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THE APPLICATION OF NUCLEAR ENERGY TO AGRICULTURE

contract AT (30-1)-2043

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Tropical Training and Research Center
Turrialba, Costa Rica

THE APPLICATION OF NUCLEAR ENERGY TO AGRICULTURE

Annual Report

to the

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Tropical Training and Research Center

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RESEARCH

A. MUTATION BREEDING AND RADIATION BOTANY

1. Induction of Mutations in Manihot
(C. C. Moh and J. J. Alan)

As mentioned in the previous report, Manihot (common name: yuca or manioc in Latin America, but not the yucca plants grown in the deserts of New Mexico and Arizona), is a major food crop in the low lands of the American Tropics, is rich in carbohydrates, and has a phenomenal yield. Since the introduction of Manihot to Africa in the 16th Century, Manihot has widely spread throughout the area and also has become an important staple foodstuff over large parts of the African continent. Now, Manihot has become a major supplier of food and feed calories to the tropical world (3).

Although Manihot has a wide adaptability to the areas from high rainfall to semi-arid, it cannot stand frost. Therefore, the frost-line is a natural boundary limiting Manihot distribution (4), and the plants can rarely be seen in the temperate zone except preserved specimens which can be found in some herbariums or a few plants in tropical botanical gardens.

This report first describes briefly the Manihot botanical characters, an understanding of which is essential for inducing mutation work, and second, the progress work on the induced mutations.

a. Botanical characters of Manihot

Most of the cultivated forms of Manihot are perennial shrubs (Fig. 1). The tuberous roots produced by the plants provide

Fig. 1. A six-month old Manihot esculenta plant (Cultivar No. 68) showing its general morphology.

food for human consumption (Fig. 2). The roots can be harvested after eight to twenty-four months of plant growth, depending upon the cultivars. The plants can easily be propagated by cuttings but less commonly are propagated by seeds. Manihot is monoecious. Unisexual flowers are born in inflorescences. A few pistillate flowers occur at the base of the inflorescence, and many staminate flowers above. The males do not open until all the females have bloomed (Fig. 3). Because of this flowering character, Manihot is chiefly pollinated by insects, and cross pollination occurs more frequently than self-pollination. This gives rise to a great probability of inter- and intra-specific hybridization in the natural population which contributes to a vast morphological variability and genetic heterozygosity found among the cultivars today.

b. Radiosensitivity of Manihot shoot apex

Foot-long cuttings of mature woody stem from 8-month old plants were irradiated with acute gamma radiation (1426 r/minute), from 2 to 5 kr. The irradiated cuttings were grown in a nursery for growth observation. After 5 months, the growth results were recorded (Table 1).

Table 1. Radiosensitivity of shoot apex of Manihot esculenta (cultivar No. 68) to acute gamma irradiation.

Radiation dose (kr)	No. of cuttings irradiated	No. of shoots emerged	Average plant height in 5 months (cm)	% of growth reduction
0 (ck)	7	19	79	0
2	7	16	76	3.8
3	7	18	73	7.6
4	7	19	50	36.7
5	7	4	21	73.5

Fig. 2. The mature roots from a Manihot esculenta plant (Cultivar No. 68). The roots of this plant weighed 33 lbs.

Fig. 3. The male and female flowers of Manihot esculenta showing a marked protogyny of the flowering habit. The male flowers do not open until the last female flower on the inflorescence has bloomed.

It can be seen that at a dose of 2 or 3 kr, the plant growth, as expressed by its height, was not reduced to a significant degree, as compared with the control (0 kr). At a dose of 4 kr, however, the growth reduction was very prominent, a 36.7% reduction. At a dose of 5 kr, not only was the growth severely inhibited (73.5% growth reduction), but also the number of shoot emergence was significantly reduced. Fig. 4 demonstrates how severely the plant growth was inhibited at different radiation doses after two months of growth.

These results show that 4 to 5 kr is the dose range that critically affects the growth of the Manihot shoot apex. A brief review of the literature reveals that the critical dose inhibits the growth of shoot apex (buds or germinating seeds) of many plant species falling within or below this dose range.

c. Somatic mutations in Manihot

The usefulness of induced somatic mutations as a tool for improving vegetatively propagated plants needs little stress. It provides an unique means to breed the plants without a sexual cycle. The induced mutations, if desirable, can be used directly without further breeding process. The advantageous heterozygotes can be maintained vegetatively and also be used as a "pure" line for agricultural practice.

The induction of somatic mutations has its difficulties. Since the somatic tissues of higher plants are diploids or polyploids, and most of the induced mutations are recessive, the recessive

Fig. 4. The sensitivity of Manihot esculenta cuttings to gamma radiation. The plants were one-month old. A dose of 5 kr induced severe growth inhibition.

mutations can hardly express their mutant characters in the somatic generation, unless by coincidence, both allelic loci are changed in the same manner. This prevents the possibility of obtaining almost all the recessive mutations. Secondly, it is known that many mutations are the result of chromosomal aberrations. Although, for somatic cells, chromosomal aberrations are not a critical factor for the cell death, those aberrations leading to loss of chromosomal fragments essential for the cell development usually are (2). As a result, growth retardation or death of mutated cells in somatic tissues also limit the possibility of the mutation production. Thirdly, the meristematic tissues of higher plants generally are multicellular systems and mutation is a single cell event. The mutated cell usually gives rise to a narrow sector of chimera which also limits the mutant appearance. All these above factors would result in a low somatic mutation rate.

Among the 90 Manihot cultivars in our collection, we selected two for the preliminary experimental trials. One (cultivar No. 49) has chromosomal structural exchanges involving at least three pairs of homologs. Thus it produces trivalents, quadrivalents or hexavalents during meiotic division, and the pollen sterility is 66 percent (Table 2). This cultivar represents a rather high degree of heterozygosity in the somatic cells. The other (cultivar No. 68) has normal chromosome pairing, forming 18 bivalents during meiosis, and the pollen sterility is very little, if any (Table 2). This cultivar represents a "homozygous" line (at least normal cytologically). Both cultivars produce good quality of roots for consumption,

Table 2. Chromosome pairing and pollen sterility of two Manihot cultivars

Cultivar No.	No. of pollen mother cells observed	Average No. of chromosome pairing				Pollen sterility		
		bivalents	trivalents	quadrivalents	hexavalents	total counted	abnormal No.	Sterility %
49	47	17.66	0.04	0.11	0.02	369	243	65.6
68	80	18	0	0	0	767	12	1.5

however. Cuttings (one-foot long, including an average of 7 to 8 buds) of these two cultivars were irradiated with an acute gamma dose of 3 or 4 kr. After irradiation the cuttings were grown in the field nursery for somatic mutation observation.

Although there are 7-8 buds in each cutting, an average of 1-3 shoots will develop from them under ordinary conditions. Each shoot develops from a bud and is independent of the others in a cutting. Therefore, each shoot can be considered as a plant unit for determining the mutation frequency. Table 3 shows the induced somatic frequency in cultivars No. 49 and No. 68 after four months of growth.

Since there is no report on the mutations of Manihot found in the literature, it is necessary to describe briefly the mutants commonly induced in the present experiments. The chlorophyll type of mutants so far found were yellow or yellow-green mottling type. The albino or other chlorophyll mutations have not been found. Most of the mutations were changes in leaf morphology, plant form, or growth habit (Fig. 5). This is quite a contrast to the mutations induced in cereals in which more than 90 percent are of chlorophyll deficient types.

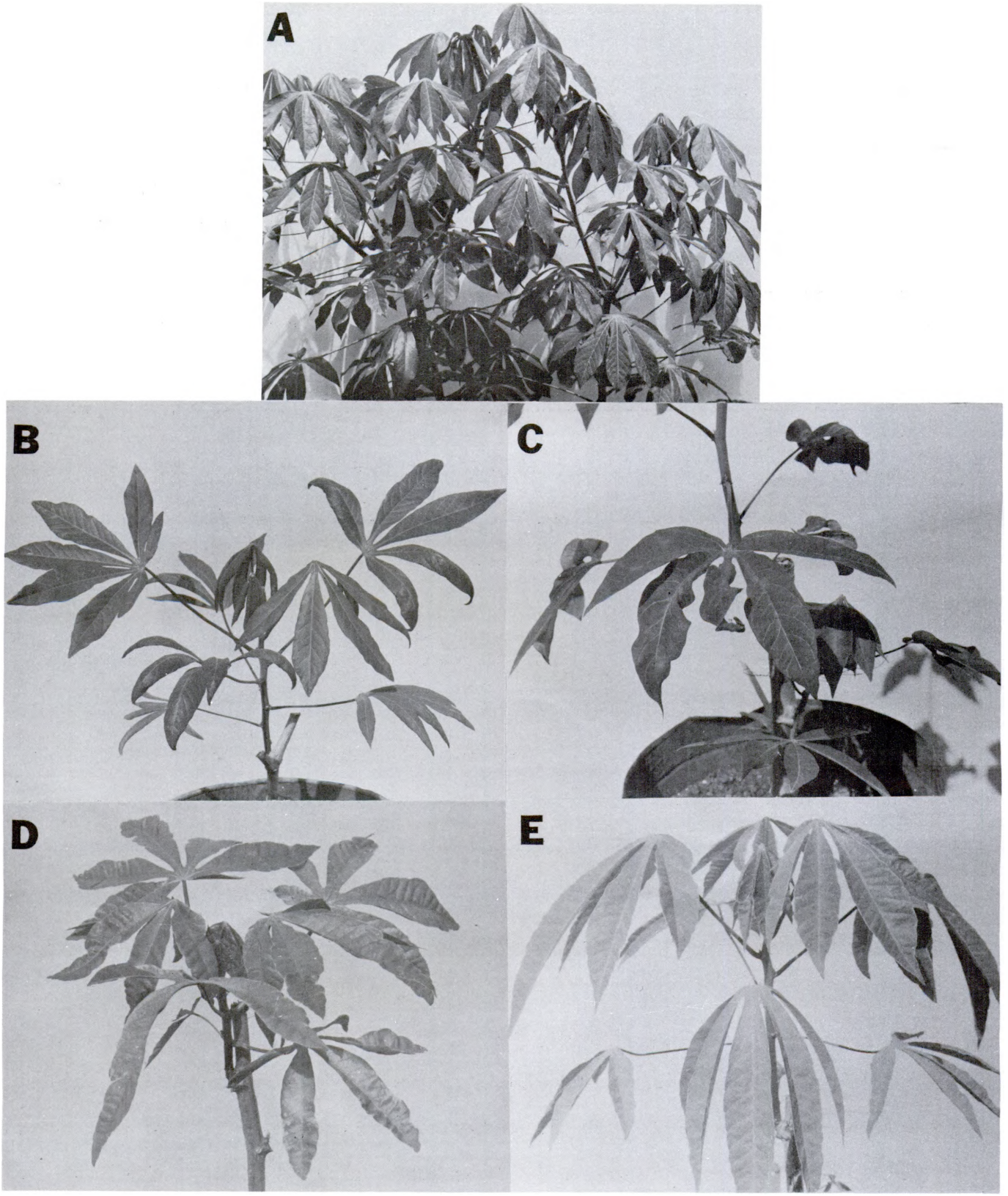

Fig. 5. Induced somatic mutations in Manihot esculenta. Mutations with a change in leaf morphology are common. (A) Normal leaf, (B) Narrow leaf lobe, (C) Curly, (D) Wrinkled, (E) Elongated yellow-green leaf.

It is clear from Table 3 that cultivar No. 49 gave rise to a significantly higher number of noticeable somatic mutations than cultivar No. 68. This is probably due to the difference in the genetic constitution between these two cultivars, as indicated by the cytological evidence that it is abnormal in one and normal in the other (Table 2). It appears that a higher degree of heterozygosity may exist in cultivar 49. A similar situation was encountered in Dahlia in which the flower color mutations can easily be induced in some varieties but none induced in the others (1).

d. Persistance of the induced somatic mutations

Some of the induced somatic mutations from cultivar 49 were isolated and purified in the R_2 generation. Cuttings from these purified mutant plants were propagated in subsequent generations. All the induced mutant characters were perpetuated from one generation to the other. So far, no sign has been noted that the isolated mutants have been changed back to the original parental types.

Table 3. Somatic mutation rate induced by gamma radiation in the cuttings of two Manihot cultivars

Cultivar No.	Treatment (kr)	No. of cuttings irradiated	No. of shoots emerged	No. of mutations	Mutation percent
49	0	48	163	0	0
	3	117	224	25	11.1
	4	36	62	13	21.0
68	0	42	91	0	0
	3	47	84	2	2.4
	4	57	102	3	2.9

e. Inducing mutations by pollen irradiation

Because of the production of chimeric sector in the meristematic tissue after irradiation and the marked protogyny nature of flowering habit in Manihot (the male flowers do not open until the last female flower on the inflorescence has bloomed), it is not possible to bring out the induced recessive mutations by self-fertilization. Therefore, seed irradiation is not an effective means for inducing mutations in Manihot.

We propose to use pollen irradiation as an alternative method for mutation induction. The plant developed from the R_1 seed of irradiated pollen is totally heterozygous without chimera formation and permits self-fertilization among the flowers of different branches. Or, the cuttings of the plant may be planted at different times to coincide with the opening of male and female flowers for crossings.

Cultivar 68 was used as an experimental material, because of its low somatic mutation rate. Mature pollen were irradiated with 2, 3, and 4 kr and pollinated to the female flowers. The fruits were set in all the doses applied. Some R_1 seeds have been obtained in 2 kr treatment. The surprising fact was that there was a great variation in plant morphology, and growth habits among the R_1 plants (Fig. 6). A continuous study will be carried out, as compared with the control (self-fertilization with non-irradiated pollen), to determine whether the variation is due to the pollen irradiation.

f. Haploid induction

As pointed out in the proposal last year, haploid is

Fig. 6. R_1 progenies from pollen irradiation (2 kr) of the Cultivar No. 68 showing the morphological variations among the progenies.

an ideal material for inducing somatic mutations as well as for mutation breeding, especially for the plant species without a sexual cycle, with self-incompatibility, or having a long life cycle. Since there is no Manihot haploid available, various experimental methods are being employed for haploid induction. Hopefully, once the haploid is obtained, the efficiency of somatic mutation induction and mutation breeding method can be increased greatly. This year, attempts were made to induce haploids by using the following methods:

1) Inter-generic hybridization. In Euphobiaceae, Hevea sp. (rubber), Jatropha sp. (frailecillo), and Ricinus sp. (castor oil) are the commonest genera grown in the tropics. Pollen of these species were pollinated to the female flowers of Manihot cultivar 68. It is hoped that the egg of Manihot can be stimulated to develop parthenogenetically. Many crosses were made, and, as expected, many flowers were dropped within a week or two after pollination. However, a number of fruits were also developed from the pollinated flowers (crossed with Ricinus, 34 fruits; with Jatropha, 12 fruits). So far, these fruits have been developed for more than six weeks. When the fruits mature, study will be made to determine whether it is a case of parthenocarpy or a parthenogenetic development.

2) Pollen irradiation. Two cultivars, No. 56 and No. 68, were selected for the pollen irradiation experiments. Cytologically both have normal chromosome pairings of 18 bivalents. Morphologically, No. 56 has green leaf petioles, and a prostrate growth habit. In contrast, No. 68 produces reddish petioles and

erect stems. Since the red color and erect growth are a dominant character in many other plant species, we used No. 68 as a male parent for pollen irradiation and No. 56 as a female. The green and prostrate R_1 progenies would be the suspected haploids. Pollen of cultivar 68 were irradiated with 2 and 3 kr, and 45 and 57 R_1 plants were respectively obtained from the crosses. The R_1 plants are five months old and show an unexpectedly wide morphological variation (Fig. 7). This large variation offers a good opportunity for plant selection in Manihot breeding. Cytological study will be made on those R_1 progenies with strong maternal characters to determine whether they are haploids.

g. Vigorous growth from irradiated cuttings

Occasionally but not consistently, some shoots grown from the irradiated cuttings had very vigorous growth (Fig. 8). The reason for that is not yet understood. Cuttings from the vigorous plants are being propagated to determine whether it is a transmittable character.

References

1. Broertjes, C. and J. M. Ballego. Mutation breeding of Dahlia variabilis. Euphytica 16:171-176. 1967.

2. Glucksman, A. Biological levels of the radiosensitivity of somatic cells. British Journal Radiology 27:660-669. 1954.

3. Jones, W. O. Manioc in Africa. Stanford University Press. 1959. 315 p.

4. Rogers, D. J. Some botanical and ethnological considerations of Manihot esculenta. Economic Botany 19:369-377. 1965.

Fig. 7. A wide morphological variation among the R_1 progenies from the cross between Cultivars No. 56 and No. 68 whose pollen were irradiated with 3 kr.

Fig. 8. Vigorous growth from an irradiated Manihot esculenta cutting. Left, the control (Cultivar No. 68); Right, the vigorous plant grown from the cutting irradiated with 5 kr. Both plants are seven months old.

2. Mutation Breeding in the Common Bean (C. C. Moh, J. J. Alan and J. C. Guerra)

After a few years of work on inducing mutations in the common bean, we found that the induced mutation method is extremely efficient in inducing certain desirable agronomic characters of this crop. For example, mutations of changing the seed-coat colors can be obtained within six to nine months (2 to 3 growing cycles) (3). Since seed-coat colors in beans are determined by the interaction of many color factors and modifiers, to improve seed-coat color by the conventional breeding method requires many generations of back-crossing. The number of crosses would be increasingly larger as the backcross generation advances. Obviously, the conventional method is tedious, time consuming, and costly. Other beneficial mutations, such as the erect type and semi-dwarf type, can also be induced by mutation breeding method with relative ease.

During the past year, we investigated the nutritional aspect of the seed-coat color mutants, further refined the screening technique, and helped other countries to develop seed-coat color mutants in their locally adapted varieties.

a. Toxicity of black beans

Bressani, et al. of the Institute of Nutrition of Central America and Panama (INCAP) reported that when the uncooked black bean was fed to rats, it caused death of rats in less than 14 days (1). Cooked black beans had no such effect. This clearly implies that there is a highly toxic compound (a trypsin inhibitor or

hemagglutinin?) in the raw black beans and cooking can destroy this toxic property. Most black bean varieties as well as the seeds of many leguminous species have this toxicant (2).

In cooperation with INCAP, seeds of the white mutant line (NEP-2) and of its original black variety were sent to INCAP to determine whether the raw bean of the white mutant has the same toxic effect as its black parental line. Feeding experiments are being performed in the laboratory of INCAP. The results will determine whether the induced seed-coat color mutants have additional advantage over their parent.

b. Correlation between seed-coat colors and other seedling characters--further refinement of the screening technique

The previous report has shown that there is a correlation between the seed-coat color and the hypocotyl color in beans. This correlation is used as a screening technique for isolation of the possible seed-coat color mutants induced from a black bean variety. However, we found that some red bean varieties produce green hypocotyls. Thus, it is not possible to isolate the seed-coat color mutants from the red beans by using the present screening technique. Further observation on more than 270 varieties in our bean collection revealed that two other seedling characters, the color of cotyledon and the color of leaf vein, are also correlated to the seed-coat colors. The results presented in Tables 4 and 5 demonstrate these relationships.

These results not only provide a method for isolation of the possible white seed mutant from red bean varieties, but also give an added assurance in selecting the seed-coat color mutants from the

Table 4. Correlation between seed-coat color and cotyledon color in the common bean*

Seed-coat color	Cotyledon color		Total varieties observed
	Red mottling	Green or yellow-green	
Black	93	0	93
Bayo	31	28	59
Red	27	49	76
White	0	43	43

* A test of independence shows that: $\chi^2=141.01$, D.F.=3, P<.01

Table 5. Correlation between seed-coat color and leaf vein color in the common bean*

Seed-coat color	Leaf vein color		Total varieties observed
	Red	Green	
Black	93	0	93
Bayo	40	19	59
Red	57	19	76
White	0	43	43

* A test of independence shows that: $\chi^2=141.00$, D.F.=3, P<.01

black seed varieties.

c. Inducing seed-coat color mutants for other countries

Since breeding of seed-coat colors can be considered as a simple task by using induced mutation technique, countries in Latin America and Africa have either requested our mutant seeds for

experimental trials or sent their locally adapted varieties for the mutation induction. A student from Peru is carrying out the mutation breeding work to fulfill the requests.

References

1. Bressani, R., et al. Effect of cooking and of amino acid supplementation on the nutritive value of black beans (Phaseolus vulgaris L.) Brit. J. Nutr. 17:69-78. 1963.

2. Liener, I. E. Hemagglutinins in foods. In Toxicants Occurring Naturally in Foods, Publication 1354. National Academy of Sciences, Washington, D. C. 1966. pp. 51-57.

3. Moh, C. C. Mutation breeding in the seed-coat colors of beans (Phaseolus vulgaris L.) Euphytica 20:119-125. 1971.

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

This year, radiosensitivity of the species in two plant families, Euphorbiaceae and Solanaceae, was investigated. This included a total of four species representing four genera in Euphorbiaceae and a total of nine species representing three genera in Solanaceae. Young plants of these species were grown radially from the cesium-137 source in the gamma field and exposed to chronic gamma radiation for 20 hours per day. This permits us to determine the maximum daily dose level at which the plants can complete their life cycle by producing viable seeds.

Fig. 9 demonstrates the growth response of the tested species at different daily dose levels after various durations of exposure in the gamma field. While information on some species has not been completed because of their longer life cycle, the data on flowering and fruiting capability definitely show the radiosensitivity trend.

In Euphorbiaceae, Ricinus (castor oil) is capable of producing viable seeds at 183 r per day, and Jatropha (frailecillo) and Manihot (yuca) can produce flowers and fruits from 200-300 r per day. All these species can be classified into the resistant group. In Solanaceae, undoubtedly there is a wide range of radiosensitivity among the species. While Solanum topiro produced flowers and fruits at 25 r per day, Solanum nigrum completed their life cycle at a daily dose of 248 r.

In addition, we have completed the radiosensitivity study on the species of Convolvulaceae and Leguminosae which was carried on from last year because of the longer life cycle of the plants. The results are presented in the latter part of Fig. 9. The added results do not alter greatly the conclusion drawn from last year that the species of Convolvulaceae are generally radioresistant and the species of Leguminosae have a wide range of radiosensitivity.

A summary of the radiosensitivity range of the species in the plant families so far studied is presented in Table 6.

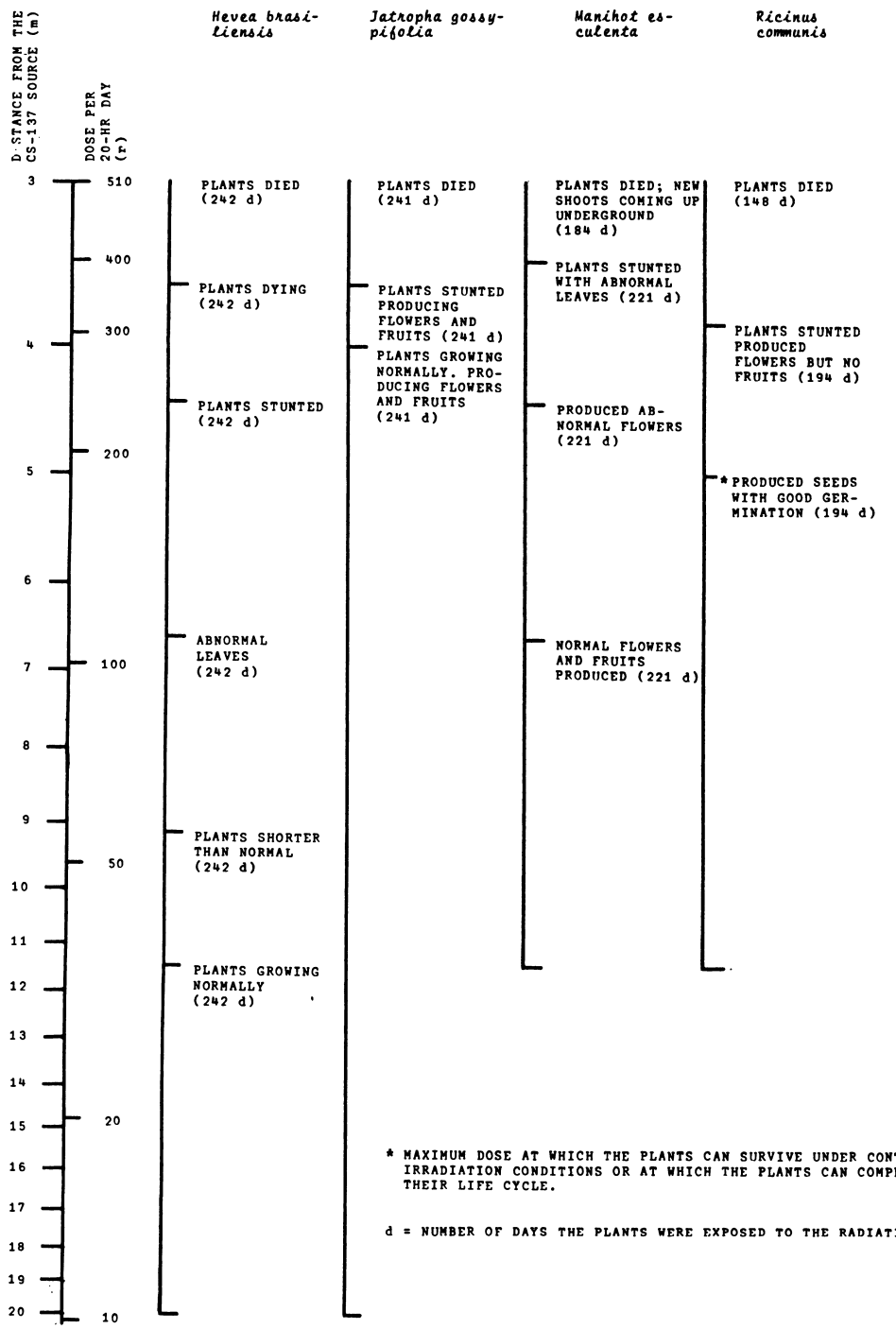

FIG. 9. GROWTH RESPONSE OF THE TROPICAL SPECIES IN CONVOLVULACEAE, EUPHORBIACEAE, LEGUMINOSAE AND SOLANACEAE TO CHRONIC GAMMA RADIATION. DISTANCE FROM THE CESIUM-137 SOURCE AND THE DOSIMETRY IN THE GAMMA FIELD ARE IN LOG SCALE.

FIG. 9. CONTINUED...

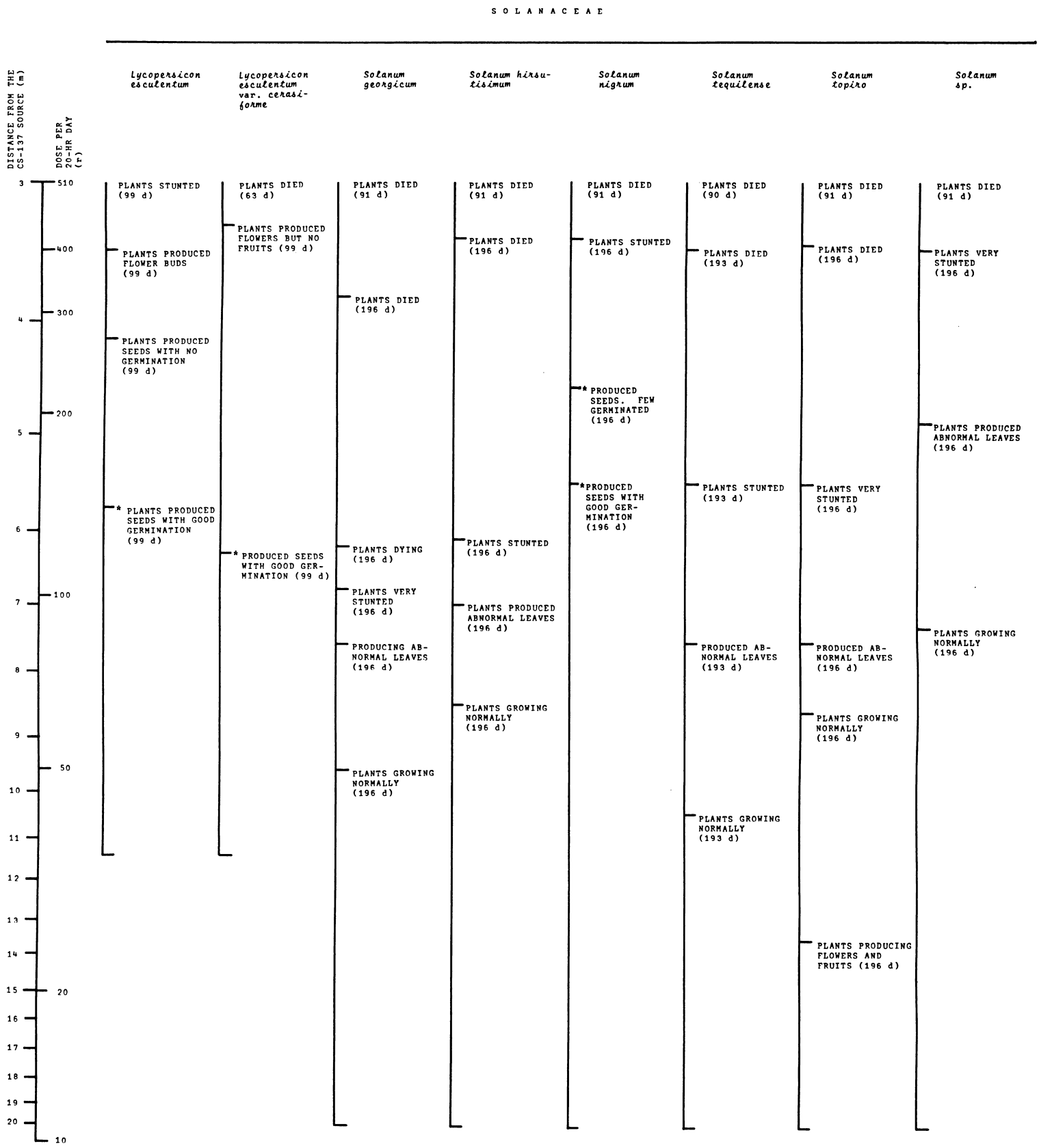

FIG. 9. CONTINUED...

