zinc has been considered to be the element with the most widespread deficiency in tropical regions. This deficiency of zinc is very common in coffee plantations in Costa Rica and Brazil (3,4). Stanton and Burger (5,6) reported also the deficiency of zinc in citrus at South Africa. They studied the effect of iron oxides on zinc availability. They showed that Zn adsorbed by iron oxides through phosphate is not available to the plant. The effect of phosphate has been discussed in many instances and the results

have been somewhat controversial (1). Research was initiated to study the interaction between zinc availability in three volcanic soils of Costa Rica. The objective was to study the interaction between zinc concentration, phosphate and free iron and aluminum sesquioxide content. To measure zinc absorption by corn, radioactive Zn-65 was used in a factorial experiment.

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The soils used in the present experiment were chosen according to zinc deficiency symptoms in coffee in the Meseta Central, Costa Rica, and based on preliminary analyses of zinc and sesquioxide content. The characteristics of soils used are indicated in Table 21.

Table 21. Characteristics of the soils used in corn experiments with zinc-65.

Soil	pH H ₂ O	Zn ppm		R ₂ O ₃ %	P _{ppm} Mehlich	CEC (NH ₄ OAc)
		EDTA*	HCl**			
1	5.4	1.70	7.5	10.3	1.5	42
2	5.2	1.20	4.5	12.2	0.8	47
3	5.3	0.64	2.0	22.3	tr	48

* 0.01 M

** 0.1 N

The soils indicate low level of extractable Zn and different values of free iron and aluminum sesquioxides. Soluble P by Mehlich procedure is very low due to high P fixing capacity of these soils.

Table 22 presents the data of soil zinc analysis after receiving different zinc and P treatments. The treatments were numbered as follows: the first number indicates the soil (1, 2 and 3); the second number indicates Zn concentration and the last number the P level.

Zinc available analyzed by EDTA and HCl 0.1 N increased as a function of zinc applied. With increases in the P applied, the values for zinc extracted with EDTA also increased. This is not observed when HCl is the extractant. Samples were analyzed before incubation and after 15 days. Additional analysis should be carried out at the end of 30 days.

Corn plants were grown in 1 kg of soil for 30 days. The dry weight is also shown in Table 22.

The dry weight of corn plants is markedly increased as the P level increases. An increase is also observed in dry weight as the zinc level increases for soil 1 (lowest in free sesquioxides) followed by soil 2. There was no visible effect of zinc on dry weight of corn on soil 3 (the highest in free sesquioxides of Fe and Al). This seems to be related to high fixation capacity for zinc as shown in Figure 7. This preliminary data was obtained by shaking the soil with known concentration of Zn-65 and analyzed at different periods. A rapid increase in retention is observed during the first period. The specific activity remains constant during the initial 6 hours of shaking period and increases afterwards. No explanation is given for the moment, since further

Table 22. Zinc content of the soil as a function of different treatments and the dry weight of corn plants.

Treatment (P ₂ O ₃ :Zn:P)	Before incubation		15 days incubation		Corn dry wt. g
	EDTA	HCl	EDTA	HCl	
	-----ppm-----				
Soil 1	1.70	7.5			
100	2.15	12.00	1.90	11.75	
101	2.54	12.50	2.20	11.25	11.9
102	2.63	9.50	2.44	11.50	13.8
110	2.63	16.75	3.20	15.25	4.9
111	4.36	14.25	3.26	15.25	12.2
112	5.20	14.75	4.30	15.00	14.6
120	5.80	20.00	4.60	19.50	-
121	5.92	21.00	5.06	20.50	12.8
122	6.68	21.50	5.64	20.50	16.0
Soil 2	1.20	4.5			
200	1.00	5.75	1.01	9.50	-
201	1.20	8.25	1.20	8.75	8.6
202	1.52	7.75	1.45	8.50	11.4
210	3.32	17.50	2.24	15.50	3.1
211	3.22	11.00	2.40	13.75	8.9
212	3.96	12.63	3.42	13.75	12.6
220	4.70	18.00	3.42	15.25	-
221	4.80	15.00	4.00	16.50	8.9
222	5.72	16.75	5.20	16.50	13.3
Soil 3	0.64	2.0			
300	0.42	4.25	0.25	5.75	-
301	0.50	5.00	0.40	5.75	5.1
302	0.80	3.00	0.72	5.75	9.7
310	1.68	9.25	1.12	11.75	2.4
311	2.14	10.50	1.36	10.25	6.0
312	3.20	8.50	2.00	10.75	9.3
320	3.06	12.50	1.86	15.00	-
321	3.87	15.00	2.20	13.75	9.3
322	4.52	11.00	3.54	15.00	9.9

Dosis: phosphorus - 0 ZnSO₄ - 0
 ppm - 400 - 15
 - 1000 - 30 lb/a

Fig. 7. Zinc retention in high sesquioxide soil (Soil 3) as a function of time.

studies will be carried out. Nevertheless, the total activity and total zinc concentration in equilibrium solution decreased significantly with time.

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TRAINING

1. During the year July 1, 1969 to June 30, 1970, the following staff members of the Nuclear Energy Program gave the following training lectures or seminars to the students and teachers from Latin America:

Kozen Igue

1. Use of radioisotopes in agricultural research.
2. Use of radioisotopes in plant physiology.

Oscar Hidalgo

Instrumentation, radiochemistry, and radiation protection.

Carl C. Moh

Cytogenetics

2. At the request of FAO/IAEA, an entomology training course on the use of radiation in controlling insects was sponsored jointly by the IAEA and the Institute. The Institute is the AEC contractor. The course was held at the Training and Research Center, Turrialba, Costa Rica, from April 20, to May 29, 1970. A total of 20 students from various Latin American countries, most of them professionals, participated in the course. Staff members of the Nuclear Energy Program assisted in the organization and offered lectures for the course. The radioisotopes laboratory and the gamma pool were also provided for the training purpose

3. Three students received their training from the Nuclear Energy Program. Luis Delgado and Ricardo Pineda went back to Peru,

and are teaching in the University of the Altiplano and in the University of Piura, respectively. Victoriano Rios went back to Panama and is taking a position with the Agriculture Ministry of Panama.

CONSULTING, MEETINGS AND TRAVELING

Dr. Elemer Bornemisza

From July 6 to 13, 1969, Dr. Bornemisza attended the panel meetings on Volcanic Ash Soils in Latin America held at the Training and Research Center, Turrialba, Costa Rica. He also served as the secretary in the soil chemistry and physics section of the panel.

Dr. Kamta Katiyar

From September 1 to 5, 1969, Dr. Katiyar attended a research coordination meeting on "The Application of the Sterile Male Technique for Control of Insects with Special Reference to Fruit Flies" held in Vienna. The meeting was organized by FAO/IAEA.

Dr. Carl C. Moh

From August 14 to 20, 1969, Dr. Moh went to Washington, D. C., to discuss the projects in the Nuclear Energy Program of the Institute with the USAEC, and also went to the annual meetings of the Genetics Society of America in Madison, Wisconsin.

From November 17 to 23, 1969, he represented the Institute at the 7th Meeting of Interamerican Commission of Nuclear Energy held at the Pan American Union, Washington, D. C. He also went to the AEC office to discuss the future research projects with the AEC personnel.

PUBLICATIONS

Bornemisza, E. Experiencias del Centro de Enseñanza e Investigación del IICA sobre el aprovechamiento de mesas redondas en la programación de cursos de suelos. In Informe, Seminario Internacional de Profesores de Suelos, Maracay, Venezuela, Junio, 1969. IICA-ZA, 10 p.

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