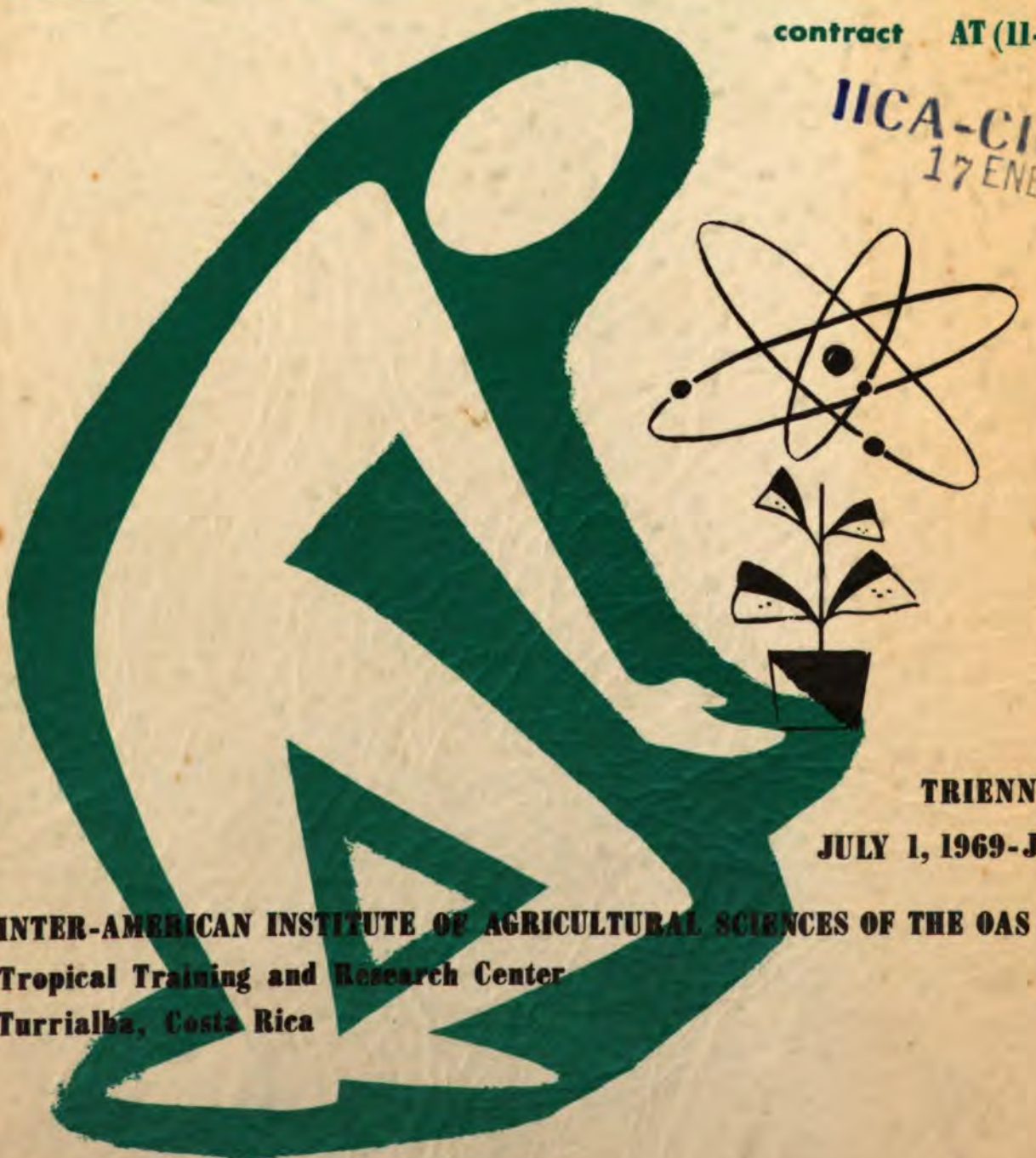
COO-3217-05

THE APPLICATION OF NUCLEAR ENERGY TO AGRICULTURE

contract AT (11-1)-3217



TRIENNIAL REPORT
JULY 1, 1969-June 30, 1972

INTER-AMERICAN INSTITUTE OF AGRICULTURAL SCIENCES OF THE OAS 1 JULY 1972
Tropical Training and Research Center
Turrialba, Costa Rica

THE APPLICATION OF NUCLEAR ENERGY TO AGRICULTURE

Triennial Report

to the

United States Atomic Energy Commission

Contract No. AT(11-1)-3217

Prepared by

Carl C. Moh

Interamerican Institute of Agricultural Sciences of the OAS
Tropical Training and Research Center
Nuclear Energy Program
Turrialba, Costa Rica

1 July 1969 - 30 June 1972

CONTENTS

PERSONNEL OF THE NUCLEAR ENERGY PROGRAM

Professional Staff

Carl C. Moh, Ph.D.	USA	Cytogeneticist, Head of Program
Elemer Bornemisza, Ph.D.[1]	Costa Rica	Soil Chemist
Oscar Hidalgo, Ph.D.[2]	Nicaragua	Insect Pathologist
Kozen Igue, Ph.D.[3]	Brazil	Soil Chemist
Kamta P. Katiyar, Ph.D.	India	Entomologist
Juan Jose Alan, M.S.[4]	Costa Rica	Jr. Cytogeneticist
Raul Fuentes, M.S.[5]	Mexico	Jr. Soil Chemist
Eddie A. Ramirez, M.S.[6]	Venezuela	Jr. Entomologist

Non-professional Staff

Lucia Lopez	Costa Rica	Secretary
Ritha Legarda[7]	Colombia	Laboratory Aide
Emma Roda[8]	Costa Rica	Laboratory Aide
Alvaro Castillo	Costa Rica	Laboratory Aide
Luis Mora	Costa Rica	Field Overseer
Rafael Mora	Costa Rica	Gamma Field Overseer
Manuel Picado[9]	Costa Rica	Laboratory Aide

---

1. Resigned July, 1969, and went to IICA-Andean Zone
2. Joined staff, January, 1970.
3. Joined staff, December, 1969. Resigned February, 1972, and went to CEPLAC, Itabuna, Bahia, Brazil.
4. Re-joined staff, August, 1969.
5. Position terminated as of November, 1971.
6. Resigned December, 1970, and went to Shell Service for Agriculture, Venezuela.
7. Joined staff, January, 1971. Resigned June, 1971.
8. Joined staff, September, 1970.
9. Replaced R. Legarda, July, 1971.