C O N V O L V U L A C E A E

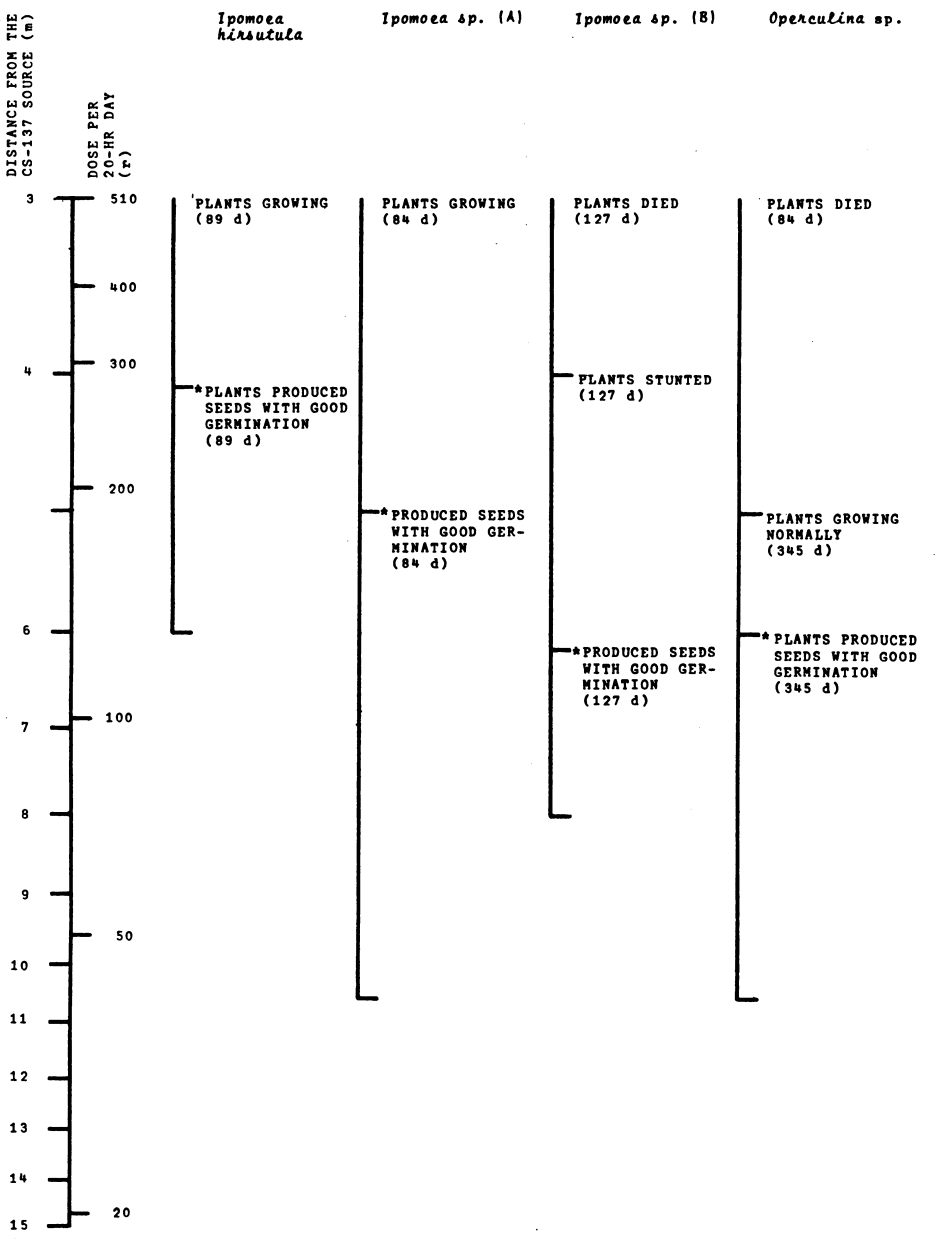

FIG. 9. CONTINUED...

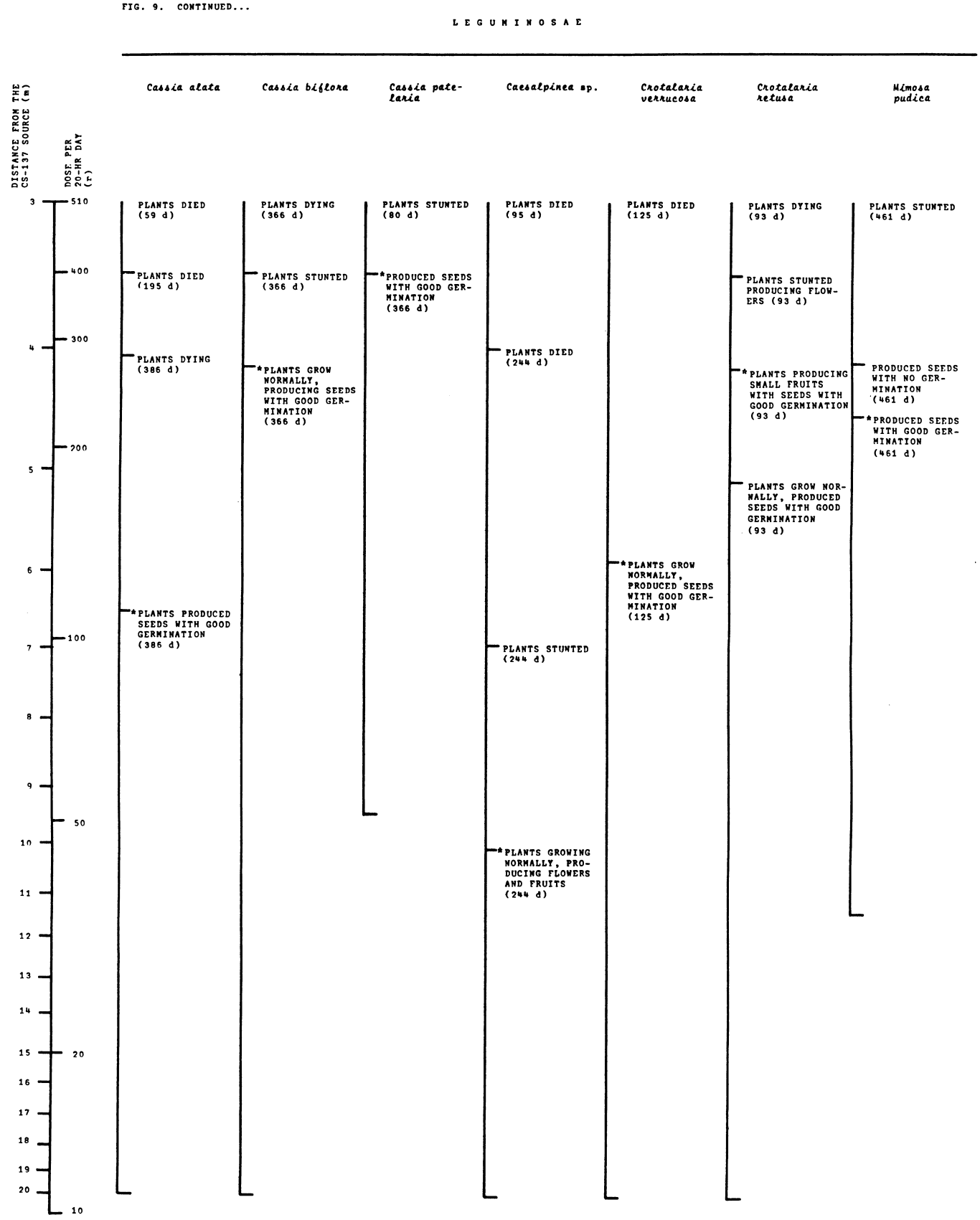

Table 6. Radiosensitivity range of the tropical plant species in Gymnosperms and other plant families

Plant group or families	No. of genera	No. of species	Range of maximum dose the species can tolerate or complete the life cycle (r per day)	Radiosensitivity classification
Gymnosperms	5	5	5 (*Pinus*) -- 27 (*Cycas*)	sensitive
Dicotyledons:				
Convolvulaceae	4	7	116 (*Ipomoea* sp.) -- 248 (*Ipomoea hirsutula*)	resistant
Euphorbiaceae	4	4	183 (*Ricinus communis*) -- 210 (*Jatropha gossypifolius*)	resistant
Leguminosae	15	29	25 (*Vicia faba*) -- 400 (*Cassia patelaria*)	wide range
Solanaceae	3	9	25 (*Solanum topiro*) -- 248 (*Solanum nigrum*)	wide range

B. CONTROL OF INSECTS BY MALE STERILIZATION METHOD

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

a. Effect of gamma irradiation on mating competitiveness and sexual maturity of the Medfly males.

Purpose and Methods

Last year experiments carried out in the laboratory in small cages (1 ft^3), indicated that irradiation of the Medfly males (in late pupal stage) with 6, 8, or 10 kr, did not delay the rate of sexual maturity of treated males. When irradiated males (6, 8, or 10 kr) and normal males were simultaneously confined in cages with normal females, irradiated males mated less frequently than normal males. This year the experiment was repeated in the field in large cages (9' x 9' x 7') to determine under outdoor conditions: 1) the mating competitiveness of Medfly males sterilized with different doses; and 2) the effect of irradiation on the rate of sexual maturity of the treated males.

The male Medflies used in the tests were irradiated in the late pupal stage (24 hr prior to adult emergence). Effects of three sterilization levels (6, 8 and 10 kr) on mating competitiveness and rate of sexual maturity of treated males were studied. All the flies used in the experiments emerged between 5:00 AM - 3:00 PM and the sexes were separated within 27 hr after emergence.

One hundred twenty-five unmated males of each treatment (0, 6, 8 and 10 kr) were released with 250 virgin females in a cage with

2-3 coffee plants. Before fly release, the leaves of the coffee plants were thinned out to facilitate quick location of mating pairs. At the time of release, adults were approximately 48 hr old. Release was not affected with flies younger than 48 hr because mating frequency of Medflies less than 2 days old is very low.

Fly release was affected between 8:00 - 8:30 AM. Mating pairs were collected individually in small shell vials. Time of capture of each mating pair was noted on the vials. The males of different treatments were marked with different fluorescent powder dyes by mixing the dye powder with the pupae. Later on males were identified in the laboratory under a dissecting microscope using ultraviolet light to illuminate the dye color mark present on the ptelenum.

In order to eliminate 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. In all the tests normal females were always marked with the dye color used to mark the normal males, since normal males and normal females used in any one test came from one single batch of pupae.

Mating pairs were constantly collected by two persons for three days as follows: first day for 8 hr (8:00 AM - 4:00 PM), second day for 8 hr (7:00 AM - 3:00 PM) and the third day for 4 1/2 hr (7:00 AM - 11:30 AM). An attempt was not made to collect mating pairs before 7:00 AM. Preliminary observations made before actual tests, indicated that Medflies do not mate until after 7:00 AM under our outdoor

conditions. The roof of the cage was covered with white transparent polyethylene sheet to portect the flies from rain. The experiment was carried out on five different times, each time using a different batch of pupae.

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Table 7 presents the total number of males of each treatment mated during a 3-day pariod. As found under laboratory conditions, results of field experiments showed that the irradiation of Medfly males at pupal stage (24 hr before emergence) adversely affects the mating vigor of the treated males. Mating vigor of irradiated males is reduced approximately by 24-35%. Males irradiated with 6, 8 and 10 kr mated 65.3, 75.6 and 69.5%, respectively, as frequently as normal males.

Table 7. Effect of different sterilization doses on the mating competitiveness of the Medfly males[a]

	Number of matings[b] by different age males				
	2-day	3-day	4-day	Total	% matings
Normal	69	156	37	262	100.0
6 kr	36	102	33	171	65.3
8 kr	49	120	29	198	75.6
10 kr	35	118	29	182	69.5

[a] Irradiated at pupal stage 24 hr prior to emergence.

[b] Based on total of 5 tests. In each test, 250 virgin normal females were released in screened outdoor cages (9' x 9' x 7') with 500 unmated males (125 males of each treatment).

Table 8 presents the hourly mating frequency data of males of each treatment (0, 6, 8, and 10 kr) for the 3-day test period. The results indicate that irradiation of Medfly males up to 10 kr, does not delay the rate of sexual maturity of treated males. Throughout the test period of three days, the hourly mating frequency pattern of irradiated (6, 8, or 10 kr) males is similar to that of normal males.

On the second day of the test, when the majority of the males had reached sexual maturity, 59.5% normal male matings took place compared to 64.6% 10 kr sterile male matings. Hourly mating frequency of different type males for this day (i.e. when males were three days old) is presented in Fig. 10. The curves clearly indicate that the mating frequency pattern of normal males and irradiated (6, 8, and 10 kr) males is similar. The curve for normal males is higher (in position) than irradiated males since more females mated with normal males than with irradiated males. Peak mating frequency took place between 8-9 AM in normal as well as in irradiated males. A gradual decline in mating frequency for all treatments is noticed after 9 AM.

b. Induction of visible Medfly mutants by gamma irradiation

Purpose and Methods

Availability of a good visible genetic marker is very useful in the present campaign of Medfly eradication by sterile insect releases. In the past two years efforts were made to breed a visible Medfly mutant by treating the males with ethyl methanesulfonate

Table 8. Mating competitiveness data of normal and irradiated (6, 8, and 10 kr) Medfly males released simultaneously with normal females in field cages[a]

Age of males	Observation time	Percent male matings[b] observed during 3-day periods in various types of males					
		N	6 kr		8 kr		10 kr	
2 days	9-10 AM	0.0	(0)	0.6	(1)[c]	0.5	(1)	0.5	(1)
	10-11 AM	6.3	(17)	4.7	(8)	7.1	(14)	6.0	(11)
	11-12 AM	7.3	(19)	2.3	(4)	3.0	(6)	2.7	(5)
	12- 1 PM	5.0	(13)	6.4	(11)	6.6	(13)	3.8	(7)
	1- 2 PM	5.7	(15)	5.8	(10)	6.1	(12)	5.5	(10)
	2- 3 PM	1.5	(4)	1.2	(2)	0.5	(1)	0.5	(1)
	3- 4 PM	0.4	(1)	0.0	(0)	1.0	(2)	0.0	(0)
	Total	26.2	(69)	21.0	(36)	24.8	(49)	19.0	(35)
3 days	7- 8 AM	3.8	(10)	3.5	(6)	5.6	(11)	4.9	(9)
	8- 9 AM	16.0	(42)	16.4	(28)	19.2	(38)	18.7	(34)
	9-10 AM	11.4	(30)	11.7	(20)	10.6	(21)	11.5	(21)
	10-11 AM	10.3	(27)	14.0	(24)	10.1	(20)	10.4	(19)
	11-12 AM	7.3	(19)	3.5	(6)	4.0	(8)	5.5	(10)
	12- 1 PM	5.7	(15)	2.3	(4)	5.0	(10)	8.2	(15)
	1- 2 PM	3.4	(9)	6.4	(11)	4.0	(8)	4.9	(9)
	2- 3 PM	1.6	(4)	1.8	(3)	2.0	(4)	0.5	(1)
	Total	59.5	(156)	59.6	(102)	60.5	(120)	64.6	(118)
4 days	7- 8 AM	0.4	(1)	1.8	(3)	0.5	(1)	0.0	(0)
	8- 9 AM	4.6	(12)	5.3	(9)	3.0	(6)	5.5	(10)
	9-10 AM	5.0	(13)	7.6	(13)	6.6	(13)	6.6	(12)
	10-11 AM	4.2	(11)	4.7	(8)	4.5	(9)	3.3	(6)
	11-11:30	0.0	(0)	0.0	(0)	0.0	(0)	0.5	(1)
	Total	14.2	(37)	19.4	(33)	14.6	(29)	15.9	(29)

[a] Cages (9' x 9' x 7') were erected over 2-3 coffee plants in the field

[b] Based on total of five tests. In each test, 250 virgin normal females were released with 500 unmated males (125 males of each treatment). In each column total matings for 3 days = 100%.

[c] Figures in parentheses are total number of matings for which percentages are calculated.

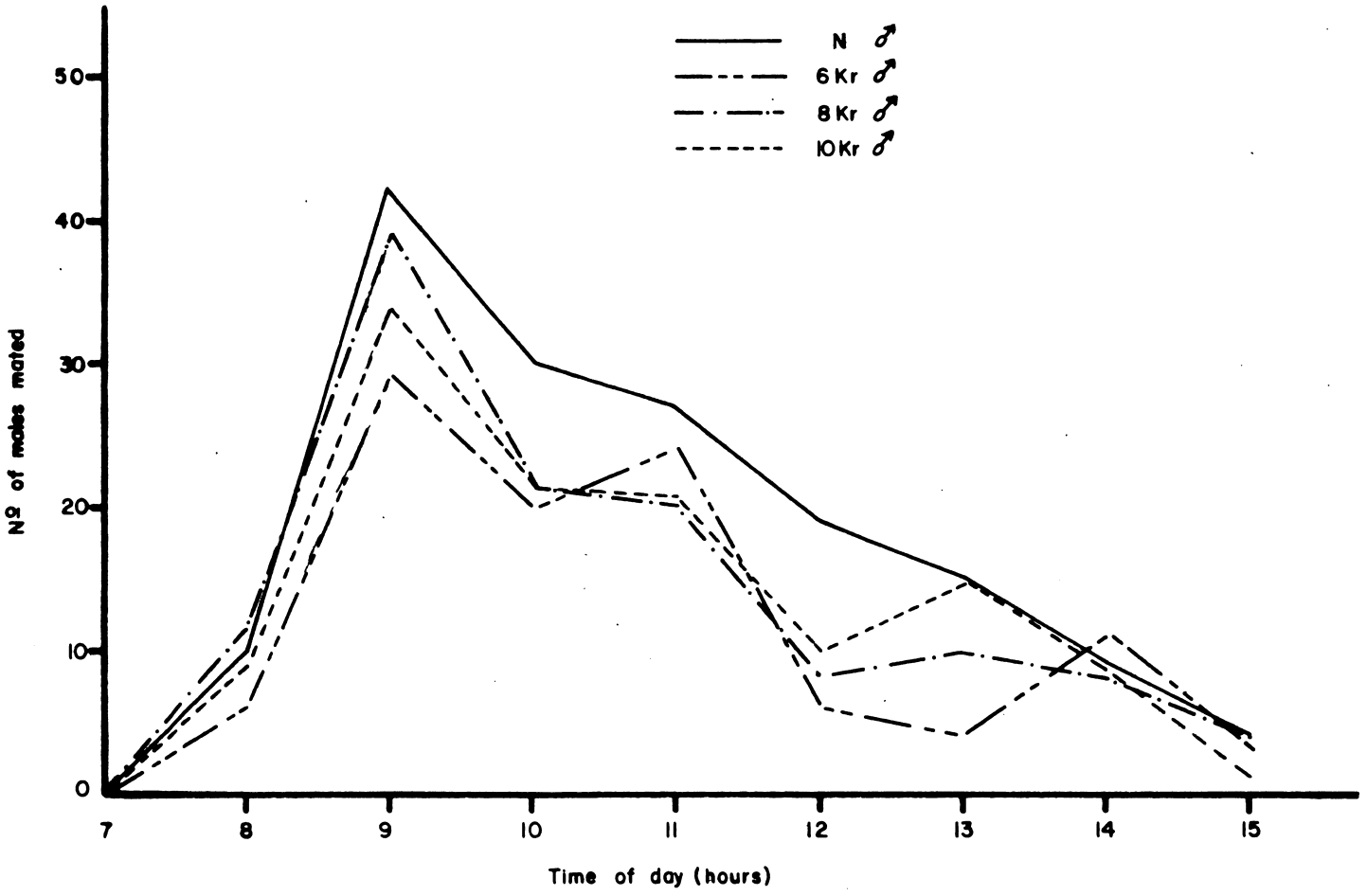

Fig. 10 Mating frequency pattern of 3 day old normal and irradiated Medfly males

(EMS). We were unable to get successful results. Therefore, we discontinued the use of EMS and since March, 1971, we started using gamma irradiation to breed for visible Medfly mutants.

The test procedure is as follows. Approximately 200, 2-day old males are irradiated with 2500 kr (inducing about 50% sterility in treated males). Three to four days after irradiation, treated males are allowed to mate in mass with untreated virgin females of the same age. One hundred mating pairs are removed and placed individually in cages to obtain F_1 flies. F_1 and F_2 adults of a single pair are allowed sib matings in mass. F_3 flies are examined for visible mutants.

Progress Report

We have examined F_3 flies from 50 individual pairs and so far no visible mutants have been found. We will continue this work next year.

2. Studies on the Biology and Sterilization of the Coffee Leaf Miner, Leucoptera coffeella (Guérin-Méneville)

a. Gamma irradiation of the coffee leaf miner (K. P. Katiyar and J. A. Reyes)

1) Effects of sterilization on fertility, fecundity, and longevity of the coffee leaf miner adults

Purpose and Methods

Studies on the feasibility of controlling the coffee leaf miner by gamma sterile male technique have been continued. Results of the preliminary work on the irradiation of pupal and adult stages of this insect were reported previously. This year detailed experiments

were carried out to study the effects of irradiation (applied to the late pupal stage and newly emerged moths) on the fertility, fecundity, longevity and mating ability of treated moths.

The coffee leaf miner is a very destructive pest of coffee in all the coffee growing countries of the Western Hemisphere. The only satisfactory control of this insect is the use of systemic insecticides which are highly hazardous to human beings and domestic animals. Also, many insect species have acquired resistance to some of the most powerful insecticides like DDT and BHC. It is therefore highly desirable to find some alternate control method for this insect. Under this project, work is being carried out to evaluate the feasibilities of controlling the coffee leaf miner by gamma sterile insect releases.

The coffee leaf miner used in the experiment came from a stock culture collected at the IICA farm and maintained in the laboratory for 10-12 generations. The larvae were reared in the laboratory on coffee plants as reported previously (1). Uniform aged pupae used for irradiation studies were obtained by spreading small coffee twigs with leaves underneath the infested coffee plants in the morning between 7:00 and 8:00 AM. Full grown larvae left the mines and pupated on these leaves. The twigs were removed at 5:00 PM in the evening. Thus the age of these pupae varied from 0-10 hr.

Irradiation was performed in a pool-type ^{60}Co source at a dose rate of ca. 1700 r/m. Pupae were irradiated in mass in a 52-mesh screened cylinder. The adults were irradiated individually in 5 ml shell vials with screened caps to facilitate aeration within the

steel canister. The canister itself was not aerated during irradiation.

Treated moths were confined in wooden framed cages (19 cm large, 12 cm wide and 14 cm high). The top and two lateral sides of the cages were covered with fine nylon cloth. Each cage had 10 pairs of moths.

Since adult coffee leaf miners are very fragile, the late stage pupae were stored individually in shell vials with screen lids. This allowed adult sexing without anaesthetizing with CO_2 and also assured a supply of virgin moths for the experiments.

In the tests of pupal irradiation, males were irradiated 23-14 hr before emergence with doses ranging from 10-60 kr. Female pupae were irradiated 21-14 hr before eclosion with 7 different doses ranging from 2-40 kr. Pupae were not irradiated with doses higher than 60 kr because this dosage has been found lethal to the male pupae (1).

Adult males were irradiated 4-21 hr after emergence with 11 different doses ranging from 10-90 kr. The female moths were irradiated 15-23 hr after emergence with 10 different doses between 1-40 kr. The wider age range (4-21 hr) of males (at the time of irradiation) is due to the longer irradiation period required for high doses given to male moths.

To study the effect of irradiation on the fertility and fecundity of moths, the females were given daily fresh coffee leaves for oviposition. A single leaf with petiolate was put in 50 ml Erlenmeyer flask with tap water. The mouth of the flask was closed with cotton to hold the coffee leaf in position and to avoid accidental drowning

of adults in the water. Daily egg collection from each cage was made for eight consecutive days following crosses (adults were paired immediately after irradiation). The eggs were incubated for 5-9 days before checking eclosion. The ability of newly emerged larvae to establish successful mine in the leaf was used as a criterion to determine egg viability. The adults were fed 10% sugar solution in 50 ml flasks through paper cellulose wicks. The sugar solution was not changed throughout the experiment.

Daily adult mortality was recorded for each sex until all the moths were dead.

All of the experiments were carried out in the laboratory at temperatures of 25±3°C and relative humidity of 73±6%. All the treatments were replicated five times except when specified otherwise.

Results

Radiation effects on adult fertility

The results of gamma irradiation on fertility of the male coffee leaf miner (treated as late stage pupal or as newly emerged moths) are presented in Table 9 and Fig. 11. The results indicate that there is no difference in radiation sensitivity of males irradiated either as pupae or as adults.

The percent hatch of eggs from females mated to males irradiated during pupal or adult stage is similar at every radiation level tested (up to 60 kr).

Effect of irradiation on the male is negligible until 20 kr. From 20 to 60 kr the rate of sterility increased linearly with

Table 9. Fertility of male coffee leaf miner, Leucoptera coffeella (Guérin-Méneville) irradiated as pupae[a] or as adult[b] and crossed with untreated virgin females[c]. (Average of 5 replicates; 10 pairs per rep.).

Dose in kr	Pupae		Adults	
	Total eggs examined	Av. % hatch ± S d	Total eggs examined	Av. % hatch ± S d
0	3114	95.0±0.81	3132	95.4±1.30
10	3083	88.8±1.42	3408	90.0±0.82
20	2787	80.5±1.27	2865	80.1±5.02
30	3075	62.4±2.64	2732	60.8±7.31
40	2991	42.3±2.01	2805	46.4±8.59
45	--	---	2554	30.4±3.94
50	3062	24.2±2.48	2770	23.1±3.54
55	--	---	2886	16.7±2.18
60	2818	19.3±2.69	2547	10.9±2.51
70	--	---	2437	4.5±2.69
80	--	---	2676	1.2±0.72
90	--	---	2716	0.2±0.22

[a] Irradiated 23-14 hr before emergence

[b] Irradiated 4-21 hr after emergence

[c] Eggs were collected for eight consecutive days following crosses.

increase in dose. Beyond 60 kr the rate of sterility does not increase in proportion to increase in radiation dose.

The dose-response curve for induced sterility of the male coffee leaf miner irradiated either as lat-stage pupae or as newly emerged moths, seems to be of two-hit nature. A dose of 10 kr did not produce much sterility (88.8±1.42% egg-hatch when pupae were irradiated compared to 95.0±0.81% egg-hatch of the control and 90.0±0.82% egg-hatch when adults were irradiated compared with 95.4±1.3% egg-hatch for the check). Saturation caused a reduction in the rate at which the effect increased with dose as the sterility

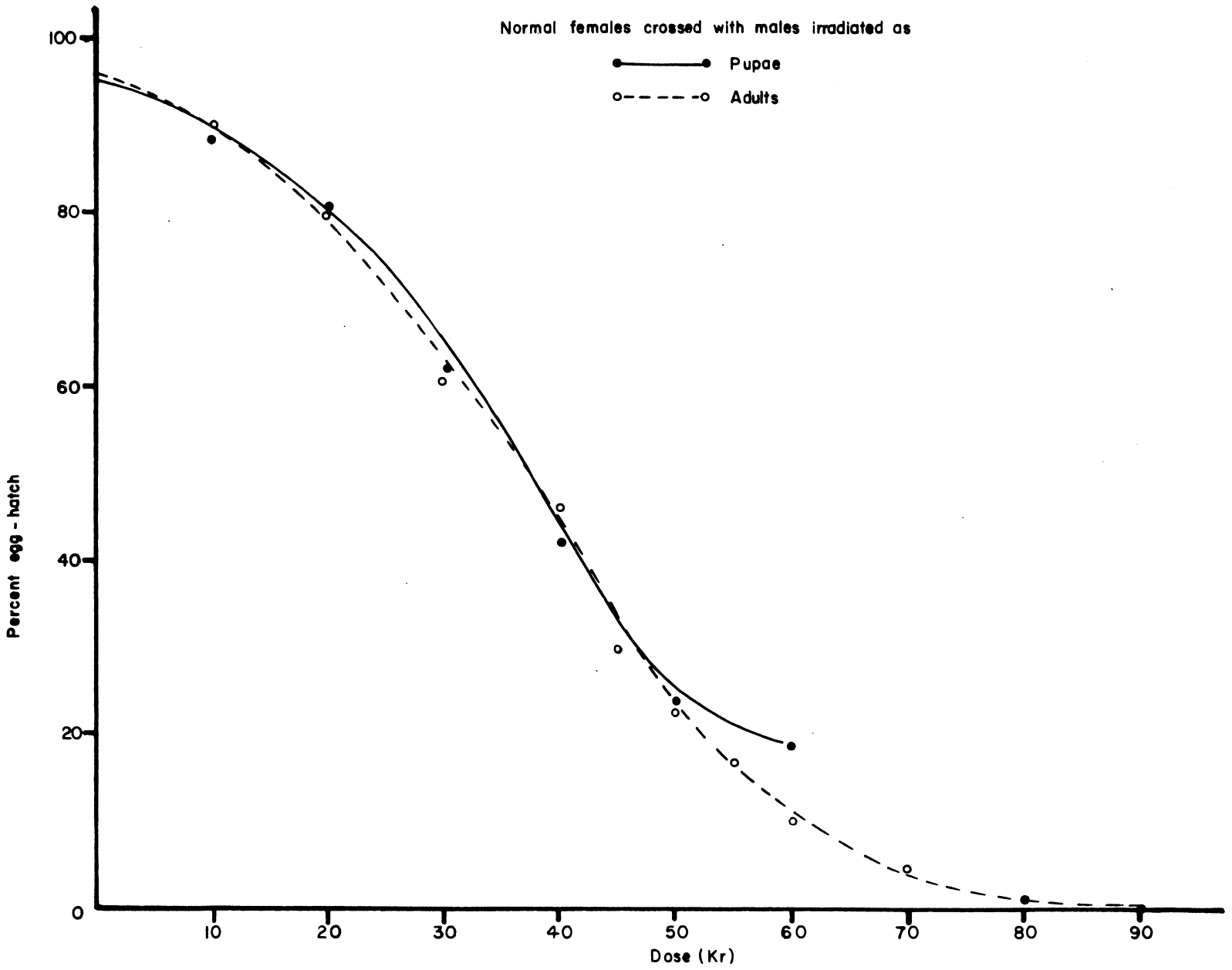

Fig. II Effects of gamma irradiation on fertility of coffee leaf miner males

approached 100%. A dose of 90 kr is needed to achieve more than 99% sterility (0.2±0.22% fertility).