I. RESEARCH ACCOMPLISHMENTS

A. MUTATION BREEDING AND RADIATION BOTANY (C. C. Moh and J. J. Alan)

1. Induction of Mutations in *Manihot*

a. Botanical characters of *Manihot*

Manihot esculenta (common names: cassava, manioc, or yuca) is a plant of American origin, and probably was first domesticated by Indians inhabiting northeastern South America. While the young shoots and leaves of *Manihot* are edible, the roots are the most important part of the plants and serve as a chief source of carbohydrates for human consumption in the low lands of the American tropics. Since the introduction of *Manihot* to Africa, *Manihot* has spread widely throughout the area and has also become an important staple foodstuff over large parts of the African continent (Jones, 1959).

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 (Rogers, 1965). Botanically, it belongs to the family Euphorbiaceae and the genus *Manihot*. Most of the cultivars are perennial shrubs (Fig. 1). The tuberous roots produced by the plants provide food for human consumption (Fig. 2). The roots can be harvested after 8 to 24 months of plant growth, depending upon the cultivars. The plants can easily be propagated by stem cuttings but less commonly are propagated by seeds.

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

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

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 characteristic, 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.

Because Manihot grows easily, is relatively free of diseases and pests, can adapt to a wide variety of different soils, and can grow from wet to semi-arid regions, it is a food crop that has a great potential to meet the need of the growing human population in the tropics. Agriculturally, Manihot is one of the few field crops which possesses phenomenal yields. Since the Manihot stem cuttings can grow easily, the traditional method for cultivation is by vegetative means. Usually, a foot-long cutting is buried in the ground and a plant will produce an average of 10 lbs. of fresh roots at maturity without much care. A production of 30 tons of fresh roots per acre has been reported by Dutch agriculturists in Indonesia.

Although Manihot is a basic food crop in the tropics, many agricultural weaknesses of this crop, such as long life cycle and hydrocyanic acid (HCN) content, need to be improved. Since manihots are normally propagated by vegetative means,

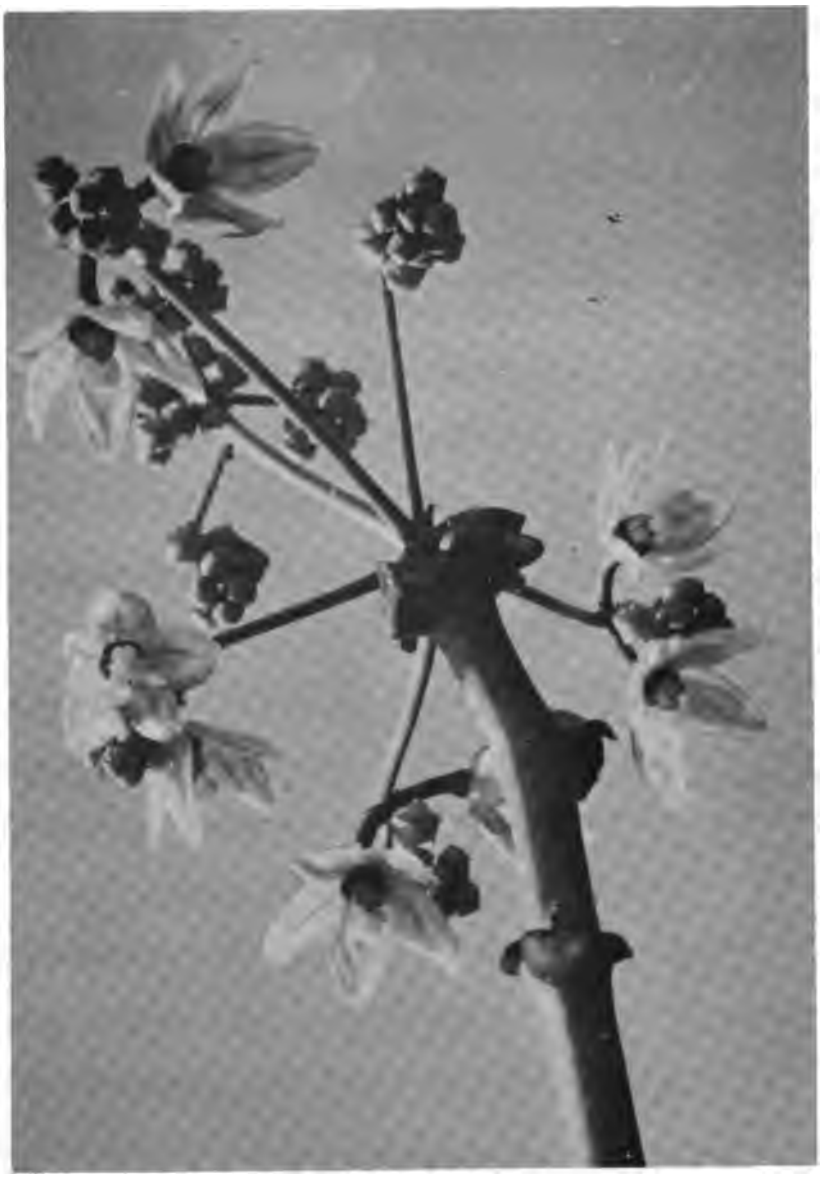


Fig. 3. The male and female flowers of Manihot esculenta showing a marked protogyny of the flowering habit. The male flowers do not open until the last female flower on the inflorescence has bloomed.

they certainly have an advantage in the process of plant breeding. The promising hybrids obtained from crosses or the desirable mutants induced by mutagenic treatments, once recognized and vegetatively propagated, can then be tested and released as improved cultivars. This greatly facilitates the breeding process without necessitating the work on purification for obtaining the homozygotes as in many seed-propagated crops. Indeed, many advantageous heterozygotes can best be maintained by vegetative propagation for agricultural practices. Because of the reproductive characteristics in Manihot, investigations were carried out to see whether induced mutation technique is an effective method for Manihot breeding. Our first approach to this study was to find an effective means to induce a reasonably high frequency of mutations in Manihot. Seed irradiation is not an effective means because of the chimera production and the protogynous flowering habit which prevented self-fertilization to bring out the recessive mutations. The possibilities of inducing mutations by irradiating the cuttings and pollens were explored and the results are presented below.

b. Induction of somatic mutations

1) Radiosensitivity of Manihot shoot apex

Foot-long cuttings of mature woody stems 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

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.

2) Somatic mutations

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,

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.

can be used directly without further breeding process. 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 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 (Glucksman, 1954). 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.

In our Manihot collection, there are more than 90 cultivars which came from Central and South America. Each cultivar and its origin is identified by a cultivar number. By using a dose of 4 kr of gamma radiation to irradiate the stem cuttings, we explored the somatic mutation rate in 8 cultivars in the collection. The mutation frequencies are presented in Table 2.

Table 2. Somatic mutation rate of eight Manihot cultivars induced by irradiating the stem cuttings

Cultivar identification number	Gamma radiation dose (kr)	No. of cuttings irradiated	No. of shoots emerged	Mutations observed in R_1	
				No.	%
9	0	25	66	0	0
	4	50	48	3	6.2
16	0	25	61	0	0
	4	50	24	0	0
23	0	25	56	0	0
	4	50	15	1	6.7
26	0	25	64	0	0
	4	50	15	0	0
49	0	48	163	0	0
	4	36	62	13	21.0
68	0	25	66	0	0
	4	50	41	1	2.4
71	0	25	64	0	0
	4	50	30	1	3.3
82	0	25	67	0	0
	4	50	48	6	12.5

It can be seen from the irradiation results that most of the irradiated cultivars produced very low frequencies of somatic mutations even though a sub-lethal dose of gamma radiation of 4 kr was given. Only cultivars number 49 and 82 had a higher mutation rate. This is probably due to a high degree of heterozygosity existing in these two cultivars. A similar situation was encountered in Dahlia in which the flower color mutations could easily be induced in some varieties but none in the others (Broertjes and Ballego, 1967).

Many mutations produced from irradiated cuttings appeared in chimeric forms. In order to obtain a homogeneous plant of the mutated character, it is necessary to grow the mutated cutting for some generations to purify the mutated sector. However, we have obtained a number of homogeneous mutated plants in the second vegetatively propagated generation by growing the chimeric cuttings.

The types of somatic mutations induced can be classified into two groups: chlorophyll and morphological. The most common types of chlorophyll mutations were yellow, orange-yellow, pale green and light green. The morphological mutations included notched leaf, curly leaf, narrow lobe and miniature (Fig. 5). Almost all these morphologically mutated characters had an effect on growth which usually showed a stunted or semi-dwarf effect.

3) Persistence of the induced somatic mutations

Some of the induced somatic mutations from cultivars 49 and 82 were isolated and purified in the R_2 generation. Cuttings from these purified mutant plants were propagated in subsequent generations. All the induced characters were perpetuated from one generation to the other. So far, no sign has been noted that the isolated mutants have changed back to the original parental types.

c. Mutations induced by pollen irradiation

1) Mutation induction

Since the somatic mutation rate induced by

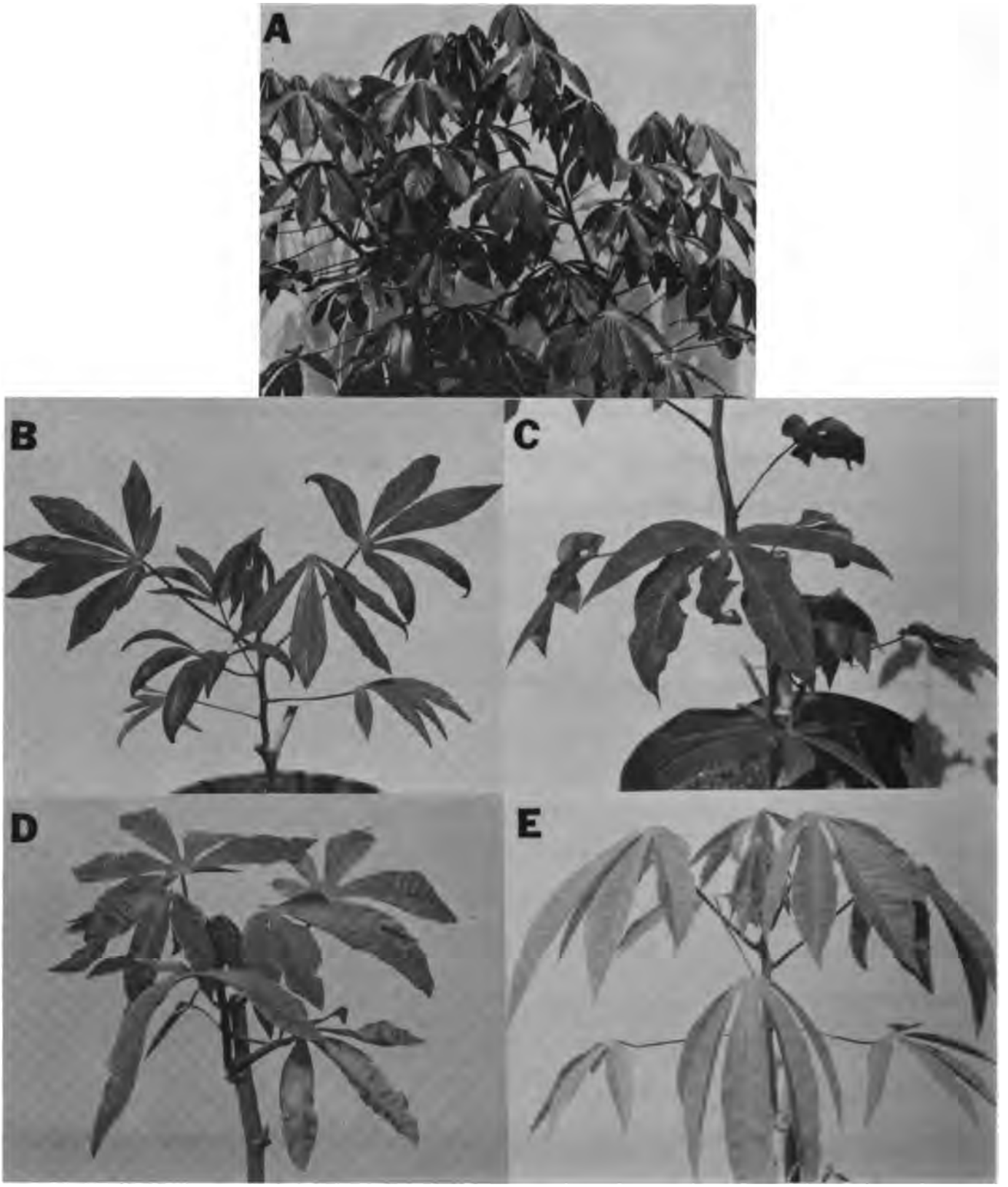


Fig. 5. Induced somatic mutations in Manihot esculenta. Mutations with a change in leaf morphology are common. (A) Normal leaf, (B) Narrow leaf lobe, (C) Curly, (D) Wrinkled, (E) Elongated yellow-green leaf.

irradiating stem cuttings was rather low and the induced mutations so far obtained were drastic changes, we were searching for other methods of inducing mutations in Manihot. Subsequently, we used pollen irradiation method which showed promising results.