Summarized results of the effects of irradiation on the fertility of the female coffee leaf miner irradiated as pupae (21-14 hr before emergence)or as adults (15-21 hr after emergence) are presented in Table 10 and Fig. 12. The dose-response curve for induced sterility in females also seems to be sigmoidal (Fig. 11). However, more data were needed between 0 and 2 kr in order to determine the real shape of the curve.

Females of the coffee leaf miner are equally radiosensitive when irradiated either as late pupae or as newly emerged moths. Dose-response curves for female fertility irradiated as pupae or as adults are very similar (Fig. 12). Percent egg-hatch (Table 10) of pupal irradiation is comparable to that of adult irradiation at every dose level tested (1-40 kr).

A dose of 30 kr induces more than 99% sterility in females irradiated either as pupae or as adults (0.5±0.33% egg-hatch when pupae were irradiated and 0.2±0.37% egg-hatch when adults were irradiated). At a 40 kr dose level females irradiated in the pupal stage seem to be slightly more radiosensitive than those irradiated in the early adult stage. Females irradiated with 40 kr during the pupal stage were 100% sterile compared to 0.2±0.13% fertility retained by the females irradiated with 40 kr during the adult stage.

Females of the coffee leaf miner are more radiosensitive (when measured in terms of fertility) than males. A dose of 10 kr

Table 10. Fertility[a] of female coffee leaf miner, Leucoptera coffeella (Guérin-Méneville) irradiated as pupae[b] or as adults[c] and crossed with untreated males. (Average of 5 replicates; 10 pairs per rep.)

Dose in kr	Pupae		Adults	
	Total eggs examined	Av. % hatch ± S d	Total eggs examined	Av. % hatch ± S d
0	3416	96.9±0.92	3328	96.9±0.77
1	--	---	1942	84.5±0.66[d]
2	3294	79.5±3.61	3222	76.0±1.92
4	2813	57.3±4.14	3163	53.8±5.86
6	2548	36.0±6.54	3361	34.9±4.25
8	--	---	2763	22.7±3.40
10	2363	13.5±3.72	3069	18.7±3.10
15	--	---	2084	7.5±7.60[e]
20	1238	1.3±0.73	2277	1.4±0.90
30	1146	0.5±0.33	1683	0.2±0.37
40	744	0.0±0.00	1741	0.2±0.13

[a] Eggs were collected for eight consecutive days following crosses

[b] Irradiated 21-14 hr before emergence

[c] Irradiated 15-21 hr after emergence

[d] Based on three replications

[e] Based on four replications

induced very little sterility in males (Table 9: 88.8±1.42% hatch in pupal irradiation and 90.0±0.82% hatch in adult radiation) and a high degree of sterility (Table 10: 13.5±3.72% hatch in pupal radiation and 18.7±3.10% hatch in adult irradiation) in females.

Radiation doses of 90 and 30 kr are required to induce more than 99 % sterility in males and females respectively.

Radiation effects on adult fecundity

Table 11 and Fig. 13 present the fecundity of untreated females

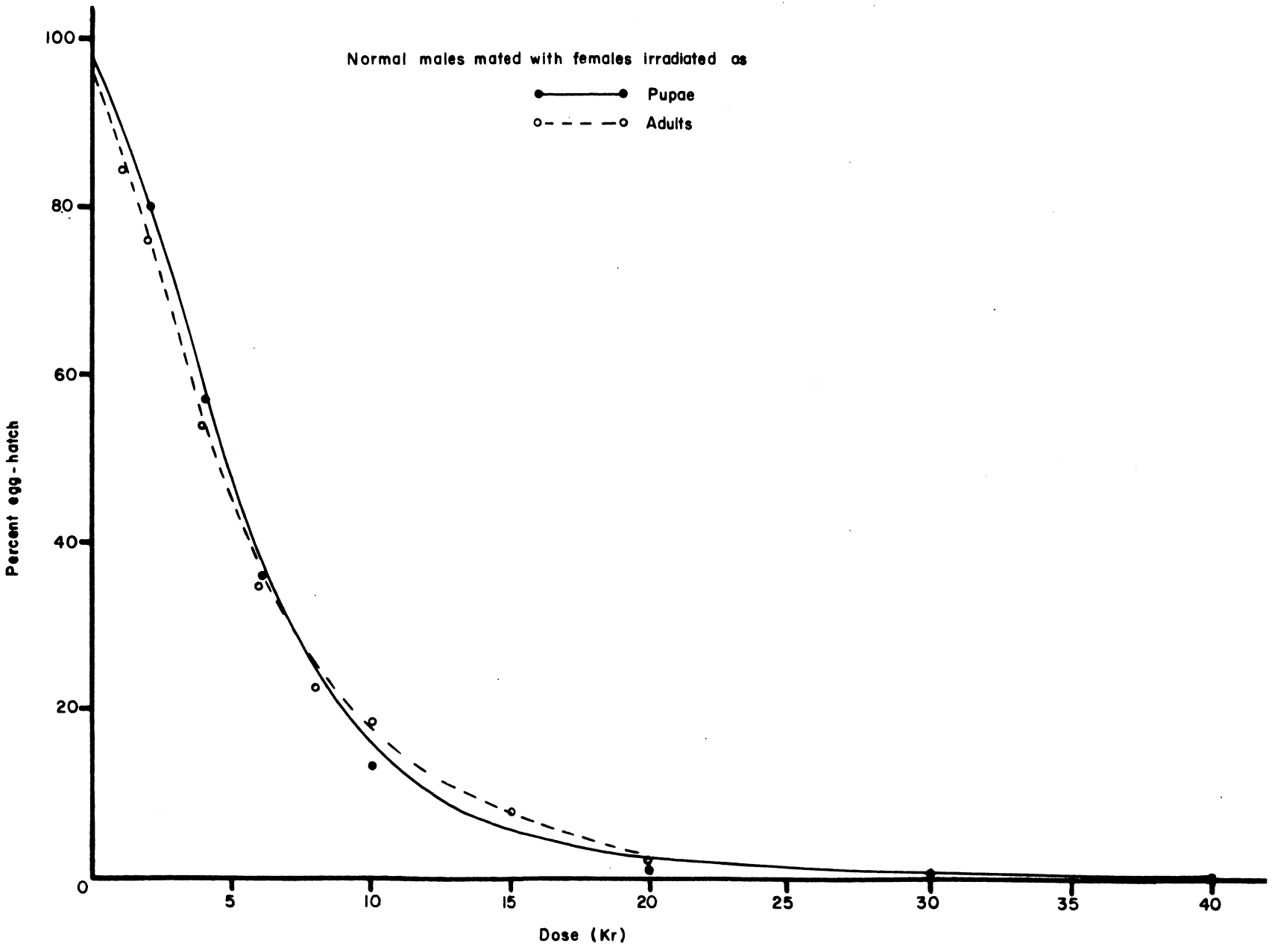

Fig. 12 Effects of gamma irradiation on fertility of the coffee leaf miner females

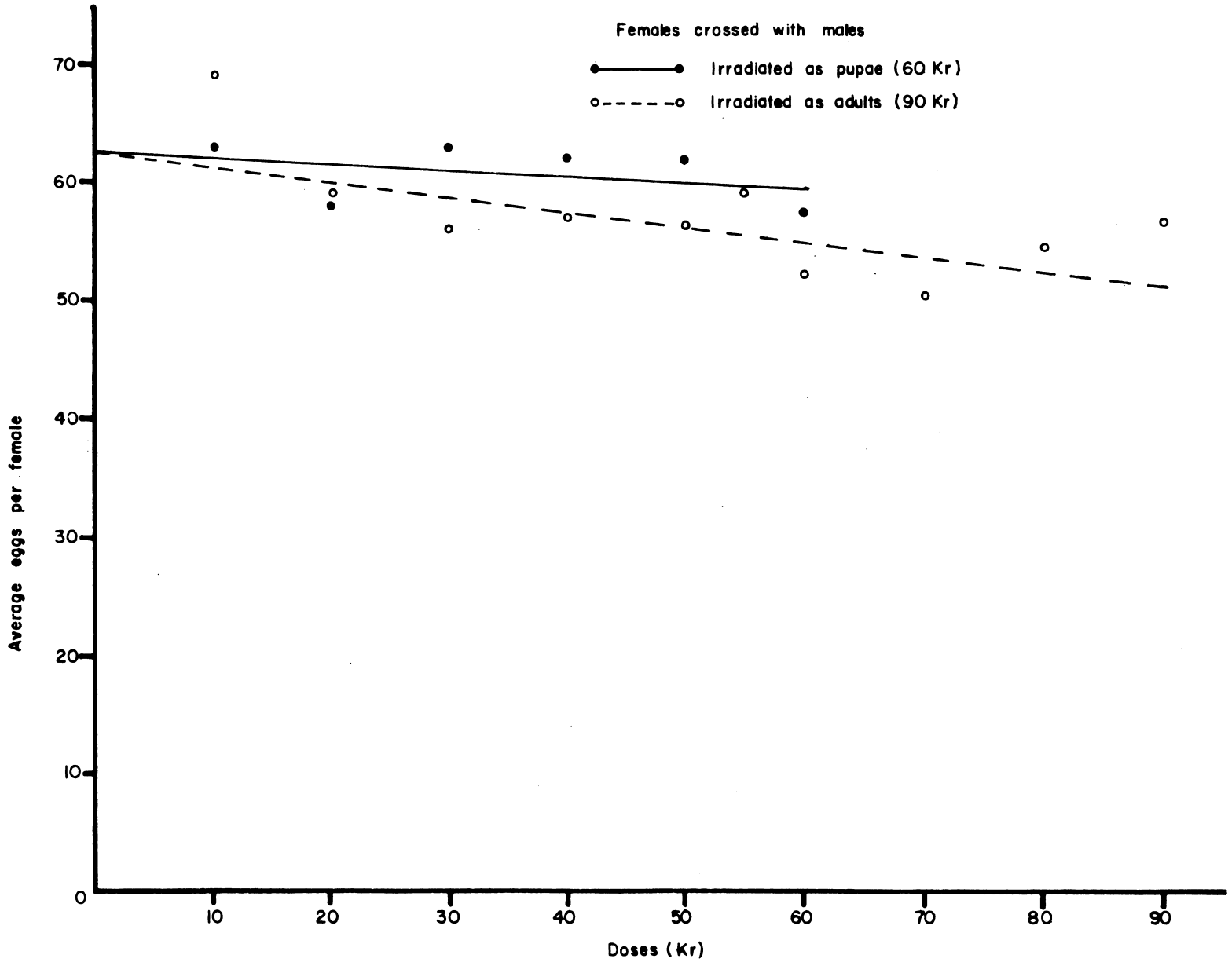

Fig. 13 Fecundity of normal coffee leaf miner females crossed with irradiated males

Table 11. Fecundity of untreated female coffee leaf miner, Leucoptera coffeella (Guérin-Méneville) when crossed with males irradiated as pupae or as adults

Dose in kr	Av. eggs/female[a] (±Sd) when crossed with males irradiated as pupae[b]	irradiated as adults[c]
0	63 ± 5.29	63 ± 7.27
10	63 ± 12.19	69 ± 14.57
20	58 ± 7.76	59 ± 12.70
30	63 ± 5.26	56 ± 19.73
40	62 ± 10.05	57 ± 13.79
45	---	52 ± 15.63
50	62 ± 8.32	56 ± 15.00
55	---	59 ± 12.76
60	57 ± 10.60	52 ± 10.21
70	---	50 ± 18.23
80	---	54 ± 7.29
90	---	56 ± 20.25

[a] Eggs were collected during 8 consecutive days following crosses

[b] Irradiated 23-14 hr before emergence

[c] Irradiated 4-21 hr after emergence

crossed with irradiated males. Irradiation of males (during pupal or adult stage) seems to have little effect on the oviposition of the females to which they are mated. A linear decrease in oviposition capacity of normal females is noticed with the increase in sterilization dose of males to which these females are mated. However, the rate of decline in fecundity is very low (0.057 eggs per female in pupal irradiation and 0.130 eggs per female in adult irradiation for each kr increase of irradiation dose). The average fecundity per female during an 8-day oviposition period was 57±10.60 and 56±20.25 eggs respectively after mating with males irradiated as pupae with 60 kr and as adults with 90 kr. Normal females mated

with untreated males, laid an average of 63 eggs per female during same oviposition period.

The effect of irradiation on the fecundity of females treated with different doses varying from 1-40 kr is presented in Table 12 and Fig. 14. The results suggest that an increase in irradiation dose caused a decrease in oviposition capacity of the treated females.

Adverse effects of irradiation on female fecundity is more noticeable when treatment is applied to the pupal stage compared to irradiating the adult stage. This seems to be due to the presence of larger numbers of radiation (40 kr) resistant eggs from newly emerged females (15-21 hr after emergence) than from females at late pupal stage (21-14 hr before emergence). Average fecundity of females irradiated (40 kr) during pupal and adult stages were 15±3.11 and 35±5.26 eggs respectively compared to 68-69 eggs oviposited by a normal female.

Radiation effects on adult longevity

It is of practical importance that irradiated insects to be released in the field should live as long as untreated insects. The daily accumulated adult mortality for males and females irradiated as pupae and as adults is presented graphically in Figures 15, 16, 17 and 18. Tables 13 and 14 present the T_{50} values (time in days when 50% of the moths are dead) of male and female moths respectively.

When pupae were treated, the lifespan of irradiated males was significantly shortened (at 5% level) at all the doses tested

Table 12. Fecundity of female coffee leaf miner, Leucoptera coffeella (Guérin-Méneville) irradiated as pupae or as adult and crossed with untreated males

Dose in	Av. eggs/female[a] (±Sd) when crossed with males irradiated as	
	Pupae[b]	Adults[c]
0	69 ± 12.36	68 ± 12.38
1	---	67 ± 2.83[d]
2	67 ± 10.71	65 ± 8.15
4	58 ± 14.35	65 ± 5.40
6	52 ± 18.77	68 ± 5.43
8	---	56 ± 4.64
10	48 ± 12.66	62 ± 2.07
15	---	52 ± 8.83[e]
20	25 ± 3.27	46 ± 4.71
30	23 ± 4.60	34 ± 4.21
40	15 ± 3.11	35 ± 5.26

a. Based on oviposition of 50 females during an 8-day period following crosses.
b. Irradiated 21-14 hr before emergence
c. Irradiated 15-21 hr after emergence
d. Average of 30 females
e. Average of 40 females

Table 13. Longevity of male coffee leaf miner, Leucoptera coffeella (Guérin-Méneville) irradiated as pupae or as adults and crossed with untreated females.

Doses (kr)	T_{50} ±S d (in days[a]) after irradiation	
	P u p a e[b]	A d u l t s[c]
0	14.3 ± 0.26	16.2 ± 0.73
10	13.6 ± 0.44	15.0 ± 0.69
20	12.4 ± 0.39	16.0 ± 0.61
30	11.6 ± 0.49	11.4 ± 0.55
40	9.4 ± 0.43	11.4 ± 0.55
45	---	12.3 ± 0.61
50	7.3 ± 0.49	12.2 ± 0.63
55	---	10.0 ± 0.53
60	4.2 ± 0.46	9.5 ± 0.46
70	---	10.3 ± 0.35
80	---	8.4 ± 0.43
90	---	7.7 ± 0.25

a. Based on 50 adults
b. Irradiated 23-14 hr before emergence
c. Irradiated 4-21 hr after emergence

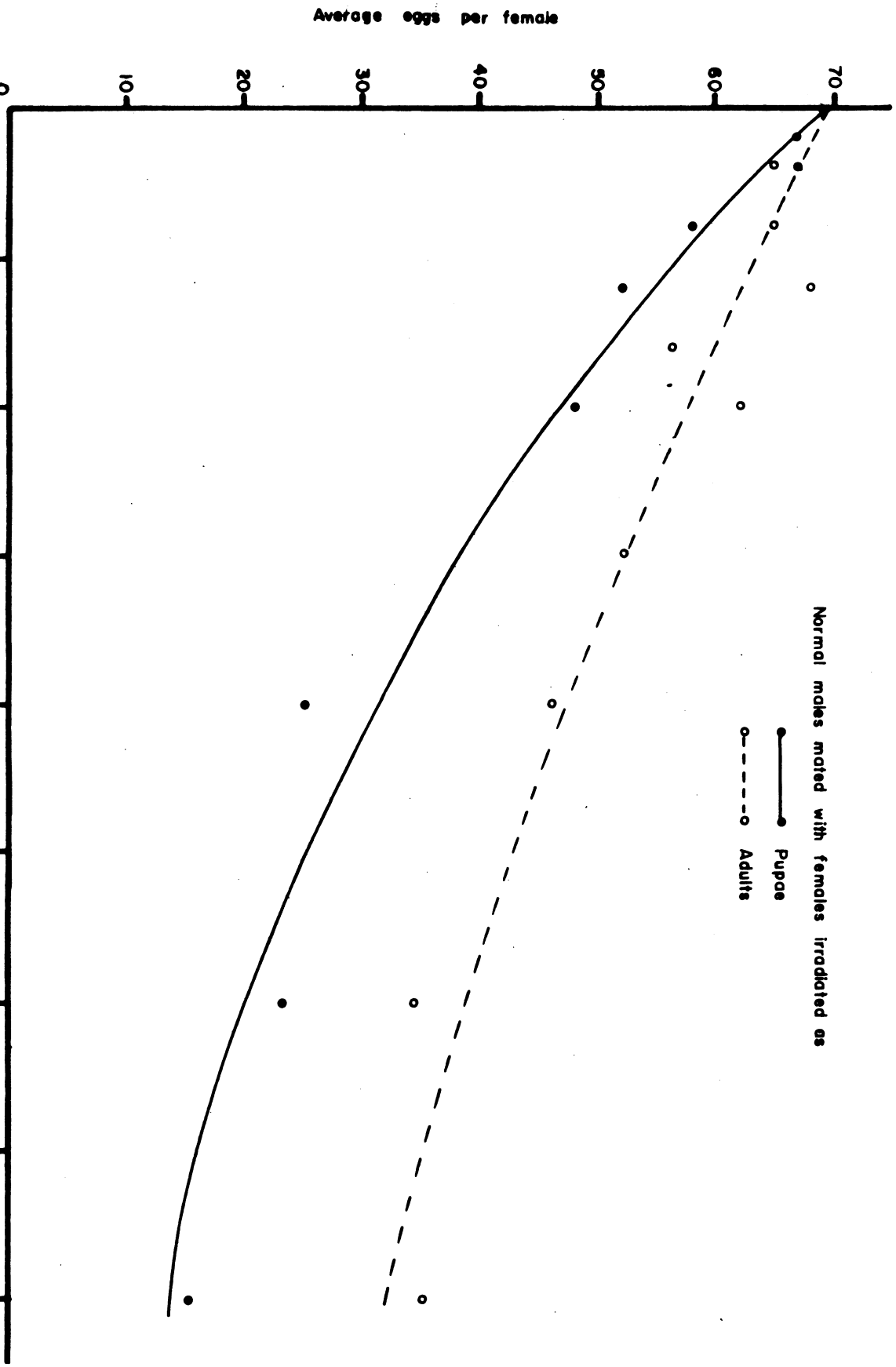

Fig. 14 Fecundity of irradiated coffee leaf miner females

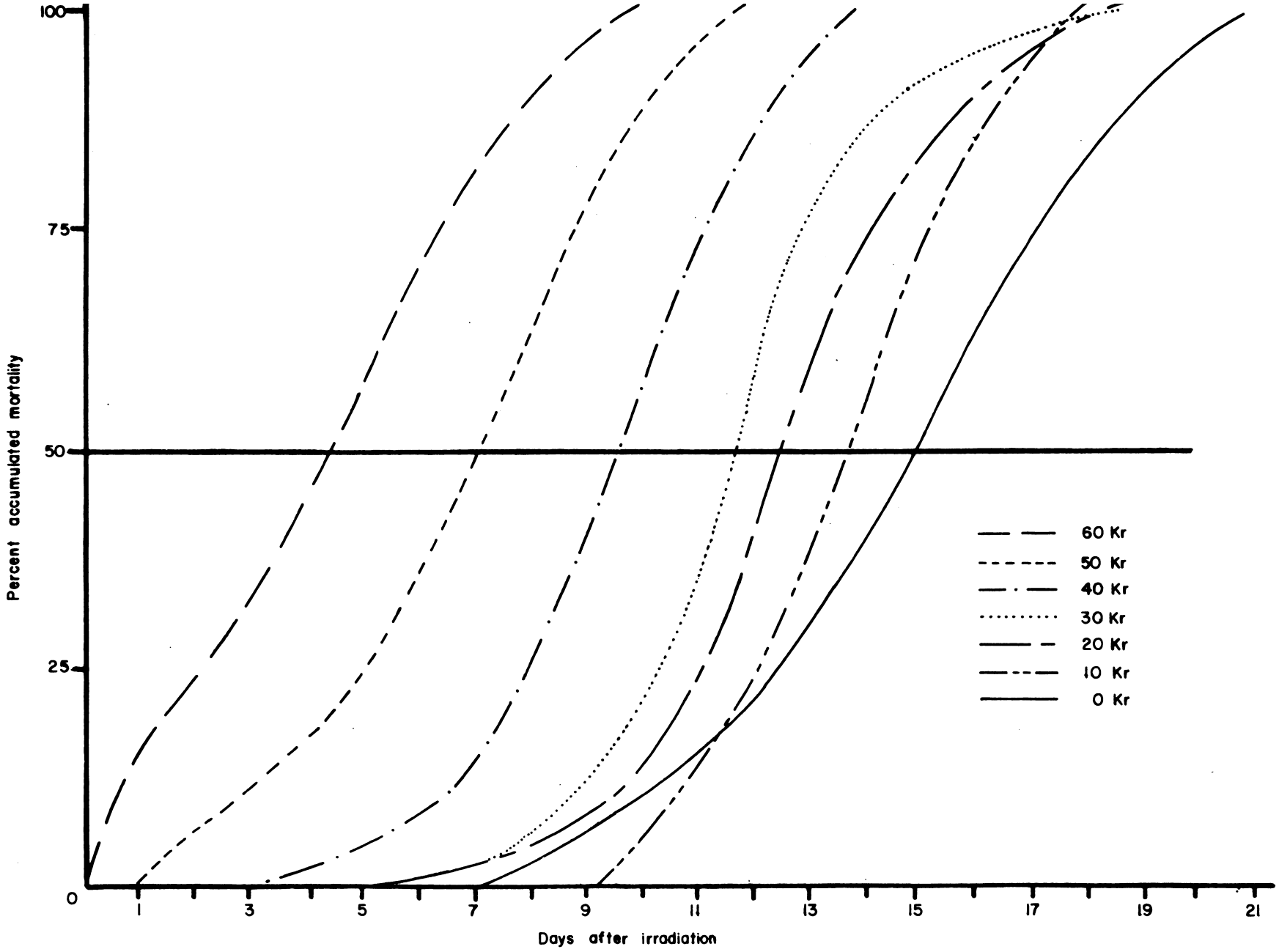

Fig.15 Accumulated mortality rates as a function of time in coffee leaf miner males irradiated as pupae

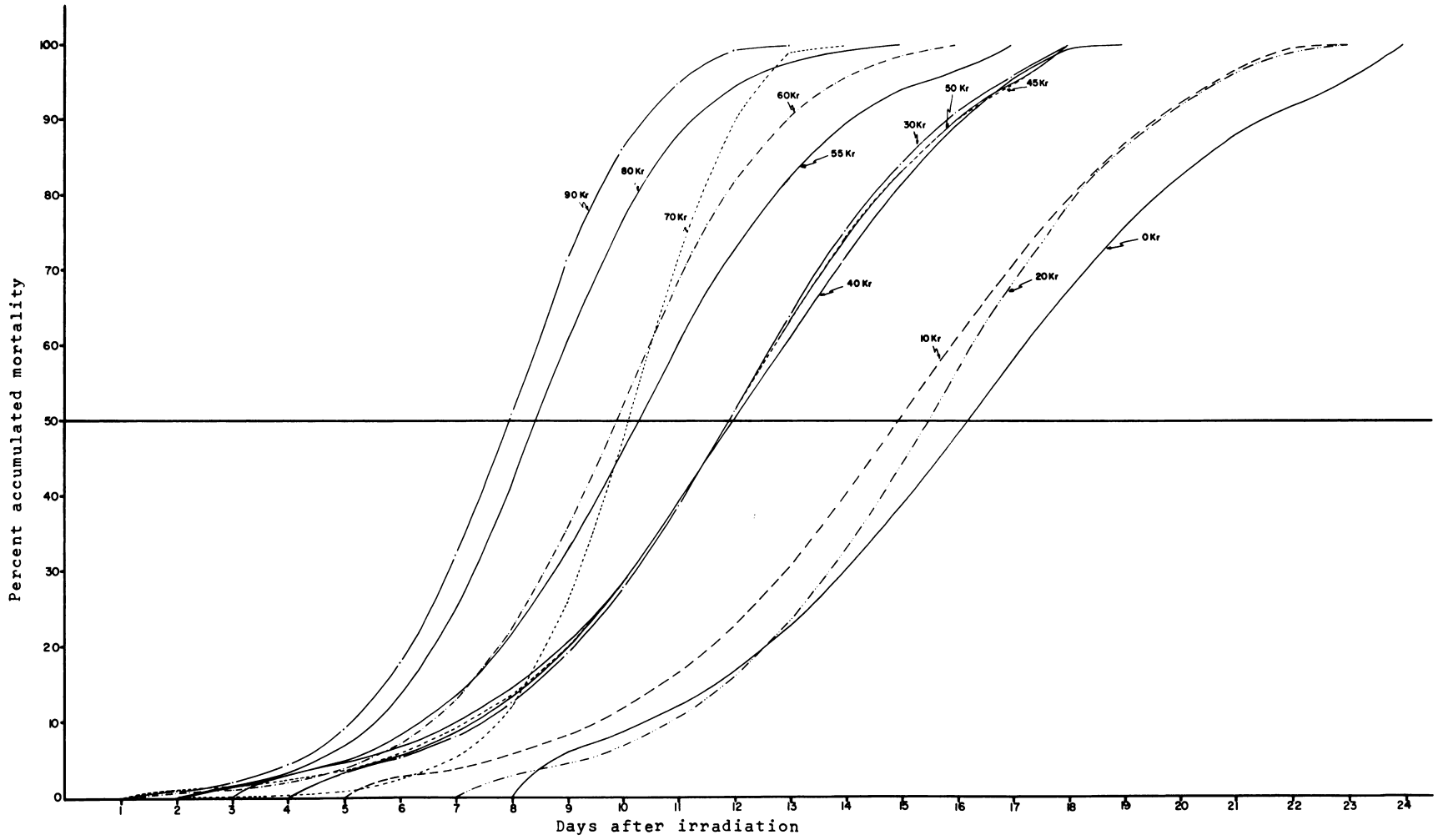

Fig. 16. Accumulated mortality rates as a function of time in coffee leaf miner males irradiated as adults

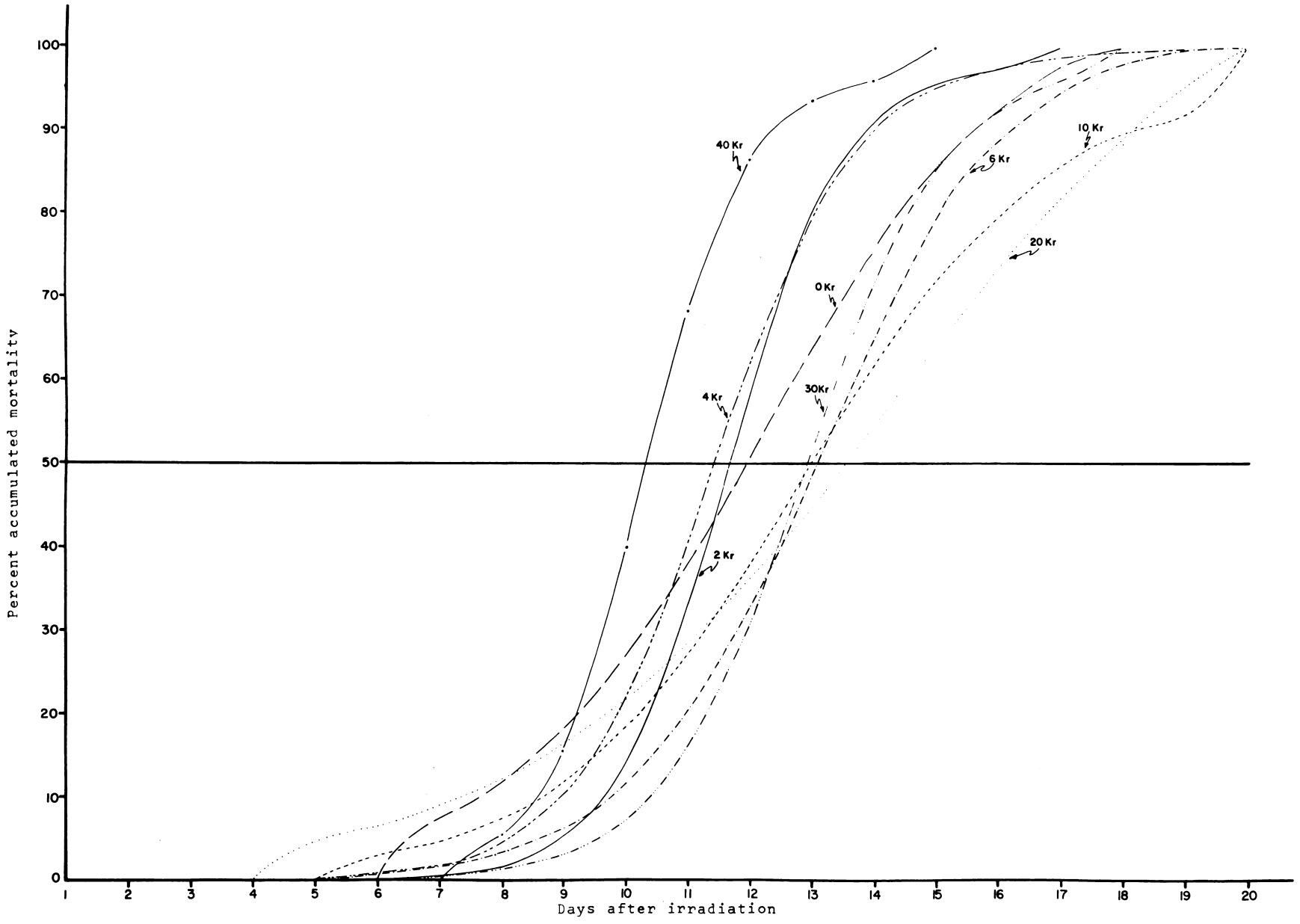

Fig. 17. Accumulated mortality rates as a function of time in coffee leaf miner females irradiated as pupae