The cultivars used for the present investigation were No. 56 and No. 68 in the collection. Number 56 has a good root quality with yellow root core, but the growth habit is prostrate, and is male sterile. Number 68 has a high root production, an erect growth habit, and no floral sterility. The roots of both cultivars take about 8 to 10 months for harvesting.

Mature pollen were collected from cultivar No. 68 at anthesis and were irradiated with an acute radiation dose of 2 and 3 kr from a cobalt-60 source. The pistillate flowers of cultivar No. 56 were protected with a bag made of nylon mosquito netting several days before the flowers opened. This process prevented the possible out-crossing made by insects. The irradiated pollen were immediately pollinated to the pistillate flowers which were then again protected with the nylon bag until the fruits matured. This process was necessary and advantageous because it allowed us to collect the seeds when the fruits dehisced explosively to eject the seeds at maturity.

The hybrid seeds from irradiated pollen as well as non-irradiated pollen (serving as a control) were stored in a dry area for 4 to 8 weeks and were grown individually in small Fertil Pots in the greenhouse. The germination of the

seeds was generally low. We found that less than 30% of the seeds from irradiated as well as non-irradiated pollen could germinate and develop into seedlings. When the seedlings were 1 to 2 months old, they were transplanted to the nursery for further development.

All the mutations reported here were isolated in the R_1 generation. A mutant consistently expressing its mutated character for more than 6 months of growth was recorded as a mutation.

The mutation frequencies obtained from pollen irradiation with 2 radiation doses are presented in Table 3. While the radiation doses used for the present experiment were not high, the frequencies of mutation obtained were appreciable. Thus pollen irradiation appears to be an effective method for inducing mutations in Manihot. It must be pointed out that all the mutations reported here were obtained in the R_1 generation. The frequencies of recessive mutations in the R_2 generation for these 2 radiation doses have not been explored, because of the difficulty of self-fertilization which arises from the protogynous flowering habit in Manihot. To plant the stem cuttings from an R_1 plant at different time intervals, however, would give a possibility of synchronizing the opening periods of the staminate and pistillate flowers, and would permit self-fertilization to bring out the recessive mutations. This study is being carried out in our laboratory.

Most induced mutations found in the R_1 generation were morphological mutations. Of the 29 R_1 mutants obtained

Table 3. Frequency of mutations in the R_1 generations induced by pollen irradiation in Manihot, cultivar No. 56 x cultivar No. 68

Radiation dose (kr)	No. of R_1 plants	Mutations[1]	
		No.	%
0 (control)	72	0	0
2	45	8	17.8
3	55	21	32.8

[1]Most mutations observed were due to morphological changes which included the changes in leaf size (small or narrow leaf-lobe), leaf shape (wrinkle or curly leaf), the plant form (dwarf or miniature), and growth vigor. Chlorophyll mutations were very few, and were limited to yellow mottling and yellow-green.

from the present irradiation experiment with the 2 radiation doses, 24 (83%) were classified as morphological mutants and only 5 (17%) were chlorophyll mutants. These results give a striking difference from those obtained from seed irradiation in many plant species in which most of the induced mutations obtained in the R_2 generation are of a chlorophyll-deficient nature.

The morphological mutations induced by pollen irradiation varied considerably from mutant to mutant. In fact, we found no two mutants were exactly alike. Most commonly, however, they were changes in leaf size, leaf shape, plant form, and growth vigor. Not many types of chlorophyll mutations were induced; so far they have been limited to yellow mottling and yellow-green. Some of the morphological mutants are demonstrated in Fig. 6.

Fig. 6. 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.

2) Persistence of the R_1 mutants induced by pollen irradiation

All the induced mutant characters reported here appeared to be a permanent change rather than a temporary physiological disturbance. Stem cuttings from the R_1 plants were propagated in the field and those with the mutant characters in the R_1 also expressed the identical characters in the subsequent vegetatively propagated generation. Since all the mutations were produced in the R_1 and no mutations (which could arise from the crosses of the two cultivars due to their heterozygous conditions) were found in the control population, it can be considered that the induced mutations are of a dominant nature. However, dominant true gene mutations are extremely rare. It is likely that many of the induced mutations were the results of chromosomal aberrations.

3) Advantages of pollen irradiation

The obvious advantage of pollen irradiation method for Manihot breeding is to eliminate the chimera production. The R_1 plants can be grown and immediately evaluated for their agricultural value. Desirable mutants can be vegetatively propagated and released as improved cultivars. This greatly facilitates the breeding process without carrying out the work on purification of the chimeric sector.

Another advantage of pollen irradiation is that when a pollen is irradiated, only a haploid set of the chromosomes is affected by the radiation. The other set of chromosomes of the egg nucleus is not irradiated and thus is unaffected. Therefore, the resulting zygote, after fertilization,

would have less genetic or chromosomal damage and have a better chance of survival to reveal the mutational changes. This may explain our present results that a rather high frequency of mutations in the R_1 generation can be induced with a relatively low dose of gamma radiation. Manihots are allopolyploids (Jenning, 1963). Polyploids usually have a higher ability to tolerate chromosomal aberrations than do diploids (Nilan, 1956; Sparrow, et al., 1961) which may also be attributed to the phenomenon that a high mutation rate was induced by pollen irradiation in Manihot.

4) Possible use of the induced mutant in Manihot breeding

As has been pointed out previously, there was a wide variation in plant morphology and growth vigor among the R_1 progenies from pollen irradiation. This large variation gave us an excellent opportunity for making selections of the desirable types. Indeed, we have isolated a number of mutants with vigorous growth (Fig. 7) for our breeding program. We are searching for the early mutants which produce mature roots in less than 8 months for the semi-arid areas of the Pacific coast of Central America. If the vigorous mutants produce sizeable edible roots in 6 to 7 months, they can be considered as early mutants. These mutants are being vegetatively propagated for field trials.

Fig. 7 A vigorous mutant of Manihot among the R_1 progenies induced by pollen irradiation.

References

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

Jenning, D. L., 1963. Variation in pollen and ovule fertility in varieties of cassava, and the effect of interspecific crossing on fertility. Euphytica 12:69-76.

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

Nilan, R. A., 1956. Factors governing plant radiosensitivity. Conference on Radioactive Isotopes in Agriculture. AEC Report TID-7512, Washington, D. C. pp. 151-162.

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

Sparrow, A. H., Cuany, R. L., Miksche, J. P. and Schairer, L. A. 1961. Some factors affecting the responses of plants to acute and chronic radiation exposures. Radiat. Bot. 1:10-34.

2. A Compact Mutant in Coffee

During the early years of this AEC project at Turrialba Center, part of the work was to study the effects of ionizing radiations on coffee (Coffea arabica L.) because of its economic importance to many Latin American countries. The biological and genetic effects have been published earlier (Moh, 1962; Moh and Obregoso, 1960). The most unusual biological response of coffee to the radiations was that when the seeds were irradiated, a high frequency of morphological mutants was induced in the R_1 generation and that the mutant character appearing in the R_1 plant was not a sectorial change but rather, the whole plant produced a similar mutant character (Moh, 1961). This evidence suggests that the development of a young coffee shoot is from a single initial cell of the corpus.

In an experiment designed for inducing mutations for breeding purposes, samples of coffee seeds (Coffea arabica var. typica), 100 seeds per sample, were irradiated with a series of acute radiation doses from 2.5 to 20 kr from the ^{60}Co source in the gamma field (in 1958, the only radiation source then available in the project). The irradiated seeds were grown in the nursery and the R_1 plants were then transplanted to the field for further observation.

In 1961, the R_1 plants began to reach a full grown stage and to produce fruits. One plant in the R_1 population irradiated with 5 kr showed many desirable characteristics and has drawn much of our attention. This plant was semi-dwarf, had short internodes and compact floral clusters, and produced fruits rather heavily. Seeds of this plant were then propagated and the progenies showed a segregation from the parental type to the very dwarf type. Evidently, the semi-dwarf compact R_1 mutant was due to incomplete dominance.

Since the characteristics of this semi-dwarf compact mutant are very desirable, effort has been made to isolate a homozygous line for agricultural practice. After four generations of selfing and selection, we have obtained homozygous lines of this semi-dwarf compact type of plants. The average plant height was about 6 feet which greatly faciliated the fruit harvesting. The internodes were short, and the fruit clusters grew compactly along the fruiting branches. Figs. 8 to 11 show the morphology of the semi-dwarf compact mutant.

Fig. 8. A homozygous line of the coffee compact mutant. These plants were three years old after the seedlings were transplanted in the field. The height of the plants were about 6 feet which greatly facilitates the harvesting process.

Although the selected mutant lines appeared to have a high yielding potential, the actual productions of the fruits and seeds have not been evaluated. This year, the seeds of the isolated homozygous lines are being propagated for yield trials.

References

Moh, C. C. and Obregoso, G., 1960. The induction of angustifolia mutants in coffee in R_1 generation by ionizing radiations. Genetics 45:1000.

Moh, C. C., 1961. Does a coffee plant develop from one initial cell in the shoot apex of an embryo? Radiation Bot. 1: 97-99.

Moh, C. C., 1962. The use of radiation induced mutations in crop breeding in Latin America and some biological effects of radiation in coffee. Internat. Jour. Appl. Radiat. and Isotopes 13:467-475.

3. Mutation Breeding in the Common Bean

After a few years of work on inducing mutations in the common bean, we found that the induced mutation method is very 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). 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 more tedious. The effectiveness of gamma radiation and ethyl methanesulfonate

Fig. 9.

Fig. 10.

Fig. 9. A normal branch of coffee, variety typica, showing long internodes and lax fruit clusters.

Fig. 10. The branches of the coffee compact mutant showing a compact fruiting habit along the branches.

Fig. 11. A branch of the coffee compact mutant demonstrating the side branches of the main branch, which tends to produce fruits also.

(EMS) in inducing seed-coat color mutations and the genetic nature of the induced mutants were presented in the 1966-1969 Triennial Report and other publications (Moh, 1969; 1971).

In the past years, we have: 1) developed a screening technique which makes the mutation breeding method for the seed-coat colors more efficient; 2) studied the field performance and nutritional value of the white bean mutant; and 3) tested the mutagenic effect of cycasin on beans. The motivation and the results of these studies are described below.

a) The development of the screening technique for seed-coat color mutants

The success of induced mutation method for plant breeding is largely conditioned by the following two factors: First, it depends upon whether a plant species has the genetic potential to mutate to the desirable traits. Secondly, it depends upon the ease of detecting the desirable mutants. For the first, information on the mutability of the desirable traits and the knowledge on the effectiveness of the mutagen are essential. For the second, an efficient screening technique for isolating the induced desirable mutants is helpful. It is known that the seed-coat of beans has a wide range of color variations in the natural population. While working on the induced mutations in beans with various mutagens, it was noted that the genetic factors controlling seed-coat color were mutable.

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

hypocotyl color of early seedling stage. Essentially, the seed colors can be divided into four principal groups: black, bayo (from deep brown to light yellow), red, and white. The correlation between the seed color and the hypocotyl color can be seen in Table 4. All the black varieties observed produced red hypocotyl, and all the white varieties produced green hypocotyl. The bayo and red varieties could produce either red or green hypocotyls. This correlation provides a criterion to detect the possible seed color mutants induced from a black variety.

Table 4. Correlation between the seed-coat colors and the hypocotyl colors of 271 bean varieties*

Seed-coat color	Hypocotyl color		Total varieties
	red	green	
black	93	0	93
bayo	38	21	59
red	54	22	76
white	0	43	43
Total varieties	185	86	271

* A test of independence showed that the total $\chi^2 = 136.82$, P value is highly significant.

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 Table 5 and 6 demonstrate these relationships.

These results not only provide a method for isolation

Table 5. 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 6. 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, $P<.01$

of the possible white seed mutant from black bean varieties, but also give an added assurance in selecting the seed-coat color mutants from the black seed varieties.

By using the results, we established a screening method. The procedure of the screening method is summarized in steps for breeding practices.

1) The R_1 plants are harvested individually after the mutagenic treatment. The selfed progenies of an R_1 plant are sown in a row in a soil box. A 3x0.7 m box is sufficient for 40 rows for the selfed progeny

test.

2) When the hypocotyl hooks of the seedlings begin to emerge, normally 4 to 5 days after sowing, the mutants with a change in hypocotyl color from red to green or to light pink are marked for transplanting.