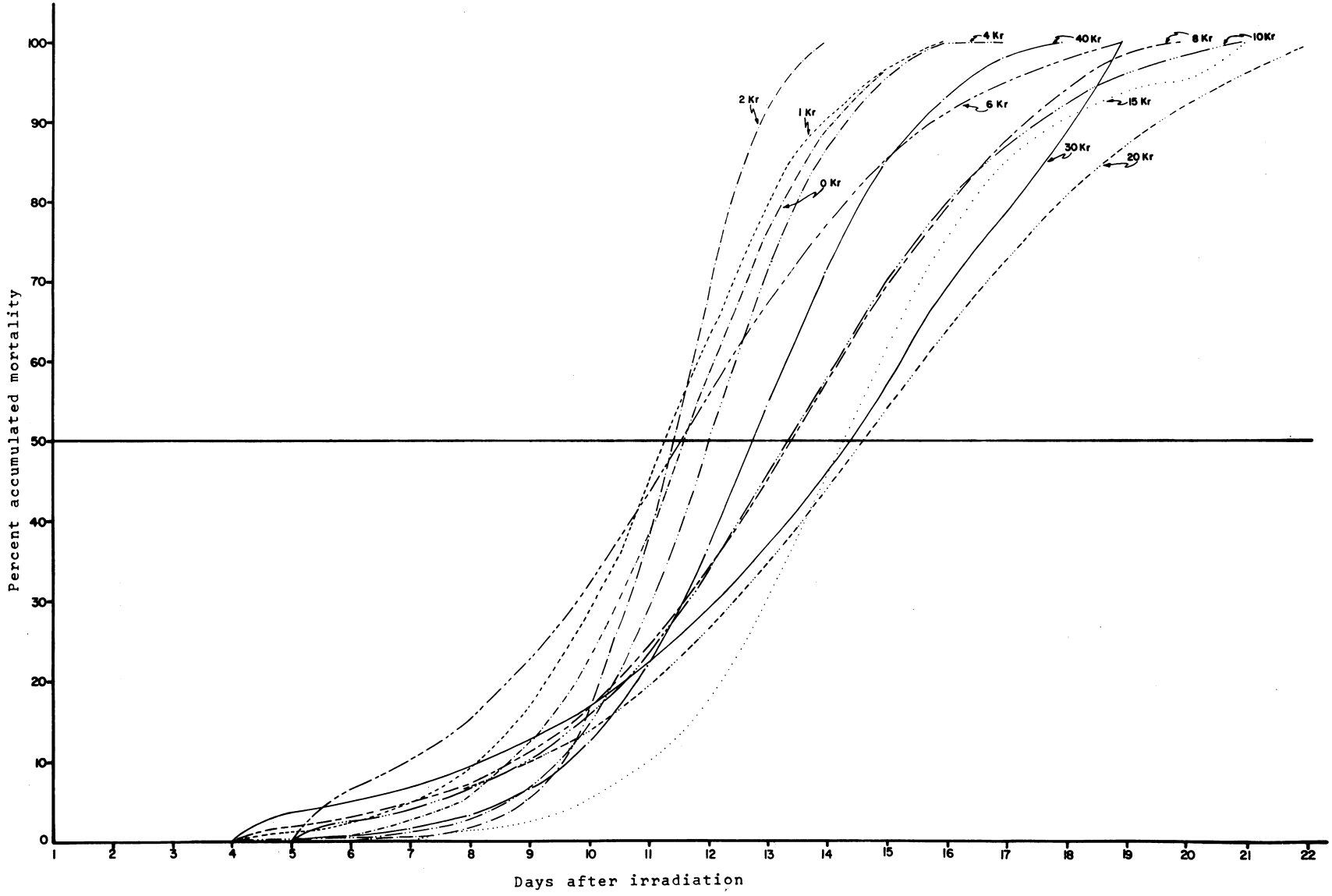

Fig. 18. Accumulated mortality rates as a function of time in coffee leaf miner females irradiated as adults

Table 14. Longevity of female coffee leaf miner, Leucoptera coffeella (Guérin-Méneville) irradiated as pupae or as adult and crossed with untreated males

Dose in kr	T_{50} ± S'd (in days[a]) after irradiation P u p a e[b]	 A d u l t s[c]
0	11.0 ± 0.49	11.5 ± 0.41
1	---	12.0 ± 0.46[d]
2	10.6 ± 0.35	11.5 ± 0.26
4	10.6 ± 0.40	12.4 ± 0.44
6	12.3 ± 0.50	11.3 ± 0.58
8	---	13.0 ± 0.57
10	11.6 ± 0.60	12.6 ± 0.60
15	---	14.3 ± 0.56[e]
20	12.2 ± 0.70	14.2 ± 0.65
30	11.7 ± 0.39	14.2 ± 0.66
40	9.5 ± 0.28	12.5 ± 0.44

a. Based on 50 adults
b. Irradiated 21-14 hr before emergence
c. Irradiated 15-21 hr after emergence
d. Based on 30 adults
e. Based on 40 adults

(Table 13). Similarly, longevity of irradiated males was significantly reduced at all the doses (except the low doses of 10 and 20 kr) when newly emerged adults were irradiated. Doses of 60 kr applied to pupae reduced the longevity of males by 70.5% (T_{50} for treatment = 4.2±0.46 days compared to T_{50} for control = 14.3±0.26 days). Similarly the sterilization dose of 90 kr applied to newly emerged moths shortens the life span of treated males by 52.5% (T_{50} for treatment = 7.7±0.25 days compared to T_{50} of 16.2±0.73 days for control). Thus the effect of irradiation on longevity of the male coffee leaf miner is more pronounced when treatment is applied to the pupal stage.

Longevity of the adult female coffee leaf miner generally is not adversely affected by irradiation (Table 14). Lifespan of females

significantly increased (by 0.5-2.8 days) with every dose except 2 and 6 kr, when irradiated during the adult stage. Longevity of females irradiated in the pupal stage was significantly reduced (at the 5% level) with low (2 and 4 kr) and high (40 kr) sterilization doses. Irradiation of females in the pupal stage with intermediate doses (6-30 kr), significantly increased (by 0.6-1.3 days) the life-span of treated moths. Irradiation has been reported to increase the longevity of treated females of several other Lepidopterous species, eg. Heliothis virescens (F.) (2) and Laspeyresia (=Carpocapsa) pomonella (L.) (3).

References

1. Katiyar, K. P. and F. Ferrer. Rearing technique, biology and sterilization of the coffee leaf miner, Leucoptera coffeella Guer. (Lepidoptera:Lyonetiidae). In Symp. on the Use of Isotopes and Radiation in Entomology, Vienna, 1967. Proceedings. Vienna, International Atomic Energy Agency, 1968. pp. 165-175.

2. Elsayed, E. I. and J. B. Graves. Effects of gamma radiation on the tobacco budworm. II. Irradiation of moths. Jour. Econ. Entomol. 62:293-296. 1969.

3. Proverbs, M. D. and J. R. Newton. Influence of gamma radiation on the development and fertility of the Codling moth, Carpocapsa pomonella (L.) (Lepidoptera:Olethreutidae). Can. Jour. Zool. 40:401-420. 1962.

Effect of sterilization on the mating vigor of the treated males

Purpose and Methods

In insect control by the sterile male technique it is very important that sterilization should not adversely affect the mating vigor of the treated males. The irradiated males should compete reasonably well in

mating with untreated males. The following experiment was designed to determine the effect of a 90 kr sterilization treatment on the sexual vigor of the treated males.

The insemination capacity of males irradiated 24 hr after emergence was compared with untreated males. The sterilization dose of 90 kr was selected because it induces more than 99% dominant lethal mutations in the sperm of treated males (Table 9).

Insemination capacity of males of each treatment (irradiated with 90 kr and untreated) was measured by confining individual males with five virgin females in a small cage for 24 hr. The females were then removed and their reproductive system was dissected in Belar solution under a stereoscope and examined for the presence of sperm under a compound microscope. The females which carried at least a trace of sperm in the spermathecae were scored as inseminated. Each surviving male was provided with five fresh virgin females for another 24 hr period. This procedure was repeated until all the males were dead. Each treatment had 10 24-hr old males at the beginning of the experiment. All the females used in the experiment were 24 hr old.

The experiment was carried out in the laboratory at 25±3°C temperature and 75±5% relative humidity.

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Table 15 presents the number of females inseminated every day by individual males (irradiated and untreated). The results indicate that 90 kr sterilization drastically reduced the insemination capacity of treated males. On an average, untreated males inseminated 35.1

Table 15. Consecutive insemination of normal coffee leaf miner females caged for 24 hr with normal males or irradiated[a] males (90 kr) in 5:1[b] (female:male) ratio

Male age	Number females inseminated by individual male																							
	Normal males (number)												Irradiated males (number)											
(days)	1	2	3	4	5	6	7	8	9	10	Total	Average	1	2	3	4	5	6	7	8	9	10	Total	Average
1	3	3	3	3	3	3	3	3	3	3	30	3.0	2	2	3	1	3	2	2	3	2	3	23	2.3
2	3	3	1	3	2	3	3	3	3	3	27	2.7	2	3	1	1	1	2	1	2	2	1	16	1.6
3	4	3	2	3	4	3	3	4	4	3	33	3.3	1	1	2	1	1	1	0	1	1	1	10	1.0
4	2	4	4	3	4	4	4	2	3	3	33	3.3	0	0	0	0	1	0	0	0	0	0	1	0.1
5	3	2	2	3	3	3	2	2	4	3	27	2.7	0	0	0	0	0	0	0	0	0	0	0	0.0
6	4	3	2	3	4	4	3	2	3	3	31	3.1	0	c	0	c	0	0	c	0	c	0	0	0.0
7	2	3	2	3	4	4	3	2	2	3	28	2.8	c	-	c	-	c	0	-	c	-	c	-	0.0
8	3	3	2	3	3	4	3	2	4	2	29	2.9	-	-	-	-	-	c	-	-	-	-	-	-
9	2	2	2	2	3	3	3	3	3	3	26	2.6												
10	3	4	3	3	3	3	3	2	2	2	28	2.8												
11	2	2	2	3	2	3	2	2	2	2	22	2.2												
12	3	2	2	3	c	2	2	3	3	c	20	2.0												
13	3	1	2	2	-	1	c	1	2	-	12	1.2												
14	2	1	1	c	-	c	-	c	1	-	5	0.5												
15	c	c	c	-	-	-	-	-	c	-	-	-												
Total	39	36	30	37	35	40	34	31	39	30	351	35.1	5	6	6	3	6	5	3	6	5	5	50	5.0

a. Irradiated as adults, 24 hr after emergence
b. In first 4 males of each treatment female to male ratio was 4:1
c. Time when male died

Females during their life period compared to an average of 5.0 females inseminated by an irradiated male. Results also indicate that untreated males inseminate females until they die while irradiated males

inseminated only during the first 3-4 days of their life.

Later matings of the treated males, indicated difficulty in the transfer of sperm from male to female. Examination of the testes of dead irradiated males showed the presence of an appreciable amount of sperm. Except for one mating on the 4th day, irradiated males did not inseminate any females after the 3rd day of the experiment.

b. Radiation induced sterility in the progeny of the male coffee leaf miner
(K. P. Katiyar and E. A. Ramirez)

1) Effects on fertility, fecundity, longevity, larval and pupal mortality and adult sex ratio of F_1 moths

Purpose and Methods

Like other Lepidoptera, the coffee leaf miner Leucoptera coffeella (Guérin-Méneville) is resistant to gamma irradiation when sterility is used as a criterion to measure the sensitivity. As reported earlier (Table 9) a dose of 90 kr is required to induce more than 99% sterility in coffee leaf miner males. This (90 kr) sterilization dose has been found to reduce (by about 86%) the insemination capacity of treated males (Table 15).

Recently, in several Lepidopteran species, it has been found that the parent moths receiving sub-sterilization doses produce completely sterile F_1 progeny. The inherited sterility of the F_1 has been reported in the Codling moth, Carpocapsa pomonella L. (1), the cabbage looper, Trichoplusia ni (Hübner) (2), the sugar cane borer, Diatraea saccharalis (Fab.) (3), the tobacco budworm, Heliothis virescens (F.) (4) and several other Lepidopteran species.

The phenomenon of F_1 sterility could perhaps add more value to sterile male control in the coffee leaf miner. By reducing the sterilizing dose of 90 kr chances are greater to improve the mating competitiveness of the irradiated males. Studies therefore were initiated last year in our laboratory to determine the presence of F_1 inherited sterility in the coffee leaf miner; and if present, to see if the use of F_1 inherited sterility can be used to increase the mating competitiveness of irradiated males.

All the insects used in the tests came from the colony established in our laboratory. The moths were reared on living coffee plants as described previously (5).

Adult males of the coffee leaf miner (2-23 hr old) were irradiated in a pool-type ^{60}Co irradiator with a dose rate of ca. 1500 r/m. Before and during irradiation period the adults were held individually in 5 ml shell vials with screened caps to facilitate aeration. The steel canister itself in which adults were irradiated was not aerated during irradiation. Sterilization doses of 30, 45, and 60 kr were used. The tests were replicated 3-4 times but all the treatments were not necessarily studied concurrently.

Irradiated males (200-300) were confined with equal numbers of untreated virgin females in fine nylon screened cages (48 cm long, 48 cm wide and 60 cm high). Inside the cage, females were allowed to oviposite on two uninfested coffee plants for two consecutive nights (one coffee plant each night). F_1 larvae and pupae were reared in screened cages to avoid any oviposition of unwanted moths present in the laboratory.

To study the fertility, fecundity and longevity of F_1 progeny, 10 F_1 adults were confined in a cage with 10 moths of the opposite sex. Oviposition and egg-hatch records were taken for each treatment for 8 consecutive days. Daily adult mortality was recorded for each sex until all the moths were dead.

All the tests were carried out in the laboratory at temperatures of 26±3°C and relative humidities of 75±5%. F_1 larval and pupal mortality was studied in temperature controlled cabinets at 25±0.5°C temperature and 75±5% relative humidity. In the present study all F_1 progenies were derived from crosses between irradiated parent males and non-irradiated parent females.

Progress Report - Fertility, Fecundity and Longevity of F_1 Adults

Fertility of F_1 progeny derived from parent males irradiated with 30, 45, or 60 kr is presented in Table 16. The results indicate that at every dose level, F_1 progeny of irradiated males are more sterile than the treated parent males. When the parent males were treated with 60 kr, egg-hatch of the F_1 crosses (normal female x F_1 male and F_1 female x normal male) were zero. The fertility of F_1 progeny (males as well as females) arising from parent males treated with 30 or 45 kr was less than 1%.

It appears that induced F_1 sterility can be achieved in the coffee leaf miner at low sub-sterilization doses. Males retaining 63.9% fertility from 30 kr irradiation, produce nearly sterile F_1 progeny (0.1±0.20% egg-hatch in F_1 male and 0.7±0.89% egg-hatch in F_1 female).

Table 16. Fertility of F_1 coffee leaf miner adults obtained from crossing normal females with irradiated males

Dose to P_1 ♂♂[a] (kr)	% egg-hatch P_1 ♂♂ x N ♀♀	% egg-hatch[b] ± S d		
		F_1 ♂♂ x N ♀♀[c]	F_1 ♀♀ x N ♂♂[c]	F_1 ♂♂ x F_1 ♀♀[d]
0	93.9	94.1 ± 0.79 (1928)	---	---
30	63.9	0.1 ± 0.20 (1094)	0.7 ± 0.89 (1035)	0.0 (248)
40	32.2	0.2 ± 0.29 (1232)	0.2 ± 0.12 (1229)	0.0 (515)
60	11.1	0.0 (1145)	0.0 (522)	---

a. P_1 males (Parent) irradiated as adult (2-23 hr) after emergence
b. Numbers in parentheses are total number of eggs (collected during 8 consecutive days) on which percentages are based.
c. Average of 4 replications, 10 pairs per rep.
d. Average of 3 replications, 10 pairs per rep.

Table 17 presents the summarized results of fecundity of F_1 progeny arising from parent males irradiated with 30, 45 and 60 kr. Sub-sterilization of the P_1 male coffee leaf miner not only reduces the fertility of resulting F_1 progeny, but it also adversely affects the egg production capacity of F_1 moths.

Fecundity of F_1 progeny, males as well as females (when crossed with untreated moths of opposite sex) was reduced by approximately 40% in all the treatments and by about 75% in F_1 females resulting from parent males treated with 60 kr.

Table 18 presents T_{50} values (time at which 50% of the insects investigated died) of F_1 adults. Irradiation of parent male with 30, 45 or 60 kr, slightly reduced the longevity of F_1 moths of both sexes. Maximum reduction (approximately 17% in males and 25% in females) in

Table 17. Fecundity of F_1 adult coffee leaf miner produced by crossing normal females with irradiated males.

Dosis to P_1♂♂[a] (kr)	Number eggs per female[b] ± S d		
	F_1♂♂ x N♀♀[c]	F_1♀♀ x N♂♂[c]	F_1♂♂ x F_1♀♀[d]
0	48.2 ± 8.60	---	---
30	27.3 ± 7.20	25.8 ± 2.43	8.3 ± 2.59
45	30.8 ± 13.25	30.7 ± 9.29	17.2 ± 6.25
60	28.6 ± 6.71	13.0 ± 1.54	---

a. P_1 males irradiated as adults (2-23 hr after emergence)
b. Based on average of 3-4 repetitions, 10 pairs per rep. Eggs collected for 8 consecutive days.
c. Four repetitions.
d. Three repetitions.

Table 18. Longevity of F_1 adult coffee leaf miner produced by crossing normal females with irradiated males.

Dosis to P_1♂♂[a] (kr)	T_{50} ± S d (days)[b]	
	F_1♂♂	F_1♀♀
0	15.0 ± 1.35	12.3 ± 1.29
30	13.1 ± 2.80	10.7 ± 1.84
45	12.8 ± 2.15	9.8 ± 1.56
60	12.4 ± 1.03	9.2 ± 1.55

a. P_1 males irradiated as adults, 2-23 hr after emergence
b. T_{50} is time in days when 50% of the moths died. Values are based on 40 moths studied in each treatment.

lifespan of F_1 adults is found in the progeny of parent males irradiated with 60 kr.

Larval and pupal mortality of F_1 progeny

Larvae that hatched from the eggs deposited by untreated females crossed with irradiated males were reared to adult stage. Post-embryonic

mortality, i. e. larval and pupal, was recorded in this experiment. The results in Table 19 indicate that post-embryonic survival of F_1 progeny is adversely affected in the larval stage. F_1 larval mortality increased as the radiation dose given to the parent male increased. The mortality of F_1 progeny in the larval stage was 10.2, 30.5, 50.8 and 63.2 percent, respectively, when parent males were irradiated with 0, 30, 45, and 60 kr.

Table 19. F_1 larval and pupal mortality of the coffee leaf miner when the P_1 males received 0, 30, 45, or 60 kr gamma irradiation

Dosis to P_1 ♂♂[a] (kr)	F_1 larvae		F_1 pupae	
	No. Observed	% mortality[b] ± S d	No. Observed	% mortality[b] ± S d
0	124	10.2± 3.76	111	7.3± 4.63
30	253	30.5± 4.52	176	8.0± 0.50
45	460	50.8± 4.50	223	15.1± 2.77
60	285	63.2±10.14	106	15.7± 7.00

a. P_1 males irradiated as adults, 2-23 hr after emergence
b. Average of three repetitions.

Only slight adverse effects on the survival of F_1 pupae are noticed at higher irradiation doses (45 and 60 kr) given to P_1 males. Pupal mortality in F_1 progeny was 7.3, 8.0, 15.1 and 15.7 percent, respectively, when parent male moths were irradiated with 0, 30, 40 and 60 kr.

Sex ratio of F_1 progeny

As reported previously considerable mortality of F_1 progeny occurs in the larval stage (Table 19). We did not determine the sex of the dead larvae and pupae in Table 19. It is quite possible

that larval and pupal mortality is greater in one sex than the other. If this is true then the sex ratio of F_1 adults will be unbalanced. Results in Table 20 indicate no significant change in adult sex-ratio of F_1 progenies derived from irradiated (30, 45 or 60 kr) parent males. In all the treatments, the ratio of F_1 males and females is approximately 1:1. Our results are based on relatively smaller observations. More experiments will be carried out to determine the sex ratio of F_1 adults (arising from sub-sterilization of parent males). Sex distortion in the progeny of irradiated moths have been reported in several Lepidopteran species, eg. codling moth, Carpocapsa pomonella (L.) (1), the tobacco budworm, Heliothis virescens (F.) (4), navel orangeworm, Paramyelois transitella (Walker) (6) and the cabbage looper, Trichoplusia ni (Hübner) (7).

Table 20. Ratio of F_1 males:F_1 females coffee leaf miner when P_1 males were treated with 0, 30, 45, or 60 kr gamma irradiation

Dosis to P_1 ♂♂[a] (kr)	Total F_1 adults observed[b]	Adult ratio Male : Female
0	102	1.0 : 1.1
30	162	1.0 : 1.1
45	188	1.0 : 1.0
60	91	1.1 : 1.0

a. P_1 males irradiated as adults, 2-23 hr after emergence
b. Total of three repetitions.

References

1. Proverbs, M. D. and J. R. Newton. Some effects of gamma radiation on the reproductive potential of the Codling moth, Carpocapsa pomonella (L.) (Lepidoptera:Olethreutidae). Can. Entomol. 94:1162-1170. 1962.

2. North, D. T. and G. G. Holt. Inherited sterility in progeny of irradiated male cabbage loopers. Jour. Econ. Entomol. 61: 928-931. 1968.

3. Walker, D. W. and V. Quintana. Inherited partial sterility among survivors from irradiation-eradication experiment. Jour. Econ. Entomol. 61:318-319. 1968.

4. Proshold, F. I. and J. A. Bartell. Inherited sterility in progeny of irradiated male tobacco budworms: Effects on reproduction, developmental time, and sex ratio. Jour. Econ. Entomol. 63:280-285. 1970.

5. Katiyar, K. P. and F. Ferrer. Rearing technique, biology and sterilization of the coffee leaf miner, Leucoptera coffeella Guer. (Lepidoptera:Lyonetiidae). In Symp. on the Use of Isotopes and Radiation in Entomology, Vienna, 1967. Proceedings. Vienna, International Atomic Energy Agency, 1968. pp. 165-175.

6. Husseiny, M. and H. F. Madson. Sterilization of the navel orangeworm, Paramyelois transitella (Walker), by gamma radiation (Lepidoptera:Phycitidae). Hilgardia 36:113-137. 1964.

7. North, D. T. and G. G. Holt. Population suppression by transmission of inherited sterility to progeny of irradiated cabbage loopers, Trichoplusia ni. Can. Entomol. 101:513-520. 1969.

2) Mating competitiveness of F_1 males

Purpose and Methods

A dose of 90 kr (applied to newly emerged adults) is required to induce more than 99% sterility in male coffee leaf miner (Table 9). This sterilization dose seriously reduces the insemination ability of treated males (Table 15). We have found that the F_1 progeny (males as well as females) of the coffee leaf miner males irradiated

with sub-sterilization doses of 30, 45 or 60 kr are more than 99% sterile (Table 16). Reduction of sterilization dose from 90 to 45 or 30 kr is expected to reduce the somatic injury of treated males which in turn should increase the mating competitiveness of irradiated males. The following experiment was carried out to find out the mating competitiveness of F_1 males obtained from crosses of normal females with irradiated males (30 and 45 kr).

Tests were carried out in the laboratory at 25±3°C temperature and 75±5% relative humidity. The parent males (2-23 hr after emergence) were irradiated with ^{60}Co irradiator at a dose rate of ca. 1500 r/m.

Mating competitiveness of F_1 males was tested by caging them with normal males and normal females. The mating competitiveness of each type F_1 male (reared from parent male irradiated with 30 or 45 kr) was tested at 3 different ratios: 1:1:1, 2:1:1, and 3:1:1 (F_1 male:normal male:normal female). The test included two controls to check the fertility of F_1 males and non-irradiated males. Each cage had 10 females per ratio. The experiment was replicated three times, each time with different batches of insects.

The daily egg collection started one day after adults were put together in the cages and it was continued for eight consecutive days.

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The percent egg viability of the mixed moth populations of F_1 males (obtained from irradiated parent males with 30 or 45 kr), normal males and normal females is summarized in Table 21. It seems that

Table 21. Mating competitiveness of F_1 male coffee leaf miner when P_1 males received 30 or 45 kr of gamma irradiation

Dosis to P_1 ♂♂[a] (kr)	Adult ratio F_1 ♂:N♂:N♀[b]	Total eggs examined	% egg hatch			
			Rep. I	Rep. II	Rep. III	Average
0	0 : 1 : 1	1538	94.1	93.7	96.4	94.7
30	1 : 0 : 1	823	0.0	0.4	4.1	1.5
45	1 : 0 : 1	1100	0.0	0.6	0.0	0.2
30	1 : 1 : 1	1696	93.3	91.2	87.3	90.6
30	2 : 1 : 1	1482	88.7	89.2	85.0	87.5
30	3 : 1 : 1	1253	77.2	92.3	82.1	83.9
45	1 : 1 : 1	1852	86.0	95.2	91.3	90.8
45	2 : 1 : 1	1331	88.1	75.0	83.1	82.1
45	3 : 1 : 1	1308	76.3	92.5	85.5	84.8

a. P_1 males irradiated as adults, 2-23 hr after emergence
b. Ten females per ratio

F_1 males of the coffee leaf miner do not compete in mating with normal males. The maximum egg-hatch reduction of 12.6% (94.7% in check to 82.1% in treatment) was found in the 2:1:1 ratio of the F_1 male obtained from P_1 males irradiated with 45 kr. The reason for total failure of F_1 males to compete in mating with normal males is not known. Detailed observations on the ability of F_1 males to transfer sperm have not been carried out. Some casual observations however, indicate that at least in some of the matings of F_1 males, sperm were transferred into females. Next year, more experiments will be carried out to determine the failure of mating competitiveness of F_1 males.

C. PATHOLOGICAL CONTROL OF INSECT PESTS

1. Rearing Method

a. Hypsipyla grandella Zeller
(O. Hidalgo-Salvatierra and L. G. Madrigal)

Purpose

Valuable lumber trees, like cedar (Cedrella sp.) and mahogany (Swietenia sp.) and other Meliaceae, are rapidly disappearing from the natural tropical forests due to intensive commercialization of these species. Restoration of the trees has been extremely difficult and many plantations have been largely abandoned. According to Entwistle (4), one of the overriding factors against the establishment and cultivation of tropical Meliaceae is the presence of the shoot borer, Hypsipyla grandella Zeller, in the New World, and Hypsipyla robusta Moore in the Old World. The larva from these insects bores down the center of new shoots causing progressive distortion, stunting and forking of the trunk.

Even though this lepidopteron has been known for over a hundred years, very little work has been done with this insect, and so far no effective and economic means of control are known.

The purpose of this investigation is to develop a procedure to rear and maintain this insect under laboratory conditions, and with materials available, so we can have test larvae at hand for our research on the microbial control and radiation sterilization of this pest.

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We have succeeded in rearing and maintaining H. grandella under laboratory conditions on a synthetic diet. The diet contains the following:

80.7	g ground soybean in 450 ml water
8	g agar in 400 ml boiling water
36	g wheat germ
6	ml KOH, 4 M
10	g W-salt mixture
1.8	g methyl-p-hydroxybenzoate
1.1	g sorbic acid
5	ml 10% formaldehyde
13.3	ml 25% acetic acid
20	g Vitamin Diet Fortification Mixture (Nutritional Biochemical Corp.)
166.7	mg aureomycine
80	g corn cob grits (65 mesh)

The colony was started in June 1970 with larvae brought from the nearby cedar and mahogany plantations. These larvae were reared at the beginning in individual plastic cups. This procedure turned out too expensive and inefficient because the larvae will usually chew their way out of the cups. After testing all the possible available containers, we ended up using glass petri dishes which are reusable and can be sterilized. A main problem, however, had to be overcome, cannibalism.

After testing different procedures to reduce cannibalism we found that the most efficient method, in our case, was to divide the petri in nine sections using aluminum sheets, protected with a special DuPont Dulux Marine Varnish and heated overnight about 110°C. This treatment protects the aluminum against corrosion.

Our general rearing procedure is as follows: ten eggs are usually placed in a small watch glass on top of synthetic diet inside a

petri dish. This is covered and sealed with silly putty to avoid the escape of the small larvae. After ten days they are tranferred to petris with divisions, where they pupate after a larval period of four weeks. The completely formed pupae are then transferred to clean petris where they emerge after 10 to 11 days.