3) When the selected mutants are a week old and the first trifoliate leaf begins to appear, they are transplanted to a soil pot for further growth observation.

4) According to our experimental records, some mutants such as some dwarfs or miniatures are also associated with a hypocotyl color change. Those considered as deleterious ones can be discarded shortly. Only those with normal appearance and growth are maintained to maturity.

5) When the mutant plants mature, the seeds are examined for their color identification.

b. Field trials and nutrition study of the white bean mutant

Rather frequently, we have been asked the following questions: What is the yielding capacity of the induced mutants, and what is their nutritional value? To seek information on these questions, experiments were carried out in collaboration with the bean program of our Institute and the Institute of Nutrition of Central America and Panama (INCAP).

1) Yield trials

The yield data of a white bean mutant (NEP-2)

as compared with its original black seed parent (S-182N), and the white varieties (San Jero and Panamito) recommended for Latin America were supplied by the bean program of our Institute (Table 7). It can be seen that the seed production of the white mutant was slightly lower than the original black parent (7 to 15%), but was much higher than the locally recommended white varieties (17 to 22%). These results were derived from the field data of one year, however. At present we can state that the production of the induced white mutant is at least as high as, or probably higher than the two locally recommended white varieties.

The latests report from Ecuador on the field trials of beans indicated that NEP-2 was one of the top producers among the 20 bean varieties tested. The field trial location was in Loja county which has an altitude of 1,750 m (5,740 ft) above sea level. The result thus showed that the induced white mutant, NEP-2, has a wide adaptability to altitude.

Table 7. Yield trials of the white bean mutant, its original black parent, and the local recommended white varieties

Variety	Origin	Yield (kg/ha)
NEP-2	the white seed mutant	2268
S-182N	the original black seed parent	2452
San Jero	a local recommended white variety for Central America	1865
NEP-2	the white seed mutant	2088
S-182N	the original black seed parent	2475
Panamito	a commonly grown variety in Peru	1612

2) Nutrition study

In cooperation with INCAP, seeds of the white mutant (NEP-2) and its black parent (S-182N) were sent to the laboratory of INCAP for nutrition studies. Cooked beans of these two lines were fed to the rats for three weeks and the results of weight gain are presented in Table 8. The white bean sample produced 36 gm weight gain and a protein efficiency ratio (PER) of 2.32. The black bean sample produced only 21 gm with a PER value of 0.84.

It was thought that the higher PER value of the white mutant could be due to its seeds being less toxic, because it is known that in many legumes, hemagglutinin and trypsin inhibitors are present (Liener, 1966; Jaffé, 1969). Studies of hemagglutinin concentration was carried out by Dr. Werner Jaffé for INCAP (Bressani, personal communication). It was found that both the white and black bean samples had the same concentration of hemagglutinin.

Table 8. Weight gain of rats fed with the white bean mutant and its black parent (From Bressani, INCAP)

Sample	Weight gain g/21 days	PER
Black parent	21	0.84
NEP white-2	36	2.32

Tests of the trypsin inhibitor activity were carried out by INCAP. Samples of the white and the black beans were fed raw to laboratory rats, in a diet in which beans provided all

of the protein. All animals died in less than 14 days which indicates the presence of trypsin inhibitors.

An analysis of chemical composition of the white and the black beans was carried out by INCAP. Results presented in Table 9 indicated that there was very little difference between the black beans and its white mutant in moisture, fat, crude fiber, nitrogen and ash content.

The protein content of the black bean and its white mutant has been determined by the laboratory of our Institute and also by INCAP. The results showed that the protein content of the white mutant tended to be slightly higher than its black parent in all three analyses (Table 10). Based on this small difference in protein content, it is difficult to explain the good growth performance of the laboratory rats fed with white mutant beans. In an agreement with INCAP, we have decided to reconfirm the biological results obtained from the feeding experiment and to look into the protein quality of the white mutant beans. Seeds of both the white and the black beans will be analyzed for the essential amino acids, especially the sulphur containing types. Bean proteins are deficient in methionine; an addition of very small amount of this (e.g. 0.15%) would increase protein quality significantly.

c. Mutagenic effects of cycasin on beans

1) Toxicity of cycads and cycasin

Cycasin is a chemical compound, methylazoxymethanol-β-D-glucoside, ocurring naturally in the cycad plants.

Table 9. Proximate chemical composition of the black beans (S-182N) and its white mutant (NEP-2) (from Bressani, INCAP)

Variety	Moisture %	Fat %	Crude fiber %	Nitrogen %	Ash %
S-182N	10.0	1.6	5.1	3.92	4.5
NEP-2	10.8	1.6	5.5	4.07	4.1

Table 10. Protein content of the white bean mutant (NEP-2) and its black parent (S-182N)

Determination	Sample	Protein content %
1) By IICA	NEP-2	28.0
	S-182N	25.1
2) By IICA	NEP-2	28.6
	S-182N	26.7
3) By INCAP	NEP-2	25.4
	S-182N	24.5

The cycads belong to the family Cycadaceae of the Gymnosperms and are widely distributed in the tropical and subtropical regions. The plants are used as ornamentals, as animal feed, as medicine, and as human food. The toxicity of cycads has long been known as in some cases the induction of illnesses or death in animals and humans can be traced to the ingestion of the cycad or its products (Whiting, 1963). In more recent years, because of the high incidence of neurologic diseases occurring on Guam and other islands of the Pacific where the indigenous inhabitants use cycads as a source of feed (Whiting, 1963), studies on the chemistry of cycads and the biological

and pathological effects have proceeded rapidly. It has been demonstrated that the tissues of cycads, or the cycasin, the compound isolated from the tissues, are toxic and carcinogenic in a wide variety of animals (Mickelsen, et al., 1964), and radiomimetic in Allium chromosomes (Teas, Sax and Sax, 1965).

Further chemical studies and biological tests revealed that the aglycone of cycasin, methylazoxymethanol, is the toxic component (Kobayashi and Matsumoto, 1965; Matsumoto and Strong, 1963). Cycasin produces its toxic effects in a biological system only when the cycasin is hydrolyzed by the enzyme, β-glucosidase, to release its aglycone. Thus, cycasin produced carcinogenic effects in conventional rats but not in germfree rats which lack the glucosidase (Laqueur, 1965). A similar explanation can be applied to the experimental results that cycasin per se induced no mutations in Salmonella (Smith, 1966) and Drosophila (Teas and Dyson, 1967), but its aglycone was a potent mutagen.