During the course of our work we found that Trichogramma sp. is an egg parasite of H. grandella (5); that caged adults will not usually oviposite fertile eggs under laboratory conditions; and that the sex of the pupae can be differentiated by the genital opening and the characteristics of the last three abdominal segments (6).

b. Spodoptera frugiperda (Smith)
(O. Hidalgo-Salvatierra and L. G. Madrigal)

Purpose

It is well known that a complex of leipdopterous larvae belonging to the genus Spodoptera have raised to major status as a pest of important crops in the Central American area. The purpose of this work is to establish a small colony of Spodoptera in order to have test material for our general work on microbial control.

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A small colony was started in August 1970 with larvae brought from a nearby cornfield. This colony has been maintained under laboratory conditions using the same diet and general procedure described for H. grandella.

The adults oviposite in paper towels in cages similar to the one described by Ignoffo (7). Masses of eggs are placed on synthetic diet

inside petri dishes. To reduce escaping of the small larvae a rubber o-ring, instead of silly putty, is used. The tendency to escape is less strong in Spodoptera than in Hypsipyla and therefore do not have to be completely sealed in. After 7 to 10 days they are transferred to petris with divisions. The same general problems were encountered with Spodoptera as with Hypsipyla and more so. After a small successful rearing period the efficiency began to decrease to the point of having a single, not so healthy, female and two males by March of this year. We suspected that bad handling procedures, contamination and maybe a not so adequate diet, were responsible for the decline.

To save the colony we introduced a more rigorous handling system, including daily disinfection of the laboratory walls and tables, and change to a new diet similar to the ones given by Burton (2, 3) for the corn earworm and the fall army worm.

The composition of the new diet is as follows:

Liquify	53	g of overnight soaked beans in 300 ml of water
Add:	6.6	g agar dissolved in 300 ml boiling water
	33	g yeast
	53	g wheat germ
	3.5	g ascorbic acid
	2.1	g methyl-p-hydroxybenzoate
	1.1	g sorbic acid
	12	ml 10% formaldehyde
	1	capsule of Kanamicine sulphate
	13	g Vitamin mixture
	53	g corn cob grits (65 mesh)

Fortunately of the thirty remaining larvae that we saved from the single female, 24 pupated and 20 emerged as healthy adults. As of today we have 9 ovipositing females.

2. Susceptibility of *Hypsipyla grandella* Zeller to *Metarrhizium anisopliae* (Metch.)

(O. Hidalgo-Salvatierra and F. Berrios)

Purpose and Methods

As mentioned before no effective means of control of the Meliaceous shoot borer is known. In 1969 Rao and Bennet (9) suggested possibilities of biological control using predators and parasites. Kandasamy (8) found that *Hypsipyla robusta* Moore was susceptible to *Beauveria tenella*. So far however, little is known on the susceptibility of *H. grandella* to bacteria, virus and fungal pathogens. The objective of this investigation was to determine its susceptibility to the fungal pathogen *M. anisopliae*.

The fungus was obtained from Dr. D. W. Roberts at the Boyce Thompson Institute in New York. It was cultured regularly on a Sabouraud Dextrose Agar medium with yeast extract added (SDAY). The spores were harvested in water with a few drops of Triton X-100 to facilitate the dispersion of the conidia. Concentration was determined by dilution and plating, and with the aid of a hemocytometer.

Inoculation was accomplished by bathing the larva for one minute with water or with a suspension of spores at a concentration of 1.2×10^7 viable spores per milliliter.

Results

It was found that 15 day old larvae of *H. grandella* are susceptible to infection by the fungus *M. anisopliae*. The symptoms of the disease are similar to the ones described by Steinhaus (10), for other insects. Larvae killed by the fungus are pale, stiff and mummified. When placed

under humid conditions a white mycellium pierces out of their bodies through the spiracles and prolegs. This growth soon covers the cadaver, and later on takes on a green color, characteristic of the spores of this fungus. The whole larva becomes a mass of spores which disintegrates with the slightest touch (Fig. 19).

Under the conditions of the experiment 50% of the larvae were killed by the fungus (Table 22). Those that survived pupated and emerged like the control larvae, Figs. 21 and 22, with a larval period from 26 to 33 days, and a pupal stage of 10 to 11 days. Highest mortality was found six days after the treatment (Fig. 20) with a range of four to nine days. Natural mortality usually extended over the entire larval period.

Table 22. Mortality of Hypsipyla grandella Zeller larvae resulting from infection with Metarrhizium anisopliae (Metch.) at concentration of 1.2×10^7 conidia/ml

No. of larvae	Total dead larvae	Larvae killed by the fungus	No. of pupae	No. of emerged adults	Lost larvae
Control					
9	1	0	6	6	2
9	1	1	6	5	2
9	2	0	4	4	3
3	0	0	3	2	0
Total 30	4	1	19	17	7
Treated					
9	5	3	2	1	2
9	6	6	3	3	0
9	6	6	1	1	2
3	0	0	3	3	0
Total 30	17	15	9	8	4

Fig. 19. Larvae of the shootborer Hypsipyla grandella Zeller dead of infection by the green-muscardine fungus, Metarrhizium anisopliae (Metch.)

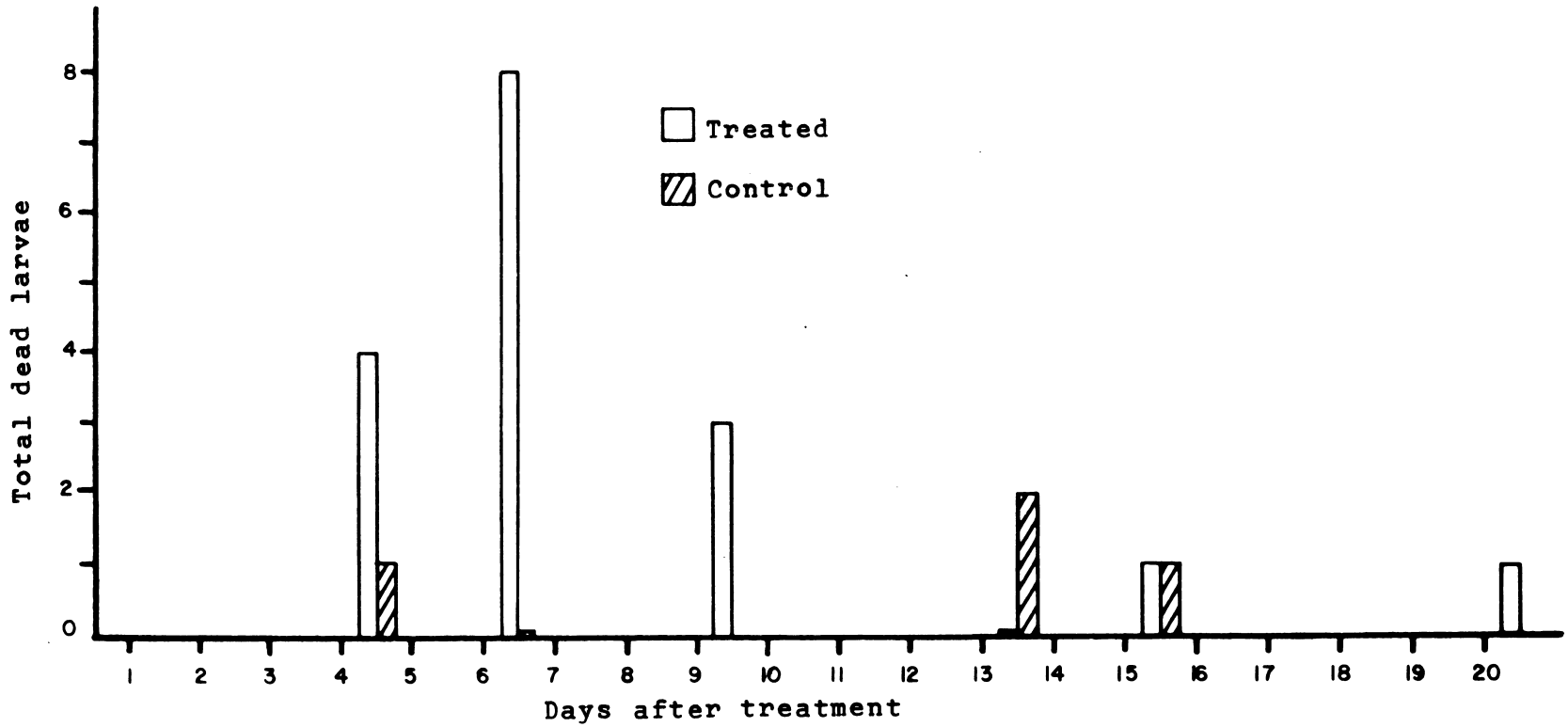

Fig. 20. Mortality distribution of Hypsipyla grandella Zeller larvae exposed to spores of Metarrhizium anisopliae at a concentration of 1.2×10^7 viable spores/ml.

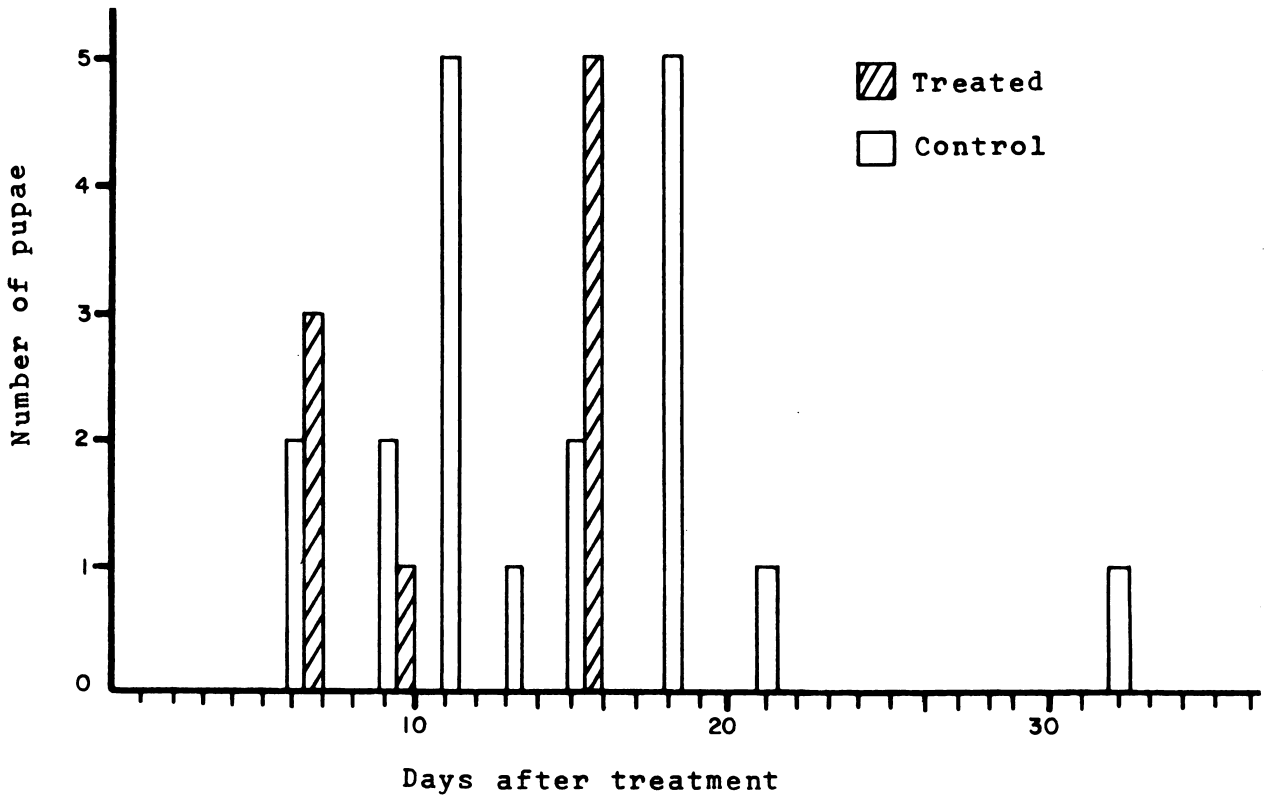

Fig. 21. Pupation of Hypsipyla grandella Zeller larvae exposed to spores of Metarrhizium anisopliae at a concentration of 1.2×10^7 viable spores/ml.

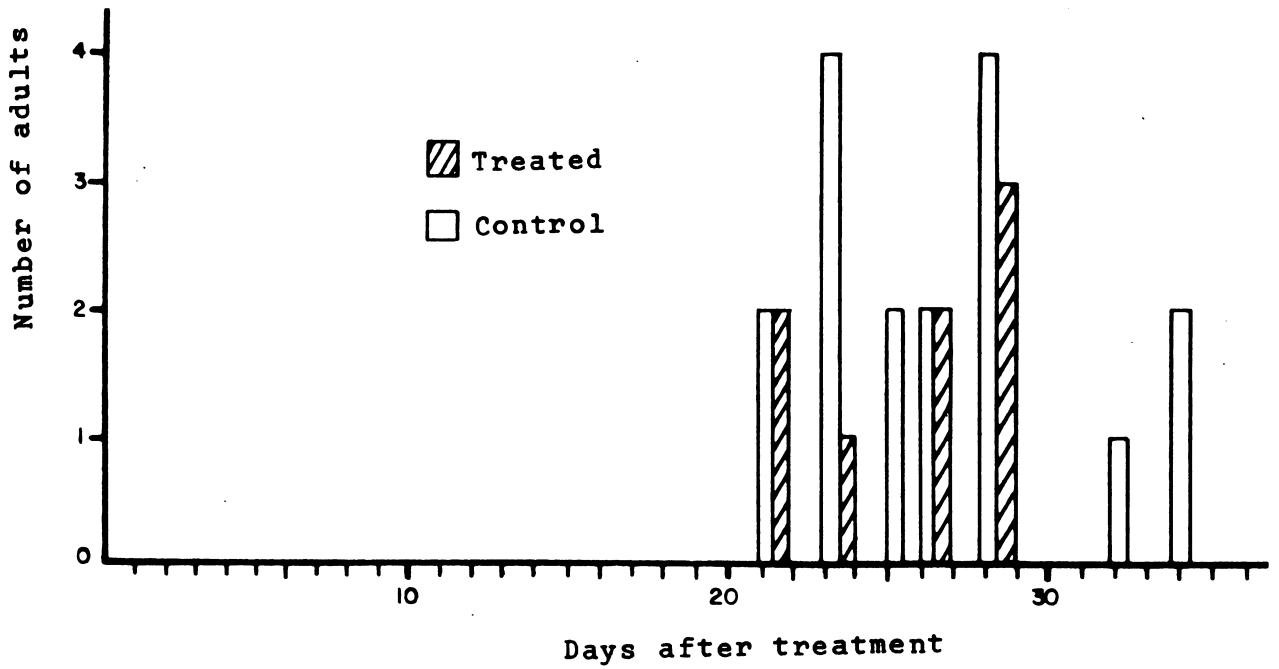

Fig. 22. Emergence of Hypsipyla grandella Zeller adults after the larvae were exposed to spores of Metarrhizium anisopliae at a concentration of 1.2×10^7 viable spores/ml.

Total mortality is shown in Fig. 23. The mortality of the control larvae was 13% and reflects causes other than the fungus under study. The cumulative mortality of the treated larvae was 57%.

In a similar set of experiments it was found that H. grandella is also susceptible to infection by the white muscardine fungi Beauveria bassiana and B. tenella (Fig. 24, 25 and 26), B. bassiana being more effective that B. tenella but less than M. anisopliae.

3. Radiation Biology and Mutagenesis of Insect Pathogens (O. Hidalgo-Salvatierra and R. Legarda)

Purpose

The objective of this investigation is "to apply radiation and radioisotopes in the field of insect pathology". Especifically, at the present time, we will limit ourselves to the radiation biology and mutagenesis of Metarrhizium anisopliae, Bacillus thuringiensis and a Spodoptera nuclear polyhedrosis (N. P.) virus. Our end with M. anisopliae is to have a mutant with the following characteristics: higher index of pathogenicity than the wild type, radiation resistance to increase the chances of survival under field conditions and a color different than that of the wild type to help in the field evaluation of the pathogen. These three characteristics can be obtained through radiation mutagenesis, among other things.

In the case of B. thuringiensis we wish to investigate the radiation repair mechanism of the bacillus and to isolate some radiation resistant strains. A mutant of this type could be very important if a preparation is to depend for its effectiveness on toxic crystals

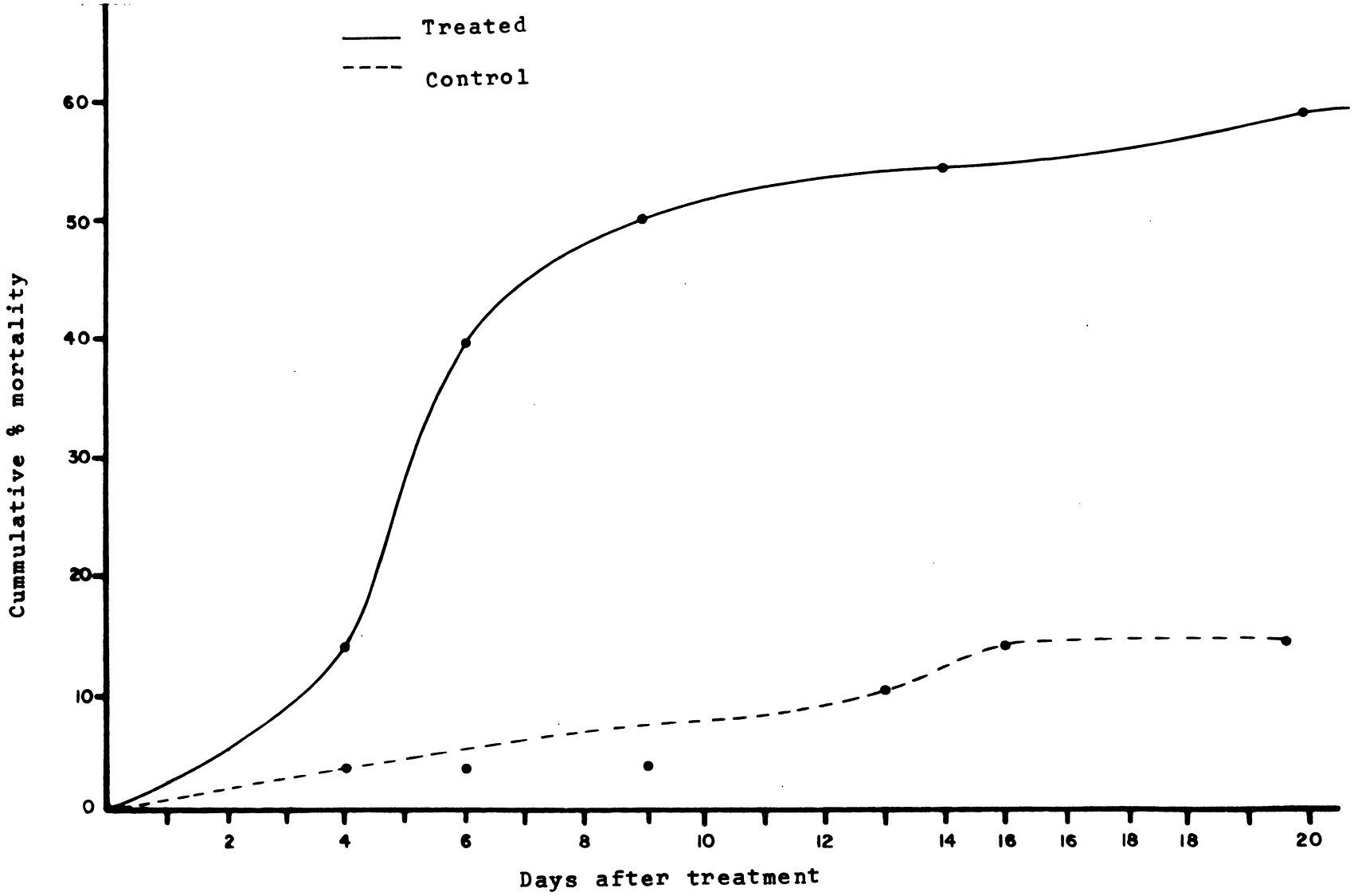

Fig. 23. Cummulative percent mortality of Hypsipyla grandella Zeller larvae exposed to spores of Metarrhizium anisopliae at a concentration of 1.2×10^7 viable spores /ml.

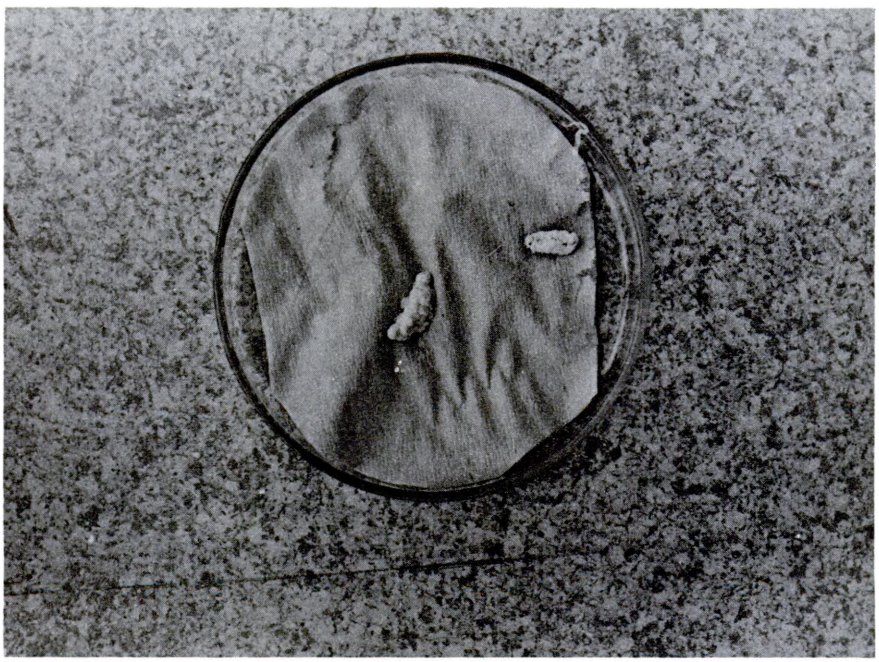

Fig. 24. Larvae of the shootborer Hypsipyla grandella Zeller dead of infection by the fungus Beauveria tenella (Delacroix)

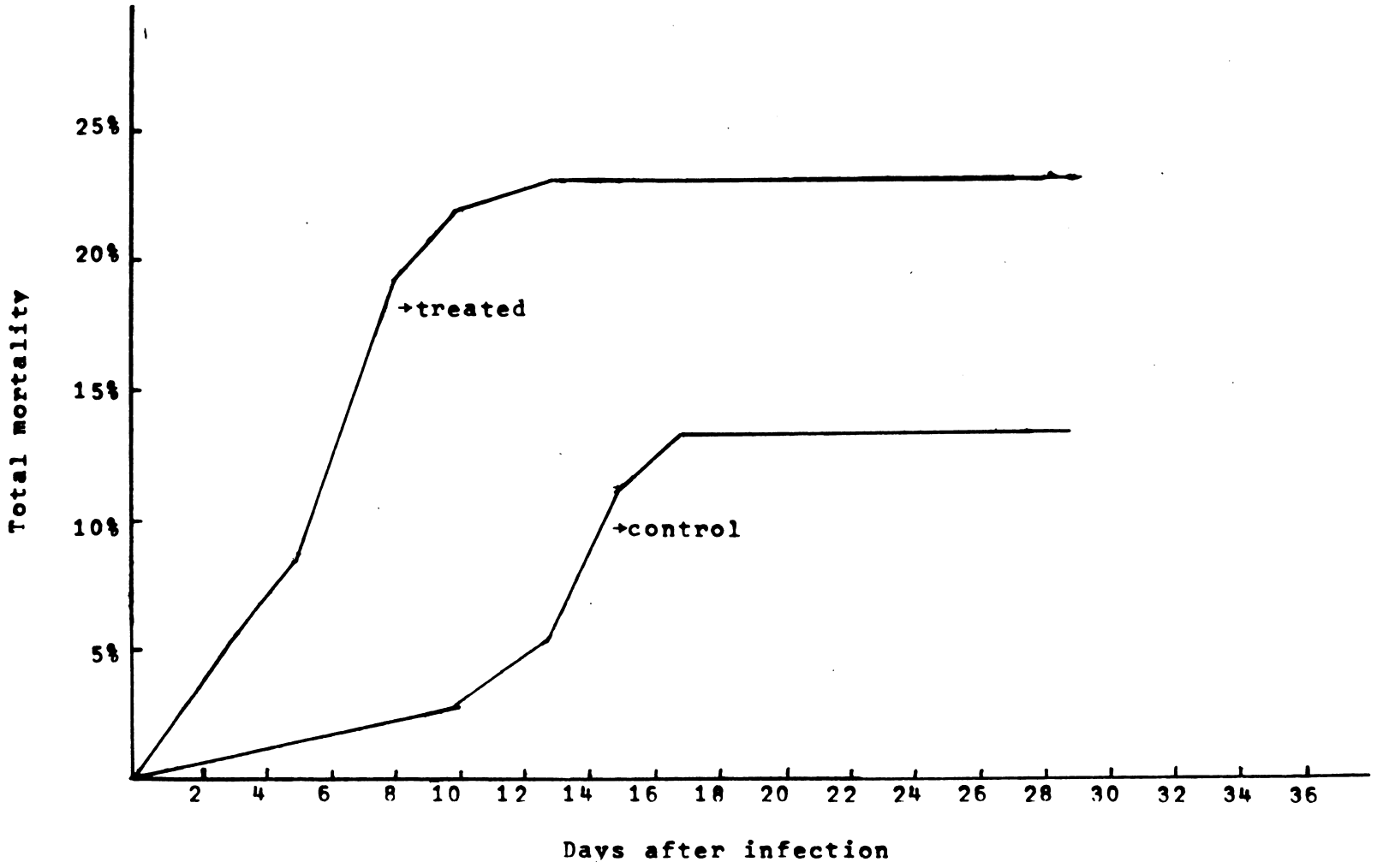

Fig. 25. Percent total mortality of Hypsipyla grandella larvae bathed with a conidial suspension of Beauveria bassiana at a concentration of 1.2×10^7 viable spores per ml.

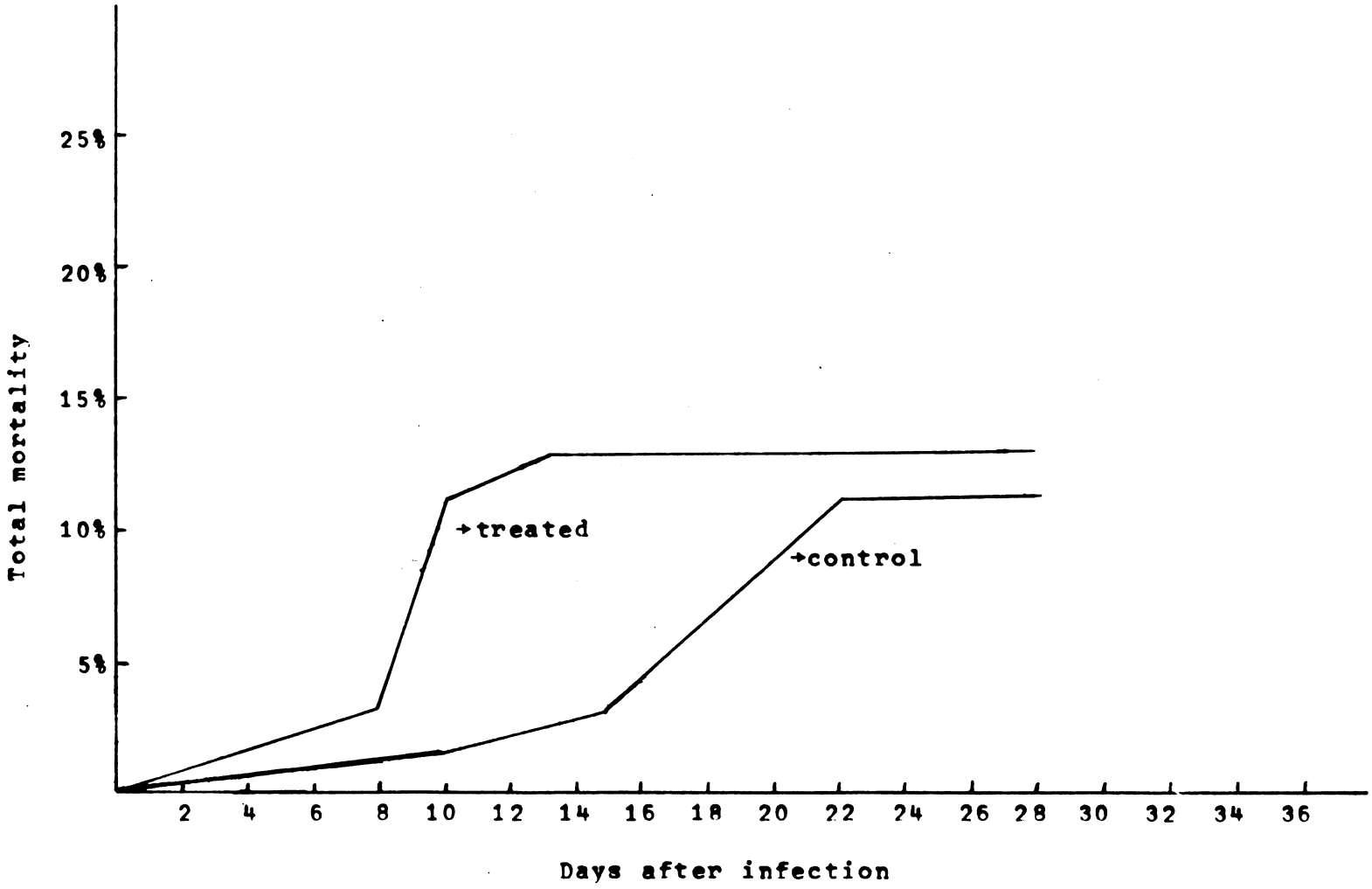

Fig. 26. Percent total mortality of Hypsipyla grandella Zeller larvae bathed with a conidial suspension of Beauveria tenella at a concentration of 1.2×10^7 viable spores/ml.

and viable spores. This may be the case in places where cheap preparations are made locally. Besides radiation resistance we will select for increased virulence with respect to our target insects.