In the present study, the mutagenic effect of cycasin on higher plants is reported. It is known that many plants contain emulsins that are capable of splitting the cycasin into the aglycone (Teas, Sax and Sax, 1965). Mutagenic effects are expected to be obtained by direct application of the cycasin to the plant materials.

2) Mutagenic effects on beans

The common bean (Phaseolus vulgaris L.) was used as an experimental material. Besides its agricultural importance in the American tropics, its short life cycle,

self-pollinated nature, and diversified genetic characters provide good botanical properties for mutation studies. The ß-glucosidase activity in the bean variety (San Fernando) used for the present experiments was tested by Dr. H. J. Teas of the University of Miami, Florida, U.S.A. Although the activity was not extraordinarily high, its presence was sufficient to warrant the performance of the experiments.

Newly harvested bean seeds, dried in an oven at 34°C for 2 weeks, were treated with cycasin solution of various concentrations at 20°C for 24 hrs. Fifty seeds were used in each treatment and the concentration of cycasin was prepared in a 10-ml aqueous solution with 0.25, 0.50, 0.75 and 1.00 mg of cycasin per seed. At the end of the treatment period, all 10 ml of cycasin solution were absorbed by the 50 seeds. The seeds were then rinsed several times with water and grown in the greenhouse. At maturity, the seeds from each treated plant were harvested separately and were grown in the soil box, one plant per row, for observation of the induced mutations. The observation was carried out for 2 weeks of the seedling period. According to our previous mutagenic experiments, most of the induced mutations, including both chlorophyll and morphological types, can be detected within this period. The pooled data on four identical experiments in which the M_1 plants were grown in the greenhouse are presented in Table 11.

In the experiment in which the M_1 plants were grown in the field, the seeds were treated in the same manner as described above, except that a total of 300 seeds were treated in each

Table 11. Survival rate and mutation frequency induced by cycasin in Phaseolus vulgaris L.

Treatment mg/seed	Number of seeds treated	M_1 surviving plants		Mutations*	
		number	% of control	number	%
		Greenhouse grown			
Control	200	183	100.0	0	0.0
0.25	200	171	93.4	78	45.6
0.50	200	99	54.1	57	57.6
0.75	200	66	36.1	41	62.1
1.00	200	44	24.0	25	56.1
		Field grown			
Control	300	240	100.0	0	0.0
0.25	300	150	62.5	72	48.0
0.50	300	35	14.6	20	57.1
0.75	300	11	4.6	8	72.7

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

concentration of 60 ml cycasin solution. The mutation study was also conducted as described above.

Table 11 summarizes the results on the survivals and mutation frequencies in the bean plants treated with various concentrations of cycasin solution. The number of survivals decreased as the concentration of cycasin increased. The LD_{50} was about 0.50 mg per seed for the greenhouse experiment and 0.25-0.50 mg per seed for the field experiment. Field grown materials usually exhibit a lower rate of survival because of

unfavorable growth conditions. In both experiments, the toxic effect of cycasin on beans, as reflected by the survival rate, is evident.

The mutation frequencies resulting from seed treatments with cycasin were very high (Table 11). As compared with our previous experiments with EMS, the mutation frequency induced by cycasin was at least as high, if not statistically significantly higher, than that induced by EMS. It appears that cycasin is a potent mutagen in higher plants and can be applied directly to the plant materials for inducing mutations providing that the plant has ß-glucosidase activity to release the active mutagenic component, the aglycone, from cycasin. Matsumoto and Higa (1966) have demonstrated that the aglycone is a good methylating agent with 7-methylguanine being formed when it reacts with both DNA and RNA. The high mutation rate may be attributed to the ability of the aglycone to methylate the genetic material.

References

Jaffé, W. G., 1969. Hemagglutinins. In Toxic constituents of plant foodstuffs. Ed. by I. E. Liener. Academic Press, N. Y. pp. 69-101.

Kobayashi, A. and Matsumoto, H., 1965. Studies on methylazoxymethanol, the aglycone of cycasin. Isolation, biological and chemical properties. Arch. Biochem. Biophys. 110: 383-380.

Laqueur, G. L., 1965. The induction of intestinal neoplasms in rats with glycoside cycasin and its aglycone. Virchows Arch. Path. Anat. 340:151-163.

Liener, I. E., 1966. Hemagglutinins in foods. In Toxicants occurring naturally in foods. Publication 1354 National Academy of Sciences. Nat. Res. Council, Washington, D.C.

Matsumoto, H. and Strong, F. M., 1963. The occurrence of methylazoxymethanol in Cycas circinalis L. Arch. Biochem. Biophys. 101:299-310.

Matsumoto, H. and Higa, H. H., 1963. Studies on methylazoxymethanol, the aglycone of cycasin: methylation of nucleic acids in vitro. Biochem. J. 98:20c-22c.

Mickelsen, O., et al., 1964. Studies with cycads. In 3rd Conference on the Toxicity of Cycads. Chicago, 1964. Sponsored by National Institute of Neurological Diseases and Blindness and National Institute of Health. Maryland.

Moh, C. C., 1969. Seed-coat color changes induced by ethyl methanesulfonate in the common bean (Phaseolus vulgaris L.). Mutation Research 7:469-471.

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

Smith, D.W.E., 1966. Mutagenicity of cycasin aglycone(methylazoxymethanol), a naturally occurring carcinogen. Science 152:1273-1274.

Teas, H. J., Sax, H. J. and Sax, K., 1965. Cycasin: radiomimetic effect. Science 149:541-542.

Teas, H. J. and Dyson, J. G., 1967. Mutation in Drosophila by methylazoxymethanol, the aglycone of cycasin. Proc. Soc. Exp. Biol. and Med. 125:988-990.

Whiting, M. G., 1963. Toxicity of cycads. Economic Bot. 17: 270-302.

4. Radiosensitivity of Tropical Plant Species

This is a continuous study of the radiosensitivity of plant species in the American tropics. The objective of this study is to obtain information on the range of radiosensitivity in the plant families. The information obtained not only serves as a guideline to use radiation doses for inducing mutations in plant breeding, but also allows us to predict the effects of radioactive fallout.

Based on our previous observations on the growth response of plants in the gamma field, temporary growth inhibition and morphological distortion have not been satisfactory criteria for measuring the radiosensitivity, since a similar morphological change, but in different degrees, can be induced by a wide range of radiation levels. Once the radiation is removed, the growth response of the plants usually goes back to normal. The criteria used for determining radiosensitivity in the present investigations are: 1) for the vegetatively propagated species, the maximum daily dose at which the plants can survive for a lengthy period or until their natural death; and 2) for the seed propagated species, the maximum daily dose at which the plants can produce seeds to complete their life cycle. These two criteria would give information on the critical dose level at which a plant species could maintain its continuity in a plant community in case of heavy radioactive fallout.