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In our preliminary experiments on the radiation biology of B. thuringiensis thuringiensis we have determined the U. V. (254 mμ) survival of an overnight culture grown in nutrient broth at 37°C. The bacteria are collected by centrifugation, washed and suspended in water or PO_4 buffer (pH 6.8, 0.05 M) for irradiation. Survival is determined by dilution and plating in nutrient agar plates incubated at 30°C.

It seems from Fig. 27 that the buffer confers some protective effect on the bacillus. We don't know, at the moment, the significance of this. We can say, however, from the presence of the initial shoulder that some repair mechanism is present in B. thuringiensis.

References

1. Berrios, F. and O. Hidalgo-Salvatierra. Estudios sobre el barrenador de las Meliáceas, Hypsipyla grandella Zeller. V. Susceptibilidad de la larva al hongo Metarrhizium anisopliae. Turrialba 21: (in press)

2. Burton, R. L. Mass rearing the fall armyworm in the laboratory. U. S. Dept. Agr., Agr. Res. Serv. 33-117, 12 p. 1967.

3. _____. Mass rearing the corn earworm in the laboratory. U. S. Dept. Agr., Agr. Res. Serv. 33-134, 8 p. 1969.

4. Entwistle, P. F. The current situation on shoot, fruit and collar borers of the Meliaceae. Ninth British Commonwealth Forestry Conference, 1968.

5. Hidalgo-Salvatierra, O. and L. G. Madrigal. Studies on the shoot borer Hypsipyla grandella Zeller. IV. Trichogramma sp., an egg parasite. Turrialba 20:513. 1970.

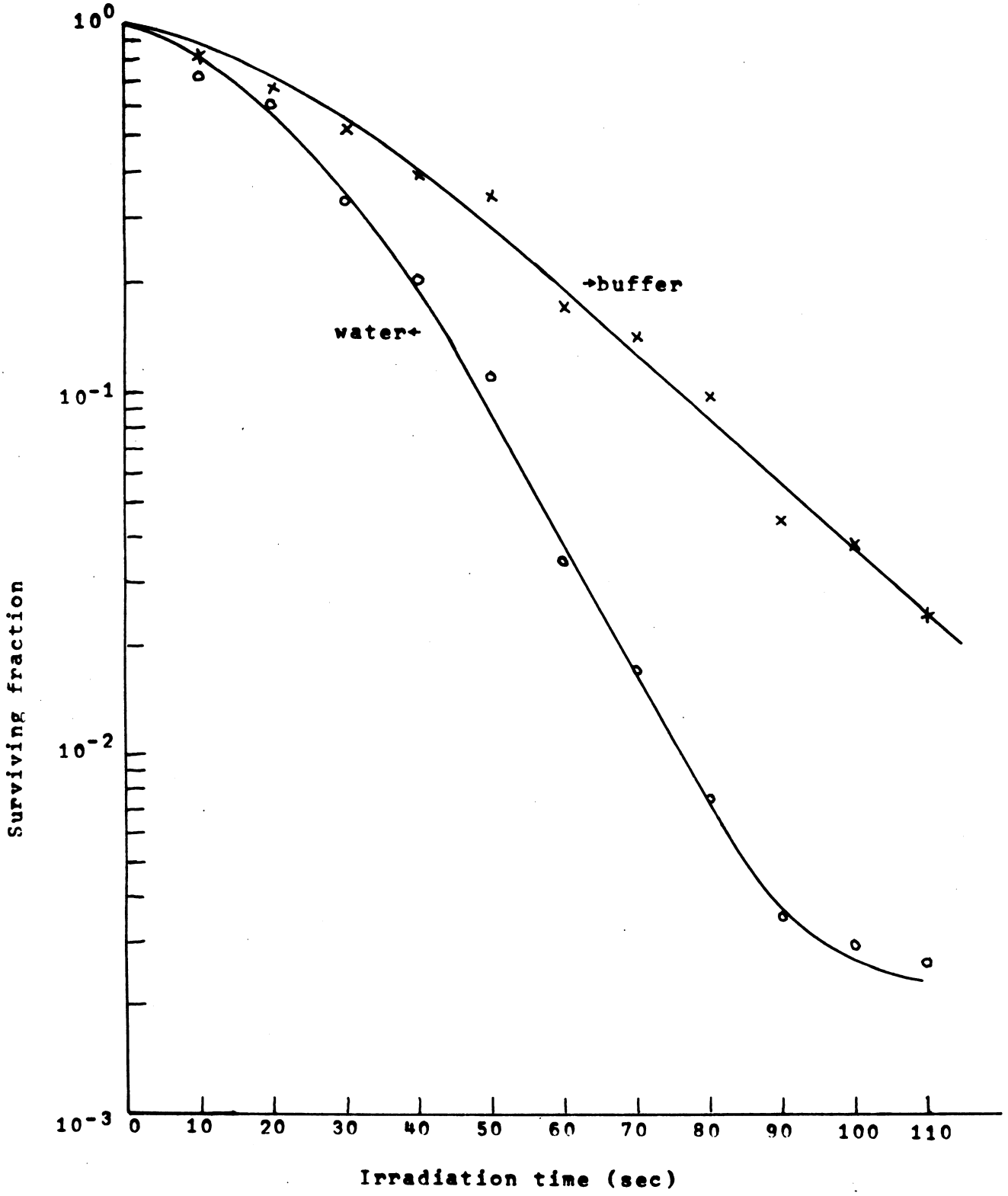

Fig. 27. Survival of Bacillus thuringiensis thuringiensis after U.V. (254 mμ) irradiation in water or in buffer. I_o = 17.5 ergs/mm^2/sec

6. Hidalgo-Salvatierra, O. Estudios sobre el barrenador de las Meliáceas Hypsipyla grandella Zeller. VII. Determinación del sexo en pupas. Turrialba 21: (in press).

7. Ignoffo, C. M. A successful technique for mass rearing cabbage loopers on a semi-synthetic diet. Annals of the Entomol. Soc. of America 56:178-182. 1963.

8. Kandasamy, D. Hypsipyla robusta Moore, a new host for Beauveria tenella (Delacroix) Siemaszko. J. Inv. Pathol. 13:149-150. 1969.

10. Steinhaus, E. A. Principles of insect pathology. Hafner Publ. Co. New York, 1967. 757 p.

D. SOIL CHEMISTRY

1. Phosphate Chemistry on Tropical Soils

a. Measurement of isotopically exchangeable P (E) and parameter (Q_o)

(K. Igue and R. Fuentes)

Purpose

The assessment of phosphate status in soils can be described in several ways. The most common procedure is the measurement of water soluble fractions, which is a measure of the phosphate potential (intensity parameter). Nevertheless, the intensity parameter itself does not indicate the ability of soil to supply the nutrient, unless the capacity factor is known. Schofield (12) emphasized that soils having identical phosphate potential may differ in their capacity to maintain the phosphate potential against removal or application of phosphate. Therefore, the phosphate potential determined at the beginning of the period of growth does not give sufficient information on the phosphate supply to plants during that period. Beckett and White (3) introduced

the phosphate potential buffering capacity (PBC) of a soil, which is defined as the amount of P to be added or removed per gram of soil in order to obtain a certain alteration of the phosphate potential. The PBC can be determined from the relation between quantity (Q_o) of phosphate and the corresponding phosphate potential (I) plot designated the Quantity/Intensity (Q/I) relationship (3).

Isotopically exchangeable P (E) has also been used to characterize the phosphorus status in the soil. Russel et al. (13) measured the fraction of soil P in equilibrium with a solution of P in N/100 $CaCl_2$. Beckett and White (3) used this procedure and found that "E" is greater than the Q_o determined from the Q/I parameter, indicating different sites for P adsorption. The interpretation of their data is somewhat difficult under highly fixing tropical soil in which the water soluble fraction is hardly detected and where the exact mechanism of adsorption is not well known. Amer (2) on the other hand, indicated the difficulty in measuring the isotopically exchangeable fraction in high P fixing soils.

This study is primarily concerned with the properties of the pool of "immediately labile" soil phosphate; to relate it with the fixation regions of Langmuir's isotherm and to obtain relationship between "E" and "Q_o" values in high P fixing acid soils.

Progress Report

Fig. 28 shows the adsorption isotherm for the three soils. The characteristics of these soils are presented in Table 23. The isotherms were arbitrarily divided in three regions (I, II, and III) following

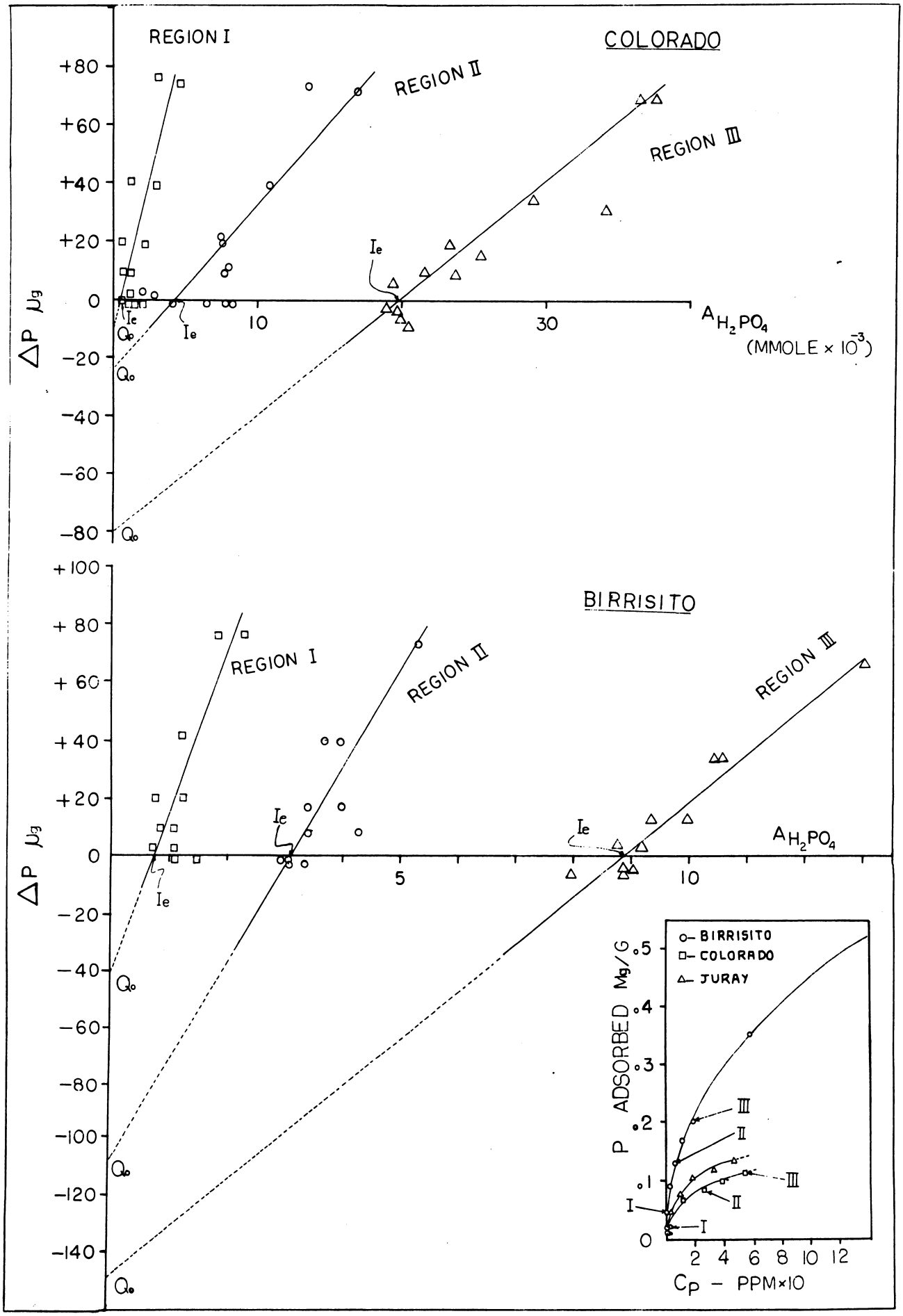

FIG 28 . QUANTITY-INTENSITY RELATIONSHIP

Table 1. Chemical, physical and mineralogical characteristics of the soil used in phosphate studies

	Juray (Distropepts*)	Birrisito (Dystrandepts*)	Colorado (Distropepts*)
Mineralogy[3]	Allophane (d) Gibbsite	Allophane (d) Gibbsite (c) Ferric gels (p)	Allophane (p) Metahalloysite (d) Gibbsite (p) Gethite/hematite (p) Ferric gels (p)
pH	6.2	5.7	5.2
Organic matter %	1	22	9
Free oxides**			
Al[1] %	12.6	26.8	31.0
Fe %	2.9	2.3	6.3
P-fractions (mgP/cm^3)			
Sol-P	tr	tr	tr
Al-P	667	143	62
Fe-P	339	64	344
Ca-P	118	35	27
Texture %			
Sand	49.5	52.0	19.0
Clay	9.5	12.0	54.0
Silt	41.0	32.0	27.0
Field*			
Dap[1]	0.83	0.55	0.90
θ[2]	64.5	102.0	35.6
Pot			
Dap[1]	0.62	0.51	1.00
θ[2]	45.2	74.9	13.6

* Classified by Knox and Maldonado (7) and Aguirre (1).
** Hashimoto and Jackson method (5). Bornemisza and Igue (4).
1. Apparent density in the field and pot experiment
2. Gravimetric water g/100 g.
3. Besoain, M. E. - unpublished data. Allophane determined according to Fieldes and Perrat (14). (d) = dominant; (p) = present; (c) = common.

the method of Muljadi et al. (10). According to these regions, Birrisito soil adsorbed 0.50, 1.50 and 2.48 mg of P/g of soil in regions I, II, and III, respectively; Colorado soil adsorbed 0.25, 0.87 and 1.32 mg P/g soil and Juray soil adsorbed 0.24, 0.78 and 1.19 mg P/g soil in regions I, II, and III, respectively.

Q/I relationship: In order to obtain the Q/I relationship the soils treated with phosphate at different regions (first P treatment) were equilibrated with ^{32}P labelled phosphate solutions with the following concentrations: 0, 0.1, 0.2, 0.5, 1.0, 2.0, and 4.0 ppm. The second equilibration time was 30 minutes to minimize diffusion process and microbial activities. Following equilibration, the amount of P adsorbed or released by the soil was determined and the plot of Q/I relationship obtained. This is represented in Fig. 28 for Birrisito and Colorado soil. As we increase the P saturation in the soil the Q_o value increased for both soils. Also, the slope of the lines decreases when P adsorbed initially goes from region I to III, which roughly is the indication of increasing potential buffering capacity (PBC). On the other hand, a lower value is obtained for the intensity parameter ($I = A_{H_2PO_4}$) in Birrisito soil as compared to less fixing Colorado soil. Nevertheless the Q_o value is much greater in the case of Birrisito soils as compared to Colorado soil. It remains to be decided later on if Birrisito presents higher capacity to supply P to the plants.

Isotopically exchangeable phosphorus (E): Table 24 summarizes the values of "E", Q_o, IDF (isotopically dilution factor), surface P, and extractable P for Birrisito and Colorado soils, respectively. The

Table 24. Relationship among "E", Q_o, and extractable P from different regions of the isotherm

Soil	Isotherm region	Isotopic Exchange P "E"[1]	P_m[2] (Quantity)	P-surface	IDF[3] (Intensity)	Q_o[4]	I_e x10^{-3}	Extractable P_{resin}[5]	P_{KCl}[6]	$P_{K_2SO_4}$[6]
		ppm					mmole	ppm		
Birrisito	I	1.25*	32	32	0.88	41	0.8	42	8	9
		145	145	170	0.73- 1.17					
	II	3.39*	85	85	2.67	118	3.1	34	25	38
		239	236	262	2.43- 3.20					
	III	6.89*	169	169	6.50	149	8.9	134	74	91
		408	395	449	0.07- 8.97					
Colorado	I	7.79*	195	193	1.37	12	0.6	20	9	11
		91	91	99	0.86- 1.79					
	II	225	256	271	1.73-11.2	24	4.4	66	39	73
	III	437	443	439	15.7-26.8	80	20.0	116	87	142

* Carrier-free - (E=B/sf, B=initial CPM/ml)

1. $E = P_o(\frac{Si}{Sf}-1)$

2. P_m - IDF x $\frac{Ai}{Af}$

3. IDF = $P_t - P_o(\frac{Af}{Ai})$ (range, in the case of the carrier method)

4. Extrapolation of Q/I to I_o
5. Five successive extractions for resin, 16 hr each
6. Ten successive extractions, 1 hr each

Si = initial specific activity
Sf = final specific activity
P_o = P initially added
P_t = P final concentration
A_i = initial CPM/ml
A_f = final CPM/ml

equilibrium concentration (I_e) where the soil neither gains nor loses phosphorus, increase from region I to region III. Isotopically exchangeable phosphorus ("E") increases also from region I to III. The "E" measured by carrier-free method is very small as compared to the carrier method. Besides, the "E" value measured at zero concentration (carrier-free) does not agree with the P_m and $P_{surface}$, and shows a rather small value. As indicated by Amer (2) exchangeable P can be overestimated for high P fixing soils when carrier free method is used. In the present case more than 95% of ^{32}P was fixed by the soil. The error can be reduced with addition of carrier. Nevertheless, under the present conditions even with the presence of the carrier the "E" value seems to be high, indicating retention of ^{32}P in non-exchangeable forms. The best measure of "E" seems to be that of equilibrium concentration where the soil neither gains nor loses phosphorus ($\Delta P = 0$), i.e. at I_e, in Fig. 28. Probably the use of carrier-free procedure presents marked error when the soil retains more than 95% of added ^{32}P. Fig. 28 also shows that extrapolation of Q/I back to I = 0 gives values for Q_o that are somewhat smaller than the "E" value. This was discussed by Beckett and White (3) indicating that Q_o measures the net-exchange sites and can be replaced by other anions, such as OH, Cl or $SO_4^{=}$, whereas the isotopic exchange sites may perhaps be held at the surfaces of phosphate compounds sufficiently well crystallized for exchange hydroxyl and phosphate to be improbable. Table 24 also shows the values of resin extractable P (Dowex-21 K-16-20 mesh, Cl^- form), and KCl, and K_2SO_4 extractable phosphorus fractions. The resin P, arbitrarily called

reserve P (11) as well as KCl and K_2SO_4 soluble fractions are closer to the Q_o value obtained from the graph, whereas "E" values are way above these values. This seems to indicate that "E" value overestimates available fraction of P in these soils, whereas Q_o, resin-P and KCl and K_2SO_4 seems to give values close to that form available for plants.

The intensity parameter - IDF (9), representing soil's contribution to P contents of equilibrium solutions were increased from region I to III. This represents the fraction of soil P that is available to the plants (9).

No attempt was made to determine "E" and "Q_o" values for phosphate untreated soil. Further measurements will be carried out for untreated soils by inverse isotope dilution method.

References

1. Aguirre, V. Estudio de los suelos del área del CTEI-IICA - Turrialba, Costa Rica. M.S. thesis, Turrialba, Costa Rica.

2. Amer, F. Determination of ^{32}P exchangeable phosphorus in soils. In Radioisotopes in Soil-Plant Nutrition Studies. IAEA Proc. Symp. Bombay. pp. 43-58. 1962.

3. Beckett, P.H.T. and White, R. E. Studies on the phosphate potentials of soils. III. The pool of labile inorganic phosphate. Plant and Soil 21:253-282.

4. Bornemisza, E. and Igue, K. Oxidos libres de hierro y aluminio en suelos tropicales. Turrialba 17:23-30. 1967.

5. Hashimoto, I. and Jackson, M. L. Rapid dissolution of allophane and kaolinite-halloysite after dehydration. In 7th Nat. Conf. Clay and Clay Minerals 102:113. 1959.

6. Hsu, P. H. and Rennie, D. A. Reactions of phosphate in aluminum systems. I. Adsorption of phosphate by X-ray amorphous "Aluminum hydroxide". Can. J. Soil Sci. 42:197-209. 1962.

7. Knox, E. and Maldonado, F. Suelos de cenizas volcánicas, excursión al volcán Irazú. In Panel sobre Suelos Derivados de Cenizas Volcánicas de América Latina. Turrialba, 1969. Turrialba, FAO/IICA, 1969. pp. 71-73.

8. McAuliffe et al. Exchange reactions between phosphates and soils: Hydroxylic surfaces of soil materials. Soil Sci. Soc. Amer. Proc. 12:119-123. 1947.

9. McConaghy, S., J.W.B. Stewart and M. Malek. Soil phosphate status as measured by isotopic exchange and other techniques.

10. Muljadi, D., Posner, A. M. and J. P. Quirk. The mechanism of phosphate adsorption by kaolinite, gibbsite, and pseudobohemite. I. Jour. Soil Sci. 17:212-247.

11. Palma, G. and H. W. Fassbender. Estudio de P en suelos de America Central. V. Uso de resinas de intercambio para evaluar la disponibilidad de P. Turrialba 20:279-287.

12. Schofield, R. K. Can a precise meaning be given to "available" soil phosphorus. Soils and Fertilizers 18:373-375. 1955.

13. Scott Russell, R., J. B. Rickson and S. N. Adams. Isotopic equilibria between phosphates in soil and their significance in the assessment of fertility by tracer methods. Jour. Soil Sci. 5:85-105. 1954.

14. Fieldes, M. and K. W. Perrot. The nature of allophane in soils. Part 3. Rapid field and laboratory test for allophane. New Zealand Jour. of Sci. 9:623-629. 1966.

b. Desorption of phosphate reacted with soil (K. Igue and R. Fuentes)

Purpose

The use of sorption isotherms may lead to a very practical way to predict P need of the soil since it takes into account the intensity and capacity factor. The rate of desorption seems to be a good measure of capacity. Fox and Kamprath (1) indicated recently that for a given level of P in a soil P solubility is greater when P is being added to the system than when it is being withdrawn.

Desorption isotherm was related to P adsorved and was shown to follow Langmuir's isotherm (1, 2, 3). Thus equilibrium solution concentration (intensity) of 0.05 to 0.1 ppm was shown to allow 95% of maximum yield (1). This concentration was attained by adding from 530 to 1100 lbs/a of P in oxisols and andosols respectively. Thus, the important question is to know how fast the "fixed" phosphate is solubilized or dissolved.

The objective of this study was to observe: a) the rate of desorption of P retained by the soil in the three regions of the isotherm in 0.02 M KCl and K_2SO_4 and anion exchange resin Dowex 21-K (16-20 mesh) in Cl^- form; and b) what fractions of soil phosphate are mostly affected by successive desorption.

Progress Report

Fig. 29 presents the results of the successive desorption carried out for regions I, II, and III of the Colorado and Birrisito soils. Phosphorus released by three extractants increased with the increase in P adsorbed by the soil. More P is extracted by K_2SO_4 and resin than KCl. The amount extracted changes little during 10 successive extractions for KCl and K_2SO_4, although in the case of resin there was a marked decrease in the regions II and III, but somewhat constat for region I, a less saturated region. For Birrisito soil the amount extracted by the resin is somewhat higher in region I than II. In general, the resin extracted more P than KCl or K_2SO_4. All three procedures bear close relation to the Q_o parameter.

Desorption studies were also carried out by saturating Colorado (Dystropepts) and Cervantes (Vitrandepts) soils by equilibrating them

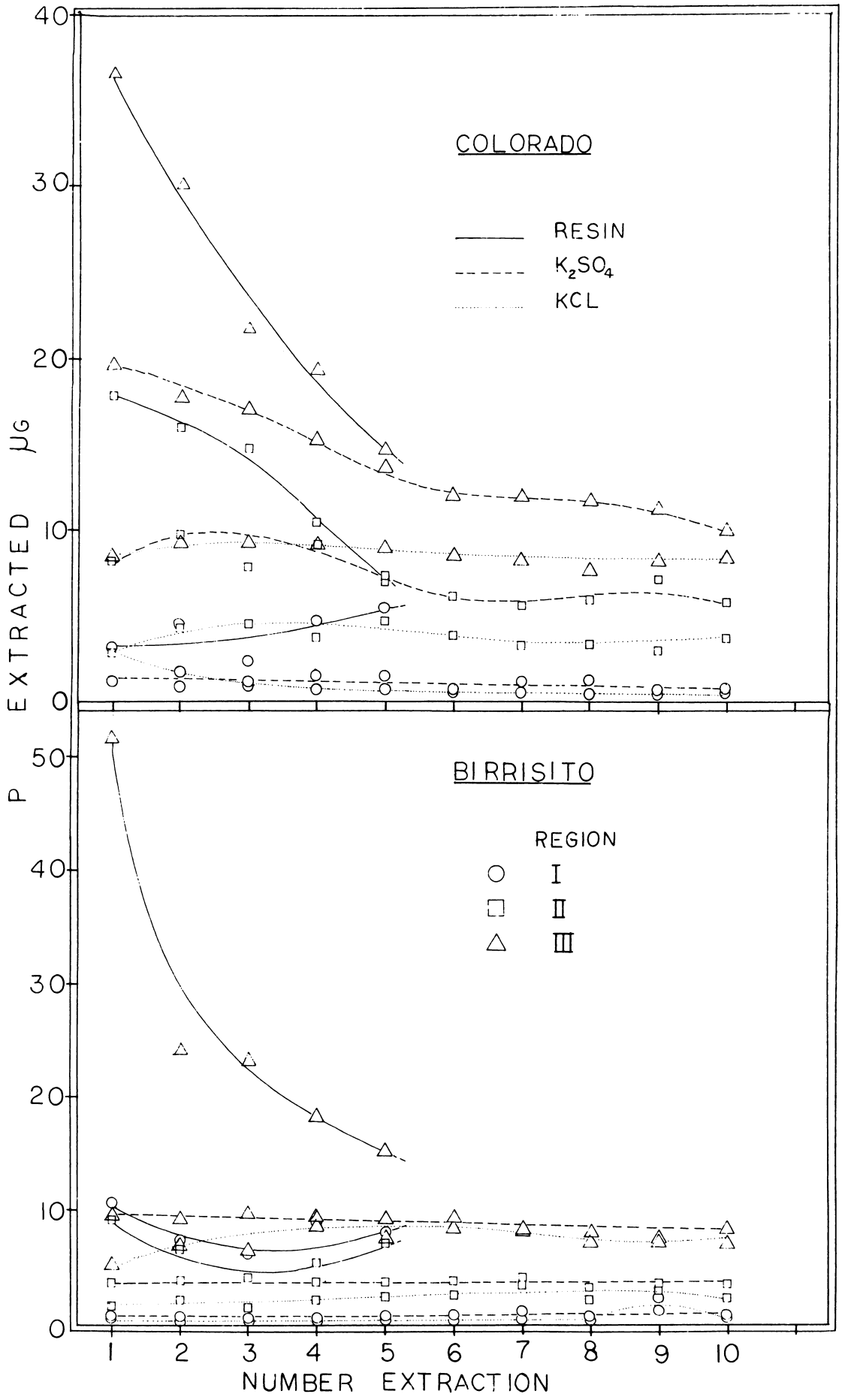

FIGURE 29 FRACTION OF EXTRACTABLE PHOSPHORUS FROM THREE REGIONS OF ADSORPTION ISOTHERM

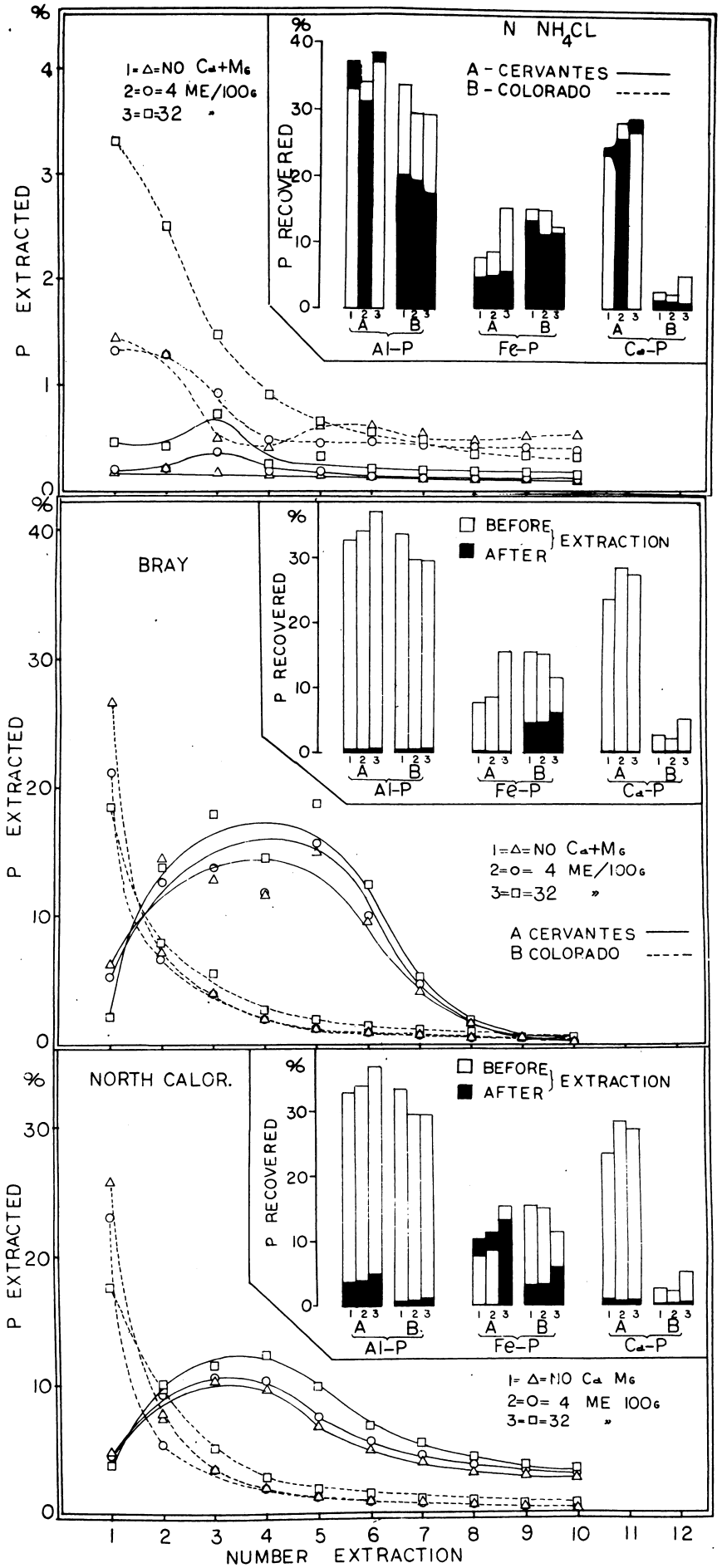

FIG 30. FRACTION OF ADSORBED P EXTRACTED BY NH_4CL, BRAY, AND NORTH CAROLINA SOLUTIONS.

with 25 ml of a 100 ppm P solution tagged with ^{32}P. Following a 6 hr saturation period, 10 successive desorptions with other commonly used extractants were undertaken and the ^{32}P was determined.

Results are presented in Fig. 30 for N NH_4Cl, 0.03 N NH_4F + 0.7 N HCl (Bray) and 0.05 N HCl + 0.075 N H_2SO_4 (NC).

The amount of dissolved P decreased with the number of successive extractions for the Colorado soil in all four extractants. In the case of the Cervantes soil, a slight increase in desorption was observed from the 3rd to the 5th extractions for NH_4Cl, Bray and NC, and then it continues to decrease. Total P released was almost comparable for all the extractants used, except NH_4Cl, in which the Colorado soil released more P initially as compared to the Cervantes*. From Fig. 30 it can be seen that the liming application increased the NH_4Cl soluble fraction in both soils, whereas for the other extractants the difference due to liming was not well-defined.

Fractionation studies indicated that NH_4Cl released more Al-P from the Cervantes and more Fe-P from the Colorado soil. In several instances there was an increase in certain P fractions, possibly due to re-fixation during the equilibration period. When the NC extractand was used almost all Al-P and Ca-P were released with the exception of Fe-P which was less soluble mainly in the Colorado soil. The Bray extractant released almost all the P-fractions from both soils, indicating, therefore, to be a very strong extractant under prevailing conditions. The resin method showed a high correlation with total plant absorbed P.

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* (Vitrandept)

1. Fox, R. L. and E. J. Kamprath. Phosphate sorption isotherms for evaluating the phosphate requirements of soils. Soil Sci. Soc. Amer. Proc. 34:902-907. 1970.

2. Hsu, P. H. Adsorption of phosphate by aluminum and iron in soils. Soil Sci. Soc. Amer. Proc. 28:474-478. 1964.

3. Veloso, A. C. Phosphate solubility in andosols and oxisols. M.S. Thesis, Univ. Calif. Riverside. 88 p. 1969.

c. Reaction and efficiency of calcium phosphates (K. Igue, D. Suárez, J. Urrutia and R. Fuentes)

Purpose and Method

The main factors governing the efficiency of a phosphate fertilizer to plants are: a) rate of dissolution, b) characteristics of the soil that limit the movement of the phosphorus dissolved due to "fixation", and c) the plant itself. Several other factors can also be indicated (5, 6, 8, 11). Bornemisza and Fassbender (1) found very low absorption of ^{32}P by corn plants from Costa Rican soils approximately 0.91 to 1.44% of fertilizer applied. De Datta et al. (3) found only 1% absorption of P from sodium pirophosphate by Sorghum in Hawaiian latosols.

These studies were undertaken with the purpose of verifying: a) the reaction of calcium phosphates near the site of application, and b) the efficiency of these fertilizers in the acid soils for corn plants.

Fertilizer material used in the studies were:

-Monocalcium phosphate monohydrate (MCP-C) 51.6% P_2O_5

-Simple superphosphate (MCP-S) 19.9% P_2O_5

-Dicalcium phosphate anhydrous (DCPA) 41.3% P_2O_5

The phosphates were labelled with ^{32}P-0.436 mCi/g P_2O_5, and manufactured

by the TVA (Tennessee Valley Authority, USA). Except for MCP-C that was prepared for -12 mesh to 1/2 inch size, the other two were in powder form (-12 mesh). The soils used in this study were already described in Table 24.

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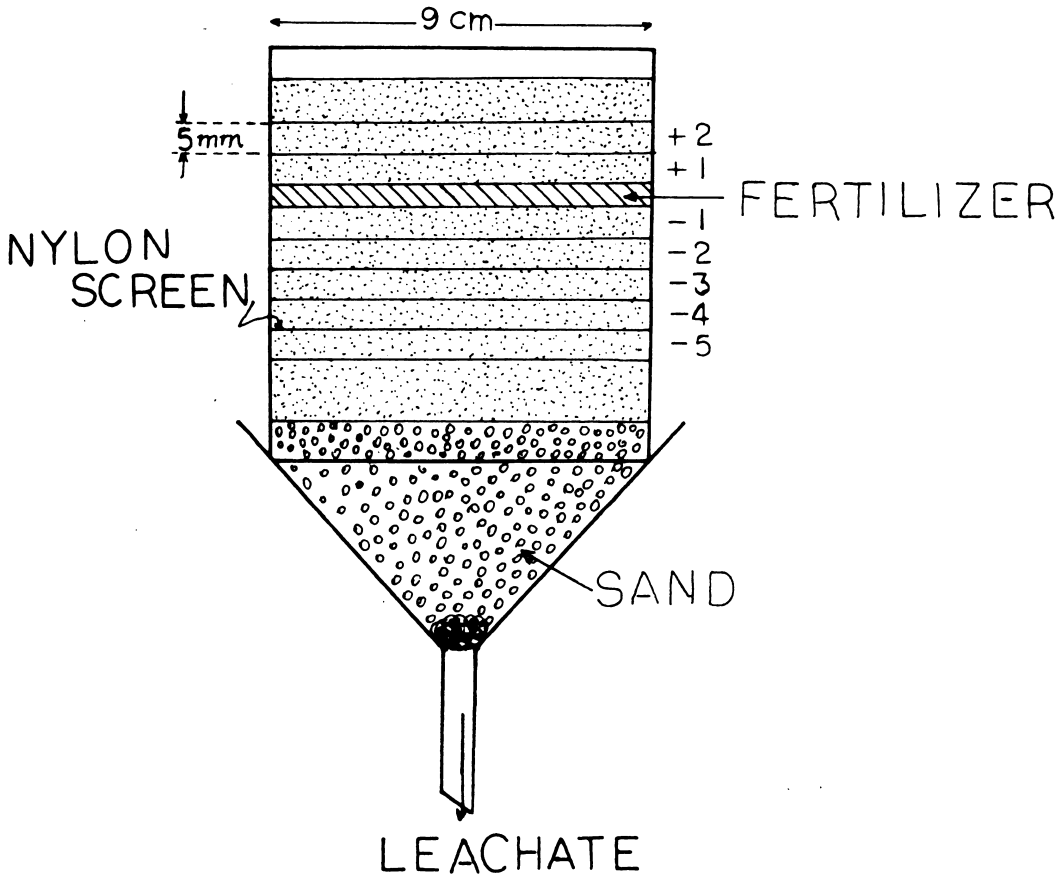
Laboratory Study: The original method described by Lindsay and Stephenson (5) which allowed to reproduce the zone near the site of fertilizer application was adapted. Fig. 31 shows the schematic diagram of the system used. Soil layers (5 mm thick) were separated by nylon screen and the columns were leached with a total of 120 mm of water in three applications at three day intervals from each other. The leachate was collected and the columns were dismantled after a 20 day period of reaction and then analyzed for ^{32}P and ^{31}P.

Table 25 shows the fraction of P leached throughout the column. Only small fraction of P was leached from MCP-C and MCP-S, whereas DCP was almost nihil. The movement and leaching of P was markedly affected by allophane content. The amount of phosphate residue left at the site of application is relatively small for MCP-S and MCP-C, as compared with DCP, in which more than 97% was left as residue.

The distribution of phosphate fractions as well as the pH and extractable Al and Fe is presented in Table 26. In general, Al-P is the predominant form in all soils; nevertheless, its relation to Fe-P decreases as the distance from the site of application increases. This seems to indicate that at low P concentration Fe is promptly reactive with P, as was the case of Birrisito and Colorado when DCP was the



SOIL BULK DENSITY

BIRRISITO - .52
COLORADO - .94 G/CM^3
JURAY - .78

FIG 31. LISIMETER USED IN STUDYING FERTILIZER REACTIONS.

Table 25. Phosphate leached from the column and residue left following application of 120 mm of water

Soil	Leachate N°	Fertilizer: MCP-C µg	MCP-C 0/00*	MCP-C Residue %	MCP-S µg	MCP-S 0/00*	MCP-S Residue[1] %	DCP µg	DCP 0/00*	DCP Residue %
Juray	1	765			472			-		
	2	297			102			15		
	3	-			100			-		
	Total	1062	0.4	4.3	674	0.3	6.1	15	-	98
Birrisito	1	86			23			-		
	2	77			556			-		
	3	107			311			-		
	Total	270	0.1	2.9	890	0.4	7.3	-	-	97
Colorado	1	24193			32170			10		
	2	21313			16400			14		
	3	17467			8400			50		
	Total	62973	26.0	5.9	56970	24.0	7.7	74	-	99

* of applied (per thousand)

1. Residue of phosphate left at the site of application after leaching.

material reacted. Yuan et al. (12) observed that P-Al/P-Fe increased with the rate pf P application. Chang and Chu (2) on the other hand, indicated that P-Al/P-Fe decrease with time, indicating that the reaction of added P is essentially surface phenomena, where activities of Al, Fe and Ca are negligible. Thus, aluminum being the dominant cation at the surface of the colloid, it will react with P; later on more stable P-Fe is formed. Calcium phosphate is only a small fraction under prevailing conditions.

The effect of fertilizer on soil chemical properties can be seen also in Table 26. The pH is markedly affected near the zone of application, due to the effect of the dissolution product of MCP-C and MCP-S (5).

Table 26. Distribution of P fractions, pH and extractable Al and Fe (ug/cm^2/layer)

	MCP-C							MCP-S							DCPD						
	column layer							column layer							column layer						
Analysis	+2	+1	-1	-2	-3	-4	-5	+2	+1	-1	-2	-3	-4	-5	+2	+1	-1	-2	-3	-4	-5
JURAY																					
Total P	1600	13390	14920	2940	968	481	256	704	9840	13190	2600	725	332	86	0	249	245	35	11	3	0
P-sol	20	765	1040	120	9	0	0	0	438	628	78	4	0	0	0	0	0	0	0	0	0
P-Al	1310	10880	11930	2400	780	376	192	564	8060	10450	2040	567	259	58	0	189	187	26	9	3	0
P-Fe	253	1350	1490	379	168	96	57	129	1220	1700	361	139	67	25	0	55	52	9	2	0	0
P-Ca	20	390	464	43	11	9	7	11	119	190	119	15	6	3	0	5	6	0	0	0	0
P-Fe occl.	-	3-	26	-	-	-	-	-	25	33	-	-	-	-	0	22	28	0	0	0	0
P-Al occl.	-	4	3	-	-	-	-	-	2	2	-	-	-	-	-	-	-	-	-	-	-
Al	270	68	68	151	321	472	545	428	137	113	167	340	518	605	614	576	532	597	603	609	642
Fe	14	4	4	9	18	29	39	26	5	4	7	20	36	43	45	37	35	42	44	48	46
pH	5.65	4.95	4.95	5.50	5.65	5.70	5.75	5.45	4.35	4.10	5.10	5.25	5.35	5.40	5.95	5.95	6.00	5.90	5.95	5.90	5.90
BIRRISITO																					
Total P	1950	12680	13430	3910	736	274	123	446	11180	13050	3130	423	157	92	2	554	452	61	25	9	1
P-sol	9	594	706	56	6	0	0	0	365	548	26	4	0	0	0	0	0	0	0	0	0
P-Al	1570	10020	10760	3270	562	199	84	325	8890	10510	2560	304	108	61	0	393	315	43	13	4	0
P-Fe	356	1630	1540	530	150	67	34	99	1650	1730	481	100	42	27	2	141	120	18	12	5	1
P-Ca	19	434	424	57	18	8	5	22	276	263	64	15	7	4	0	20	17	0	0	0	0
P-Fe occl.	0	13	12	0	0	0	0	0	15	11	0	0	0	0	0	8	12	0	0	0	0
P-Al occl.	-	7	7	1	-	-	-	-	6	5	1	-	-	-	-	-	-	-	-	-	-
Al	523	54	54	171	880	1141	1131	1048	90	90	312	860	1142	1332	1332	958	907	1320	1399	1399	1241
Fe	11	3	3	3	21	29	29	28	3	4	6	24	30	33	33	25	23	31	35	36	31
pH	5.25	4.55	4.40	4.15	5.20	5.20	5.20	5.00	3.90	3.55	4.55	4.80	4.85	4.95	5.25	5.55	5.55	5.30	5.25	5.30	5.25
COLORADO																					
Total P	3340	7700	9020	5280	2970	1870	1080	2920	8370	7790	3490	1725	858	448	0	425	457	25	10	6	3
P-sol	369	915	1110	706	400	193	92	266	645	638	401	153	53	16	0	0	0	0	0	0	0
P-Al	2290	5520	6490	3720	1920	1190	620	2050	6120	5670	2460	1100	503	233	0	233	246	7	0	0	0
P-Fe	653	1170	1310	804	620	470	355	583	1520	1410	653	454	302	199	0	192	211	18	10	6	3
P-Ca	26	91	114	45	25	14	13	26	81	69	29	17	0	0	0	0	0	0	0	0	0
P-Fe occl.	0	0	0	0	0	0	0	-	10	13	-	-	-	-	-	-	-	-	-	-	-
P-Al occl.	3	11	13	4	1	-	-	-	7	7	1	-	-	-	-	-	-	-	-	-	-
Al	69	52	58	55	64	82	116	114	107	104	104	114	138	162	245	150	145	214	247	297	266
Fe	6	5	5	6	6	7	11	9	10	11	7	9	15	20	35	16	16	32	35	35	37
pH	4.70	4.40	4.30	4.45	4.65	4.65	4.60	4.00	3.55	3.55	3.80	4.25	4.35	4.30	4.40	4.95	5.05	4.45	4.35	4.25	4.25

The DCP on the other hand, showed a rather alkaline reaction and the pH increased little near the site of application in agreement with the results published (9). Extractable Al (NH_4OAc pH 4.8) content decreased near the site of application of P, presumably due to its reaction with P. The results are quite in agreement with the mineralogical data of these soils (Table 23). The effect of phosphates upon extractable Fe showed the same pattern, decreasing at the site of application. Considering, above all, the effect of fertilizers in the soil, DCP seems to be the most adequate material.

Greenhouse Experiments

Factorial experiments were set up in order to study the efficiency of MCP-C in five different granule sizes: -12 mesh, 9-12 mesh, 6-9 mesh, 3-6 mesh, and 0.5 inch. The following doses of P were applied: 309.6, 619.2, and 928.8 mg of P_2O_5/liter of soil, corresponding to 929, 1,858 and 2,786 mg of P_2O_5/pot. Each pot received three liters of soil. The soils used were already indicated in Table 24. Corn plants were used in two successive croppings. The efficiency of P was measured as $^{32}P_2O_5$ extracted by plant/P_2O_5 added x 100. From this "A" values were calculated. Fig. 32 presents the dry weight of corn, P absorbed from the soil and fertilizer, and A value, in three soils as affected by granule size and doses. Dry weight of corn plants was influenced by dosis and granule size. Higher yield was obtained for Juray soil as compared to Birrisito and Colorado soils. Nevertheless, the effect of applied P upon absorbed P is pronounced in the case of Colorado and less for Birrisito soil. Greatest proportion of absorbed P comes from

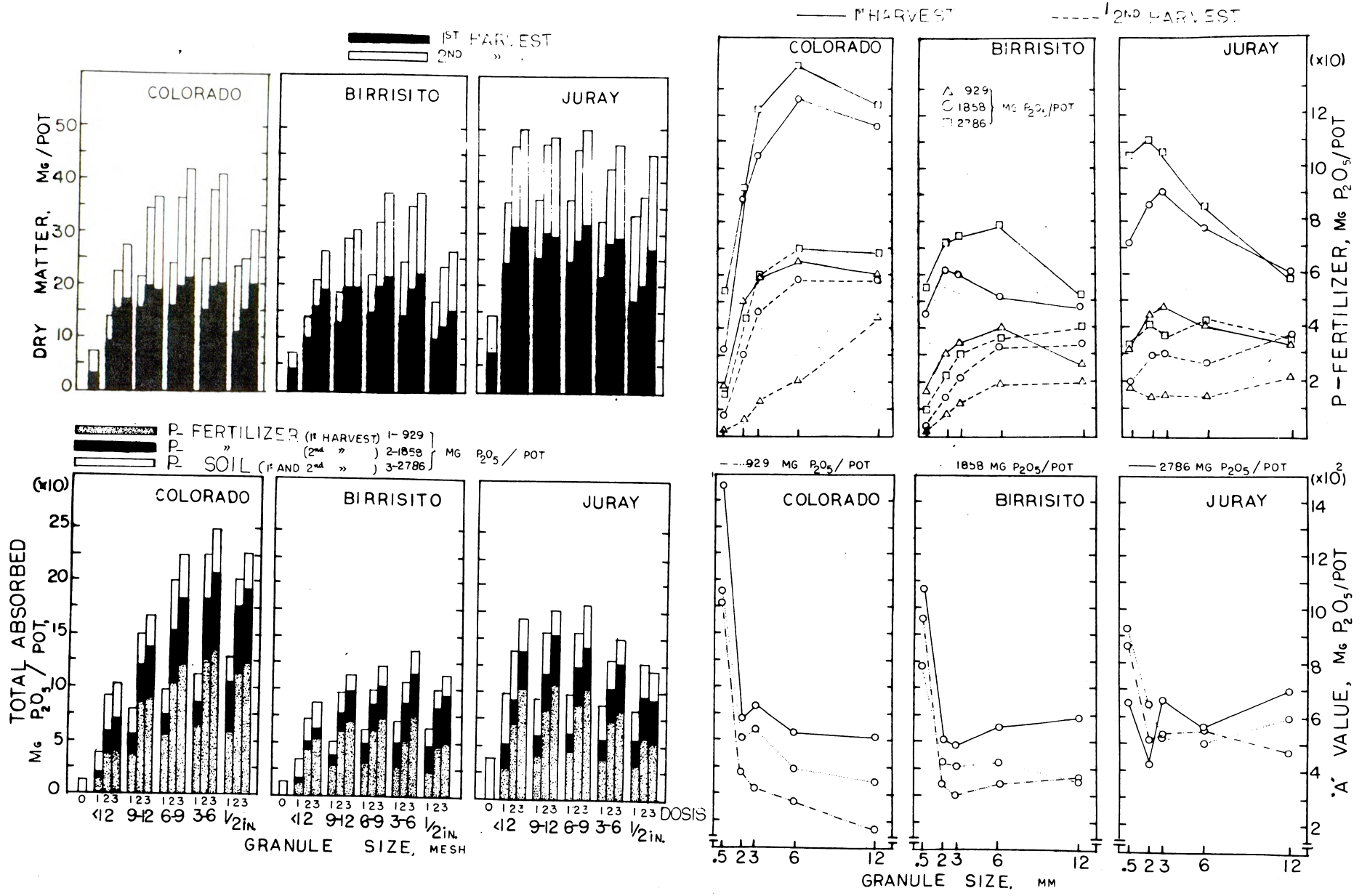


FIG 32. EFFECT OF GRANULE SIZE UPON YIELD, P ABSORBED FROM SOIL AND FERTILIZER, AND "A" VALUE

fertilizer during the successive cropping. These results agree with observation in the laboratory regarding fixation reaction, in which Birrisito showed higher retention capacity.

The effect of doses upon "A" values were different according to different granule size. For a size greater than the 0.5 mm the increase in the rate of applications increased the "A" value. In the second harvest the "A" value was increased for granule size greater than 2 mm. Granule size showed significant effect upon "A" value for all soils, different doses and harvesting. The "A" value decreased abruptly with an increase in granule size from 0.5 to 2-3 mm, with a tendency to be constant at bigger granule sizes. The variation in "A" value was indicated already by Terman and Khasawneh (11). Under the conditions studied here the "A" value can be conceptually wrong, since in the case of powder forms (-12 mesh) the MCP-C was thoroughly mixed with the soil ressembling more "L" value, whereas in the granule form it can be considered as localized, conceptually tending to "A" value. Thus, it seems to be a mix-up of "A" and "L" values (1). Nevertheless, this does not invalidate our data to analyze the proportion of plant-P originated from the soil, which is very high compared to literature data.

An experiment was also undertaken in order to verify the efficiency of MCP-C, MCP-S and DCP and its effect upon "A" value. Table 27 summarizes the data. In this case fertilizers were used in powder form (-12 mesh) and thoroughly mixed with the soil. As we can see the efficiency decrease as the doses increased for all fertilizer forms, whereas DCP tended to maintain higher efficiency than other two. This is reflected

Table 27. Comparative efficiency of MCP-C, MCP-S and DCP for corn plants*

Doses mg P_2O_5 /pot	Dry matter g/pot	P-fertilizer[1] mg P_2O_5 /pot	P-soil[1] mg P_2O_5 /pot	Efficiency** %	"A" value mg P_2O_5 /pot
MCP-C					
0	3.3	--	6.4	--	--
947	13.8	37.0	17.6	3.9	445
1894	23.1	59.5	16.9	3.1	530
2840	27.0	76.1	17.2	2.7	653
MCP-S					
947	19.2	47.8	23.4	5.0	464
1894	23.4	53.4	22.8	2.9	814
2840	29.6	85.8	26.1	3.0	852
DCP					
947	16.4	47.9	11.6	5.1	227
1894	28.1	79.1	11.6	4.2	284
2840	31.1	114.6	21.7	4.0	540

1. Plant P from fertilizer and soil.

$$- A = R\left(\frac{1-y}{y}\right) = B\left(\frac{si}{sf} - 1\right)$$

* Colorado soil - 2 kg/3 liter pot.

** $\frac{^{32}P \text{ plant}}{^{32}P \text{ applied}} \times 100$ (mg P_2O_5/pot)

in the production of dry matter. The "A" value is higher for MCP-S and is least for DCP, the most efficient form. The soil used in this experiment was Colorado. This data seems to confirm the effectiveness of DCP, the less reactive and less soluble fertilizer in present studies.

It can therefore be concluded that granule size increased MCP-C efficiency in three soils studied, showing a critical size which

maximizes the efficiency. The 'critical' granule size (for maximum efficiency) increased for second harvesting, which indicated that for long term effect, the bigger the size of the granule higher will be the efficiency of fertilizer under these conditions.

References

1. Bornemisza, E. and H. W. Fassbender. Uptake of fertilizer phosphorus from nine soils from humid tropics. Agrochimica 14: 259-268. 1960.

2. Chang, S. C. and W. K. Chu. The fate of soluble phosphate applied to soils. Jour. Soil Sci. 12:286-293. 1961.

3. DeDatta, S. K., R. L. Fox and G. D. Sherman. Availability of fertilizer phosphorus in three latosols of Hawaii. Agr. Jour. 55:311-313. 1963.

4. Fried, M. "E", "L" and "A" values. In Trans. 8th Congr. Soil Sci. Bucharest, pp. 24-29. 1964.

5. Lawton, K. and R. L. Cook. Interaction between particle size and water solubility of phosphorus in mixed fertilizers as factors affecting plant availability. Farm Chemicals 118:44-46. 1955.

6. _____ et al. Influence of particle size, water solubility, and placement of fertilizers and the nutrient value of phosphorus in mixed fertilizers. Soil Sci. 82:465-476. 1956.

7. Lindsay, W. L. and H. F. Stephenson. Nature of the reactions of monocalcium phosphate monohydrate in soils: I. The solution that reacts with the soil. Soil Sci. Soc. Amer. Proc. 23: 12-18. 1959.

8. Mattingly, G. E. and O. Talibudeen. Progress in the chemistry of fertilizer and soil phosphorus. In Grayson, M. and E. J. Griffith, eds. Topics in Phosphorus Chemistry. New York, Interscience, 1967, v. 4. pp. 157-290.

9. Moreno, E. C., W. E. Brown and G. Osborne. Stability of dicalcium phosphate dehidrate in aqueous solutions and stability of octacalcium phosphate. Soil Sci. Soc. Amer. Proc. 24:99-102. 1960.

10. Terman, G. L. and F. E. Khasawneth. Crop uptake of fertilizer and soil phosphorus in relation to calculated A values. Soil Sci. 105:346-354.

11. Terman, G. L., D. R. Bouldin and J. R. Webb. Evaluation of fertilizers by biological method. Adv. Agronomy 14:265-317. 1962.

12. Yuan, T. L., W. K. Robertson and J. R. Neller. Forms of newly fixed phosphorus in three acid sandy soils. Soil Sci. Soc. Amer. Proc. 24:447-450. 1960.

2. Cation Exchange and Retention Properties

a. Effect of liming upon the exchange capacity and the Ca and Mg movement in an andosol
(K. Igue, M. Morelli and R. Fuentes)

Purpose and Methods

In previous reports, the existence of a pH-dependent component of CEC in volcanic soils of Costa Rica was indicated. This was attributed to the presence of amorphous aluminum and iron hydroxides as well as organic-matter. Thus, the change in pH due to liming may change the CEC values of these soils, with a decrease in the exchangeable Al^{+++} and H^{+} component (3). To further study the effect of pH upon CEC, soil samples from field experiments were collected where an increasing rate of lime was applied for sugar cane. The samples were collected at five different depths from 0 to 100 cm.

The CEC was determined by unbuffered N KCl solution and N NH_4OAc buffered at pH 7.0. Exchangeable acidity was measured by displacing with N KCl and titrated as indicated by Yuan (6).

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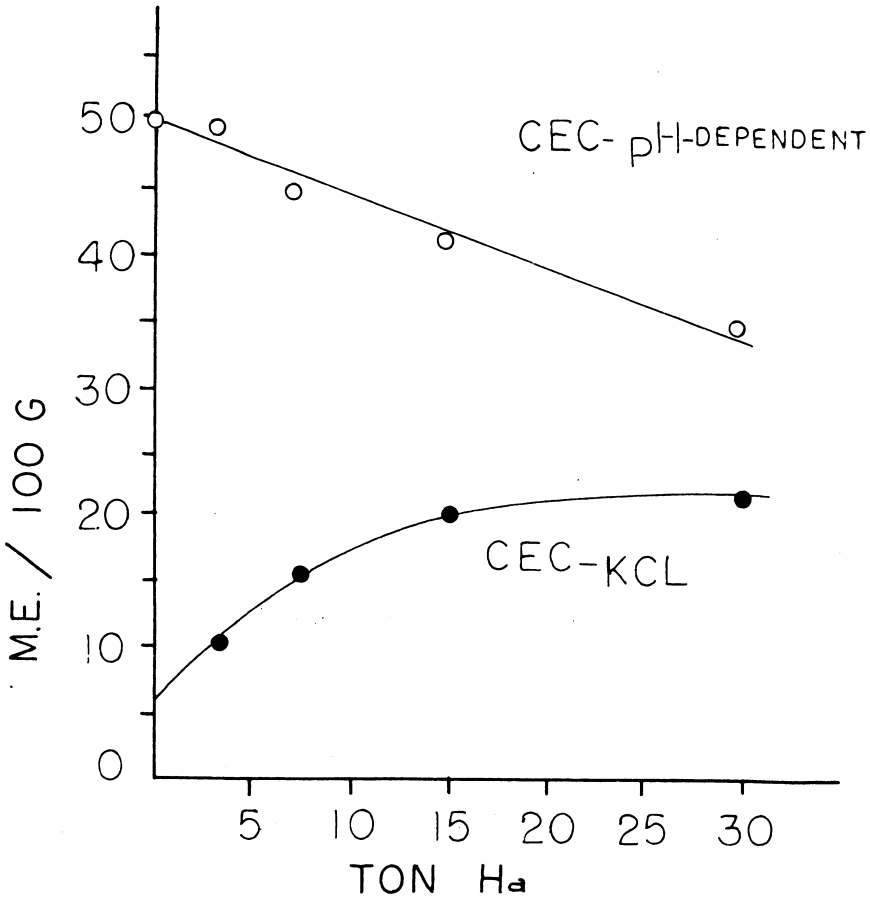
The effect of lime upon CEC measured by N KCl and pH-dependent CEC (NH_4OAc pH 7.0 minus KCl) is presented in Fig. 33. With the increase



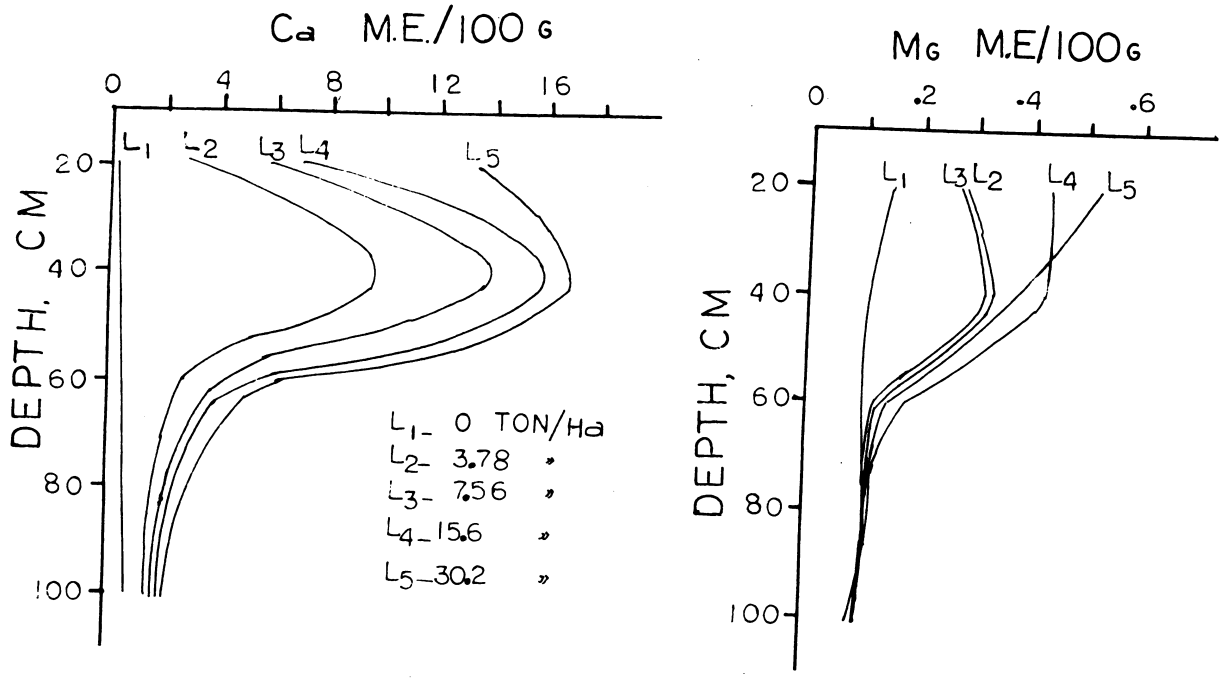


FIG 33. EFFECT OF LIMING ON CEC AND MOVEMENT OF Ca AND Mg.

in the rate of lime application the CEC-KCl was significantly increased, whereas no effect was observed for CEC-NH_4OAc. The pH- dependent component of CEC, on the other hand, decreased with liming. Due to the effect of organic matter and liming upon pH-dependent CEC, a multiple regression equation was obtained.

$$Y = 58.71 - 0.49826 + 2.26X_2 + 0.01X^2 + 0.04X_2^2 - 0.029X_1X_2 \qquad r^2 = 79.8\%$$

Y = pH-dependent CEC X_1 = lime doses X_2 = C %

The pH-dependent CEC increased considerably with the organic C content and decreased with liming.

Base saturation increased with liming and with a decrease in exchangeable acidity. The percentage base saturation is much higher when calculated on a basis of CEC-KCl. A similar tendency was shown by Mahilum *et al.* (4) for volvanic ash soils of Hawaii.

Table 28. Effect of increasing liming rate upon % base saturation and acidity (20-40 cm depth)

Ton/ha lime-stone	Ca+Mg+K	$Al^{+++}+H^+$	% Sat.	% Sat.	pH	
	-------me/100 g-----		KCl	NH_4OAc	H_2O	KCl
0	0.79	4.45	11	1.4	4.24	4.68
3.78	10.10	3.33	85	16	5.26	5.17
7.56	14.51	0.43	93	24	5.72	5.67
15.12	16.55	0.14	82	26	5.89	5.64
30.24	17.27	0.14	75	30	6.06	5.79

It is worthwhile to mention that pH in water is lower than the pH in KCl throughout all profile depth. This is expected to be so in soils with high content of amorphous material where OH^- is replaced by other anions like Cl^- or SO_4^- as indicated elsewhere (1, 5). With lime application the pH in KCl becomes lower than pH in H_2O.

The effect of liming on the movement of Ca and Mg was also studied. Fig. 33 shows the distribution of Ca and Mg throughout the profile four years after liming. It is possible to observe movement of Ca upto the 100 cm depth. The same trend is observed for Mg ,but in a smaller amount. Fox (2) showed considerable movement of Ca to a depth of 120 cm 5 years after application in andosols from Hawaii. Since the lime was applied at a 20 cm depth in the furrow the lateral movement was also observed. Thus lateral movement tended to increase with depth reaching a maximum aroud 60-80 cm.

The data presented allowed to conclude that liming andosols may increase CEC and base saturation. The CEC measured with unbuffered salt such as N KCl seems to be a reliable method under prevailing conditions. The movement of Ca and Mg is good evidence of strong leaching of bases in humid tropics.

References

1. Chao, T. T., M. E. Harward and S. C. Fong. Exchange reactions between hydroxyl and sulfate ions in soils. Soil Sci. 99:6. 1965.

2. Fox, R. L. La fertilización de los suelos de cenizas volcánicas de Hawaii. In Panel sobre Suelos Derivados de Cenizas Volcánicas de América Latina. Turrialba, 1969. Turrialba, IICA, pp. C.6.1-C.6.14. 1969.

3. Igue, K. and R. Fuentes. Exchangeable aluminum in volvanic soils. Submitted to Soil Sci. Soc. Amer. Proc.

4. Mahilum, B. C., F. L. Fox and J. A. Silva. Residual effects of liming volcanic ash soils in the humid tropics. Soil Sci. 109: 102-109. 1970.

5. Wada, K. Deuterium exchange of hydroxyl groups in allophane. Soil Sci. and Plant Nutrition 12:176-182. 1966.

6. Yuan, T. L. Determination of exchangeable H by a titration method. Soil Sci. 88:164-167. 1959.

b. Cation exchange capacity of organic matter (K. Igue and R. Fuentes)

Purpose and Methods

Under humid tropical conditions high accumulation of organic matter is observed; moreover when the soil is affected by volcanic ash. The contribution of organic fraction on exchange and retention properties deserves a more detailed study under this region of Costa Rica. High correlation has been observed between CEC and organic C content for other soils (1, 2).

Continuing our studies on CEC few soils were treated with H_2O_2 until complete destruction of organic matter was obtained (<1% organic matter). The soils freed of organic matter were tested for CEC with different methods, to observe the contribution due to the organic and mineral fraction.

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In general, the CEC of the organic fraction represents higher proportion than that of the mineral fractions, except for Colorado (Distropepts) where both mineral and organic fractions are almost in equal proportion (Table 29). The contribution of the mineral fraction increased as the pH of the solution increased, whereas the contribution of organic matter tended to decrease, again with the exception of the Colorado soil. This is due to the fact that in the case of the Colorado soil metahalloysite is the dominant clay mineral, whereas for the others allophane is the dominant one (Besoain, E. Unpublished data).

Table 29. Cation exchange capacity of mineral and organic fractions determined with N NH_4OAc at different pHs and titration method

Soil	% o.m.	pH KCl	CEC NH_4OAc N 4.0 me	%	5.0 me	%	6.0 me	%	7.0 me	%	8.0 me	%	Titration* method me	%
CERVANTES	14	4.5												
Mineral fraction...			12	26	17	32	22	34	23	35	24	--	16	64
Organic matter.....			34	76	35	66	43	66	44	65	--	--	9	36
BIRRISITO	16	5.8												
Mineral fraction...			11	25	16	29	23	38	27	40	28	--	15	60
Organic matter.....			33	76	40	71	38	62	40	80	--	--	10	40
COLORADO	10	3.6												
Mineral fraction...			11	48	14	54	15	47	18	51	18	--	14	82
Organic matter.....			12	52	12	45	17	53	17	49	--	--	3	68
CORONADO	22	5.0												
Mineral fraction...			15	32	22	38	25	34	31	40	33	--	17	65
Organic matter.....			31	68	36	62	47	65	46	60	--	--	9	35

* Method by Kamprath and Welch (1). All data is in me/100 g

Table 29 also shows the CEC determined by titration to pH 7.0 in KCl solution according to Kamprath and Welch (1). Contrary to the NH_4OAc method the organic fraction constitutes a smaller fraction. The HCl treatment of the soil decreases the pH, thus decreasing the contribution of negative charges due to organic matter.

Table 30 illustrates also the difference in CEC as a function of the three methods used with acetate as the accompanying anion. At lower pHs NH_4^+ usually gives higher values than the Ca^{++} method. This difference may be due to the effectiveness of washing excess salt,

Table 30. Comparative values of CEC measured with NH_4 and Ca acetate and isotopic method for mineral and organic fractions.

Soil	CEC me/100 g					
	NH_4OAc		$Ca(OAc)_2$		$^{45}Ca(OAc)_2$	
	Mineral fraction	Organic matter	Mineral fraction	Organic matter	Mineral fraction	Organic matter
BIRRISITO						
4.0	11	33	4.1	11	2.8	17
5.0	16	40	4.3	21	5.8	26
6.0	23	38	5.8	32	12.0	36
7.0	27	40	6.4	43	16.3	46
8.0	28	-	7.7	47	23.4	46
COLORADO						
4.0	15	31	4.1	10	1.3	16
5.0	22	36	5.1	32	3.3	40
6.0	25	47	6.2	50	5.2	61
7.0	31	46	8.3	49	11.2	57
8.0	32	-	10.0	53	21.0	54

less effective in the case of NH_4 acetate (3). Further studies will be undertaken using the $CaCl_2$ method.

References

1. Kamprath, E. J. and C. D. Welch. Retention and cation exchange properties of organic matter in Coastal Plain soils. Soil Sci. Soc. Proc. 26:263-268. 1961.

2. Pratt, P. F. Effect of fertilizers and organic matter on the CEC of an irrigated soil. Soil Sci. 83:85-90. 1957.

3. Rich, C. I. Removal of excess salt in CEC determinations. Soil Sci. 93:87-94. 1962.

3. Trace Elements in Tropical Soils

a. Factors affecting the availability of zinc in soils (K. Igue and M. Marinho)

Purpose and Methods

The importance of free sesquioxides and phosphate application upon zinc availability was emphasized elsewhere (2, 3, 4). In high phosphorus fixing soils, such as volcanic ash soils and tropical oxisols, the need for heavy phosphorus application may be of consequence in the absorption of zinc.

Experiments from last year were continued with the objective of studying the effect of the free R_2O_3 content of three volcanic soils and the effect of applied phosphorus upon available zinc.

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The amounts of available zinc in incubated soil samples are presented in Fig. 34. The amount of Zn extracted by 0.1 N HCl is higher than that extracted by Na_2-EDTA in three soils, and the extractable zinc increases with zinc application and decreases with an increase in free R_2O_3 content. Zinc extracted by Na_2-EDTA for the three soils increased with P application, although, for 0.1 N HCl there was no effect for soils 1 and 2 and a slight decrease for soil 3.

The total zinc absorbed increases with the rate of Zn application, and total zinc absorption also increases accordingly with a decrease in free R_2O_3 present in the soil. This indicates that total absorption is influenced by the content of available zinc in the soil as shown in Fig. 34. Zinc concentration in the tissue decreases with P application in all cases.

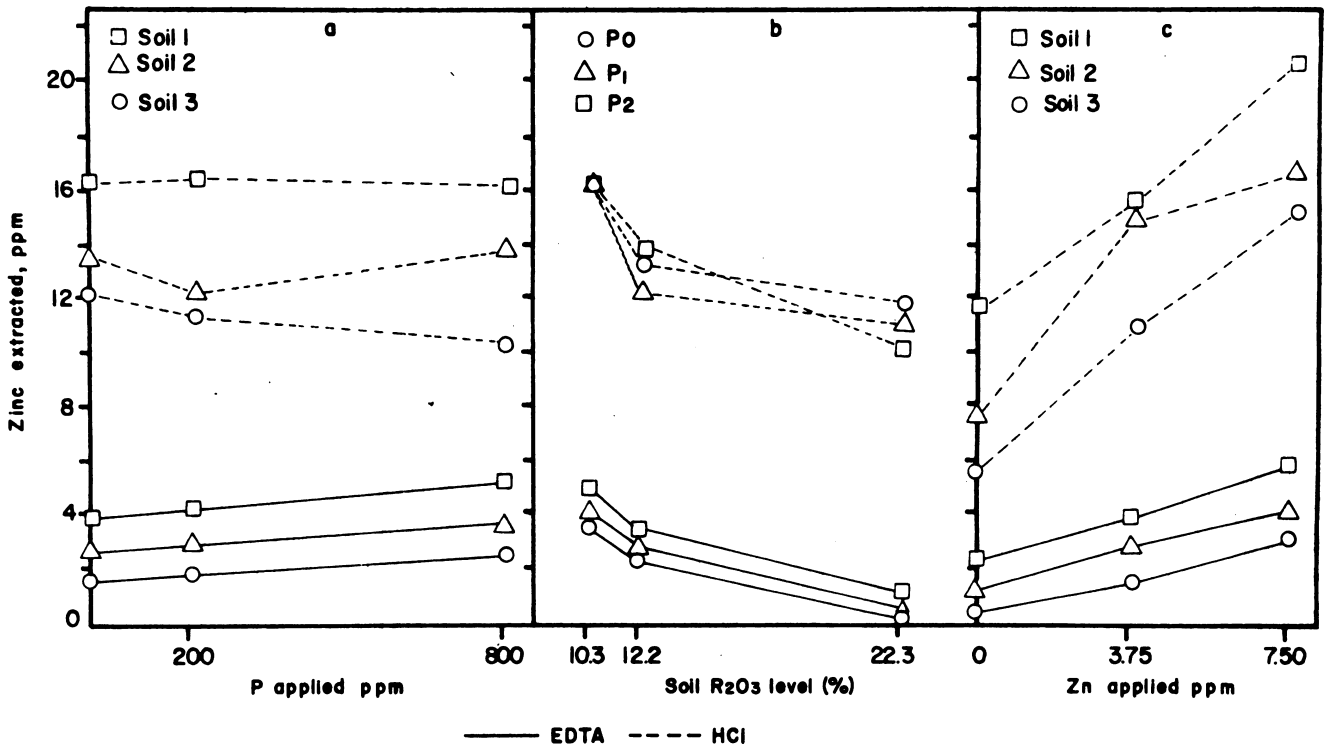

FIG 34a Zinc extracted with 0,01 M Na_2-EDTA and 0,1 N HCl as function of a) P applied, b) level of free R_2O_3, and c) zinc applied

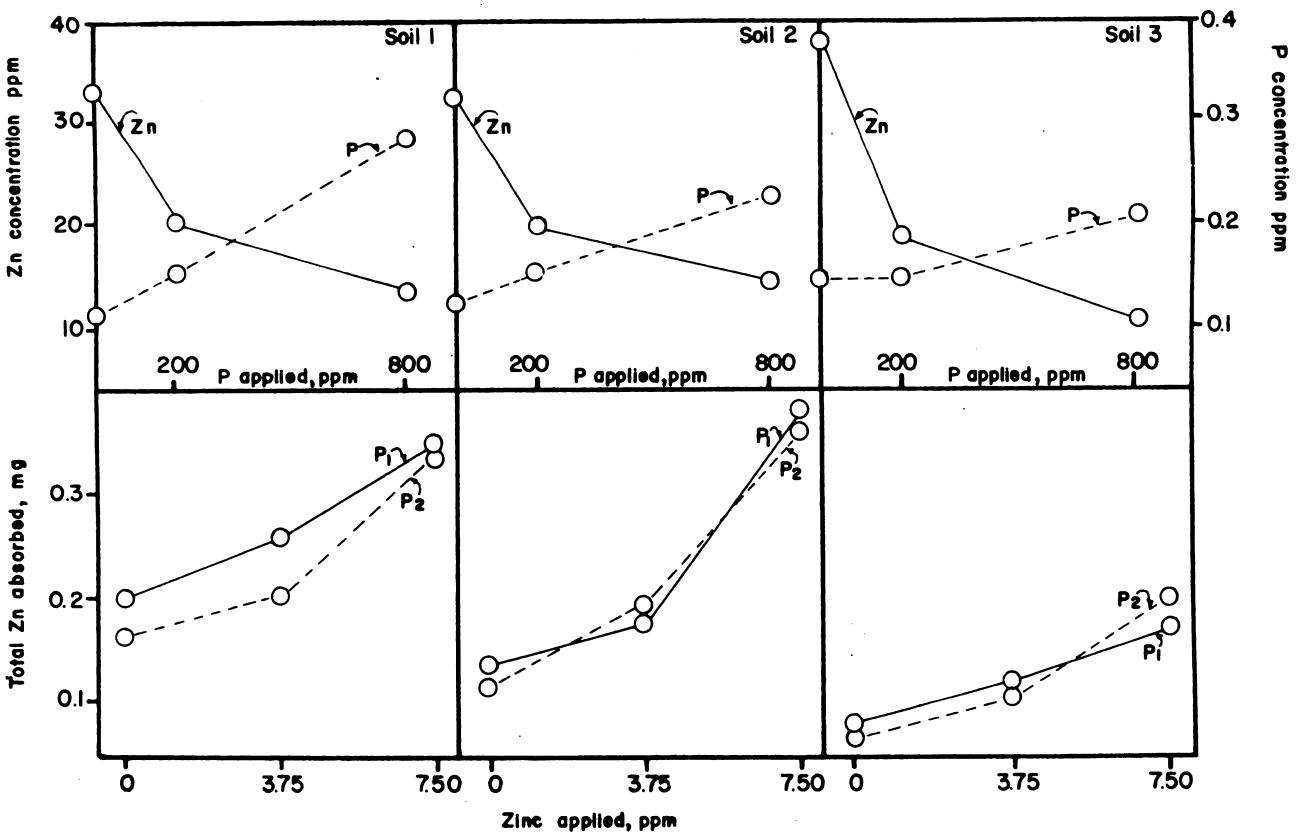

FIG 34b Effect zinc and phosphorus aplication upon total Zn absorbed and concentrations of Zn and P in the tissue

The calculated "L" value (1) is presented in Table 31. The "L" value decreases as the free R_2O_3 increases, and follows the same tendency as the extractable Zn by 0.1 N HCl and 0.01 M Na_2-EDTA. As we can also see from Table 31, the "L" value increases with Zn application, and is also affected by P application. In general, "L" value decreases as the R_2O_3 content of the soil increases.

Table 31. Calculated "L" value* for the three soils as a function of applied P and Zn

	Soil 1		Soil 2		Soil 3	
	Zn_1	Zn_2	Zn_1	Zn_2	Zn_1	Zn_2
	ppm					
P_0	23.0	-	12.5	-	13.1	-
P_1	27.3	32.5	14.6	27.8	11.3	12.8
P_2	18.7	32.5	18.9	35.3	10.8	18.5
Mean	23.0	32.5	15.3	31.5	11.7	15.6

* $L = B(\frac{Sf}{Sp} - 1)$

B = amount of Zn applied
Sf = specific activity of fertilizer
Sp = specific activity of plant zinc

References

1. Fried, M. "E", "L" and "A" values. In Trans. 8th Intern. Congr. Soil Sci. Bucharest, 1964. pp. 24-29. 1964.

2. Lengiss, E. J. et al. Factors responsible for poor response of corn and grain sorghum to phosphorus fertilization. II. Lime and P placement effects on Zn-P relations. Soil Sci. Soc. Amer. Proc. 26(6):574-78.

3. Pauli, A. W., R. Ellis, Jr. and H. C. Moser. Zinc uptake and translocation as influenced by phosphorus and calcium carbonate. Agr. Jour. 60:394-96. 1968.

4. Stanton, D. A. and R. T. Burger. Availability to plants of zinc sorbed by soils and hydrous iron oxides. Geoderma 1:13-17. 1967.

b. Survey of trace-elements in soils of Bahia, Brazil (K. Igue and C. L. Santana)

Purpose and Methods

The distribution of microelements had been emphasized in temperate and some subtropical areas, specially in the USA where Beeson as quoted by Hodgson (4) and Berger (2) prepared a descriptive map showing the deficient areas.

In Latin America this field of investigation is critical, specially in Brazil where studies of distribution of essential micronutrients is lacking. In the southeast region of Bahia, Brazil (81,000 km^2) an intensive study on natural resources had been initiated recently (7). A survey on micronutrient content of these soils was essential for the development of the cocoa research program.

The purpose of this study was to a) evaluate the total and available forms of Zn, Fe, Mn, Cu, and Mo in eight profiles from that region, and b) to relate the distribution of trace-elements with the soil components. Soil samples were collected at different depths representing different horizons. For the total content digestion with $HClO_4/H_2SO_4$ (5) and a ternary acid mixture were used as described by Ulrich (13). For the available fractions the following extractants were used: 0.1 N HCl; 1% Na_2-EDTA; N NH_4OAc at pH 7.0 and 4.8.

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The trace element contents of eight profiles studied are presented in Table 32 for Zn, Cu, Mn, Fe and Mo, respectively.

In general, the distribution of the total contents of trace elements in the profile were associated with clay distribution.

Nevertheless, in most of the soils analyzed the concentration of available forms was higher at surface horizon in association with organic matter.

Among the elements analyzed, Fe presented the highest concentration and Mo the least. The following order can be suggested: Fe>Mn> Zn>Cu>Mo.

Zinc: Total zinc concentration at different depths showed marked variation among the soils. In general, the concentration changed little with depth. The values obtained in these soils are within the limits indicated by Mitchel (8). The distribution of the available fraction was higher at the surface horizon, and decreased for the B horizon. There was no close relation between total and available zinc in most soils.

Copper: The average values for copper found in A, B and C horizons are somewhat higher than those obtained for other tropical soils (6). Nevertheless, the values obtained are within the limits indicated by Tisdale and Nelson (12), and Swaine (11). However, the average value for the A horizon is lower than that indicated by Hodgson (4) for soils from different regions. Available copper extracted by different solutions were very high and showed marked differences among the methods. The maximum concentration was found at A horizon and decreased with depth in close association with organic matter.

Manganese: Total manganese was detected within the limits indicated by Swaine (11); although Sauchelli (9) reported values from traces to 7,000 ppm in soils from different regions.

In general, the concentration of Mn in the soils studied decreased with depth, except for the hydromorphic soil where the concentration

increased for the B horizon, in accordance with Butler (3). The available forms of Mn showed maximum concentration at surface, decreasing with depth.

Iron: As was expected, the concentration of iron was very high in most soils studied. The total Fe increased with depth in association with clay and free iron oxides.

The amount of available Fe varies according to the extractant used. The distribution throughout the profile varies with the soil type.

Molybdenum: Molybdenum concentration in these soils was very low and the detection of this element was only possible by the $HClO_4/H_2SO_4$ method. The average value found was 2.50 ppm, which is little higher than the 2 ppm indicated by Anderson (1) and Swaine (10). The molybdenum concentration tends to increase for the B horizon and decrease with further depth. There was an association between molybdenum distribution and clay distribution in some of the profiles studied. In the case of Mo, further studies seem to be necessary in order to detect the available fraction. None of the methods used to measure the available fraction were able to detect Mo in these soils. Sherman (10) indicated that Mo is conveniently and effectively dissolved by digestion with $HClO_4$, 60%.

As a concluding remark, from the agronomic point of view, the relationship among the trace elements analyzed is important if it is to be used as a diagnostic tool. Further studies should be carried out to correlate the soil content and crop response.

Table 32. Total and available content as measured by the different extractants used*

Element Analyzed	Profile horizons*	Total, ppm		Available, ppm			
		$HClO_4$ 60%	Ulrich	HCl 0.1 N	Na_2-EDTA %	N NH_4OAc pH 4.8	N NH_4OAc pH 7.0
Zinc	A	40	39	5.8	4.4	2.0	0.3
	B	34	39	2.3	1.3	0.5	0.1
	C	34	32	3.8	1.3	0.7	0.1
Copper	A	15	13	2.3	2.7	0.5	1.0
	B	18	25	2.2	2.2	0.6	0.5
	C	13	17	1.5	1.2	0.6	0.4
Manganese	A	801	630	288.0	413.0	90.0	32.0
	B	392	264	224.0	99.0	18.0	7.0
	C	269	226	11.0	9.0	7.0	3.0
Iron	A	11703	16749	171.0	366.0	29.0	2.2
	B	12639	19740	104.0	122.0	21.0	3.3
	C	10480	16571	181.0	109.0	15.0	2.1
Molybdenum	A	1.98	tr	tr	tr	tr	tr
	B	2.90	tr	tr	tr	tr	tr
	C	2.33	tr	tr	tr	tr	tr

* Each horizon is the average of 2 or more subhorizons from 8 profiles.

References

1. Anderson, A. J. Molybdenum as a fertilizer. Advances in Agronomy 8:163-202. 1956.

2. Berger, K. C. Micronutrient deficiencies in the United States. Jour. of Agr. Food Chm. 10:178-181. 1962.

3. Butler, J. R. Trace-element distribution in some Lancashire soils. J. Soil Sci. 5:156-166. 1954.

4. Hodgson, J. F. Chemistry of micronutrient elements in soils. Adv. Agronomy 15:119-159. 1963.

5. Jackson, M. L. Soil chemical analysis. Englewood Cliffs, N. J. Prentice Hall, 1958. 498 p.

6. Maury, P. B. Determinación de Mo, Cu y Co, en algunos suelos del Altiplano de Pastos, Nariño, Colombia. Tesis. Universidad de Nariño, Colombia. 1959. 106 p.

7. Mitchel, R. L. Trace elements in soils. In Bear, F. E., ed. Chemistry of the soil 2nd. Ed. New York Reinhold, pp. 320-368. 1964.

8. Olmos, J. et al. Solos da região cacaueira da Bahia e do Espirito Santo. In 2nd. International Conference on Cocoa Research, Salvador, Itabuna, Bahia, November 19-26, 1967. pp. 393-403.

9. Sauchelli, V. Trace elements in agriculture. New York, Reinhold. 1969. 248 p.

10. Sherman, M. S. Colorimetric determination of P in soils. Ind. Eng. Chem. Anal. Ed. 14:182-185. 1950.

11. Swaine, D. J. The trace-element content of soils. Commonwealth Bureau of Soil Sci. Technical Communication no. 48. 1955. 157 p.

12. Tisdale, L. S. and W. L. Nelson. Soil fertility and fertilizers. 2nd. ed. New York, McMillan, 1967. 697 p.

13. Ulrich, B., K. Hempler and J. H. Benzler. Zur analitischen Bestimmung von Gesamtphosphorsäure und Laktatlöslicher Phosphorsäure in Bodenproben. Die Phosphorsäure 20:344-347. 1960.

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 of the Institute.

Kozen Igue		Soil chemistry
Oscar Hidalgo	1.	Biochemistry
	2.	Pathological control of insect pests
Carl C. Moh		Microtechnique for plant chromosomes

2. Six students received their training from the Nuclear Energy Program: three from Brazil, one from Colombia and two from Chile. After graduation, they went back to their countries and carried out teaching and research in the universities or institute.

CONSULTING, MEETING AND TRAVELING

Dr. Kozen Igue

From August 23 to 28, 1970, Dr. Igue attended the annual meetings of the American Society of Agronomy held in Tucson, Arizona.

Dr. Kamta Katiyar

From December 1 to 4, 1970, Dr. Katiyar attended the annual meetings of the Entomological Society of America held in Miami, Florida. From December 7 to 11, 1970, he also attended the meetings of the working group on the "Eradication of the Mediterranean Fruit Fly" in San Jose, Costa Rica. The panel was organized by IAEA in cooperation with OIRSA under the United Nations Development Program.

Dr. Carl C. Moh

From November 14 to 24, 1970, Dr. Moh was invited to participate in the symposium on "Induced Mutations and Plant Breeding" jointly sponsored by IAEA and Argentine Agriculture Ministry held in Buenos Aires, Argentina. His trip was paid for by the sponsors. On his trip back to Costa Rica, he stopped by Lima, Peru, to discuss the insect control project with Ing. Juan Simon at La Molina.

PUBLICATIONS

1. Berrios, F. and Hidalgo, O. Estudios sobre el barrenador Hypsipyla grandella Zeller. VI. Susceptibilidad de la larva al hongo Metarrhizium anisopliae. Turrialba 21:214-219. 1971.

2. Delgado, L. F., Moh, C. C. and Alan, J. J. Frecuencia de mutaciones inducidas por el matenosulfonato de etilo en semillas de frijol común (Phaseolus vulgaris, L.) en diferentes estados de germinación. Turrialba 21:121-122. 1971.

3. Hidalgo, O. Estudios sobre el barrenador Hypsipyla grandella Zeller. VII. Determinación del sexo en pupas. Turrialba 21:221. 1971.

4. _____ and Madrigal, L. G. Studies on the shoot borer Hypsipyla grandella Zeller. IV. Trochogramma sp., an egg parasite. Turrialba 20:513. 1970.

5. Hooper, G.H.S. and Katiyar, K. P. Competitiveness of gamma sterilized males of the Mediterranean fruit fly. Submitted for publication in Jour. of Econ. Entomol.

6. Igue, K. and Fuentes, R. Exchangeable and non-exchangeable acidity of volcanic soils. Agronomy Abstracts pp. 90. 1970.

7. _____ and Fuentes, R. Fijación y solubilización de ^{32}P en dos suelos volcánicos (inceptisoles). VIII Reunión Latinoamericana de Fitotecnia, Bogotá, Colombia, 1970. pp. 240 (abstract).

8. _____ and Fuentes, R. Exchangeable Al in volcanic ash soils. Submitted to the Soil Sci. Soc. Amer. Proc.

9. _____, Fuentes, R. and Bornemisza, E. Mineralización de fósforo orgánico. Turrialba 21:47-52. 1971.

10. Marinho, M. and Igue, K. Effect of free sesquioxides and P upon Zn absorption by corn from volcanic soils. Submitted to Agron. Jour.

11. Moh, C. C. Induced seed-coat color mutations in beans and their significance for bean improvement. In Induced Mutations and Plant Breeding, IAEA, Vienna, 1970.

12. _____ and Alan, J. J. Correlation between seed-coat color and the seedling characters in Phaseolus vulgaris, L. Turrialba 21: 173-175. 1971.

13. _____ and Alan, J. J. Bean mutants induced by ionizing radiation. VI. Unifoliate mutant. Turrialba 21:231-232. 1971.