The plants subjected to the radiosensitivity test are arranged radially from the radiation source instead of concentrically around the cesium-137 source in the gamma field. This radial arrangement gives us a continuous picture of growth responses of the plants as they are exposed to an increasing radiation dose toward the source, and provides a better means for locating the radiation level which critically affects the plant development. Generally, plants or seedlings of a species are exposed to a range of daily chronic radiation doses from 500 to 5 r. Data on growth response, death of meristematic tissue,

ability to produce flowers, fruits and seeds, and the germinability of the seeds are recorded to determine the radiosensitivity.

In this triennial report, we only present the summarized results of the radiosensitivity studies on six plant families (Table 12). These included a total of 75 species in 40 genera. The detailed growth response of each species can be found in the previous annual reports.

Because much of our effort will be devoted to the mutation breeding of tropical root crops, this radiosensitivity study is to be temporarily concluded here. A list of radiosensitivity of the species so far studied will be prepared for publication.

B. CONTROL OF INSECTS BY MALE STERILIZATION METHOD

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

This is a continuation of the cooperative porject started in 1961 between the Inter-American Institute of Agricultural Sciences (IICA) (the Institute is an AEC contractor) and the Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA) to evaluate the feasibility of eradicating the Mediterranean fruit fly (commonly known as medfly) from Central America using the gamma sterile males. In this cooperative project we are responsible for carrying out basic research needed for field trials while OIRSA has the responsibility of carrying out pilot

Table 12. Radiosensitivity range of the species in six plant families

Plant family	No. of genera	No. of species	Range of maximum dose the species can tolerate or complete the life cycle (r per day)	Radiosensitivity classification
Compositae	10	11	127 (*Sonchus* sp.) - 500 (*Emilia sonchifolia*, *Galinsoga caracasana*, *Tagetes* sp.)	Resistant
Convolvulaceae	4	7	116 (*Ipomoea* sp.) - 248 (*Ipomoea hirsutula*	Resistant
Cruciferae	2	4	151 (*Raphanus sativus*) - 500 (*Brassica chinensis*)	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

field tests to determine the effectiveness of the technique.

Towards the end of 1965, OIRSA in cooperation with the International Atomic Energy Agency, initiated an active program (funded by UNDF/SF and operated by IAEA) in Nicaragua to determine the effectiveness of the sterile male technique for medfly eradication. In 1968-1969, OIRSA carried out a field trial in a semi-isolated 48 km^2 area (6 km x 8 km) in Carazo, Nicaragua. The results from this test support the conclusion that the medfly can be eradicated by the sterile male technique in Central America.

During the last three years, like in the past, we have been actively engaged in carrying out supporting research for the Joint IAEA/OIRSA medfly project in Central America. The present report summarizes our work under this cooperative project for the period July 1969 through June 1972.

a. Insemination frequency of medfly males irradiated during pupal or adult stage

One of the basic requirements for the successful application of the sterile male technique to control an insect is that irradiated males should reasonably compete in mating with normal males. In a test designed to measure the insemination capacity of irradiated males, we found that the mating vigor of males irradiated with 10 kr at pupal stage was affected by the stage of development at which irradiation was applied. The closer to adult emergence that the pupae were irradiated, the higher the insemination frequency of the treated males. The

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

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

Insemination capacity of males of each treatment were measured by confining an individual male with six virgin females in a small cage for 48 hr. Then, females from surviving males were removed and their spermathecae were dissected out and examined for the presence of sperm under a compound microscope. The females which carried at least a trace of sperm in the spermathecae were scored as inseminated. In the medfly

female, motile sperm was found in the spermathecae 24 hr after the adult's death. Each surviving male was given six virgin females for another 48 hr period of insemination. This mating procedure was repeated until the males were three weeks old. At this time, most of the males had died in all the treatments. Each treatment had 15 males at the beginning of the test. The age of the females used in the experiment varied from 3-8 days.

The experiment was carried out in the laboratory at 25± 3°C temperature and 75±5% relative humidity. The males were given females for insemination for the first time at the age of 3 days.

Table 13 presents the average number of females inseminated per male during nine mating series by different types of sterile males (irradiated as pupae or as adults) and untreated males. The results indicate that irradiation reduces the insemination vigor of the treated males. During 3 weeks of adult life a normal male inseminated an average of 21.6 females compared to 9.9, 13.8 and 14.8 females inseminated by males irradiated as 24 hr pre-emergent, 24 and 48 hr post-emergent, respectively. The results also indicate that the males irradiated at adult stage had slightly greater insemination efficiency than those irradiated as pupae. Insemination capacity of males irradiated as adults increased ca. 1.5 fold over that of males irradiated as pupae (24 hr before emergence).

Table 13. Consecutive insemination of medfly females caged for 2 days with 10 kr irradiated males at a ratio of 6:1

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

[a] Figures in parenthesis are number of males used for matings.

b. Further studies on mating competitiveness of medfly males irradiated during pupal or adult stage

Irradiation of mature medfly pupae (1-3 days before adult emergence) with 10 kr, induces more than 99% sterility in the treated males (5). However, sterilization seems to reduce the mating competitiveness of treated males (3). We had reported previously (4) that the mating vigor (insemination efficiency) of 10 kr irradiated males is affected by the pupal stage at which irradiation is applied. The closer to adult emergence that the pupae are irradiated, the higher the insemination efficiency. However, when mating competitiveness of males irradiated during pupal or adult stage was evaluated by caging mixed populations of sterile males, normal males and normal females, we found only a slightly increased mating competitiveness of males irradiated as 24-48 hr after emergence compared to those irradiated 24 hr before emergence (6). This result is in confliction to the one obtained by ohinata et al. (7). These authors reported that treatment of male as 2-day-old adult with 10 kr gamma irradiation did not reduce their mating effectiveness. However, the same treatment applied to pupae 2 days before adult eclosion reduced mating effectiveness about 50%.

Since in the 1970 teste we compared the mating competitiveness of males irradiated 24 or 48 hr after emergence and 24 hr before emergence, we did not include irradiation of males

48 hr before emergence. Ohinata et al.(7) compared mating effectiveness of males irradiated 2 days before and 2 days after emergence. Thus, in order to compare our results with these authors with conflicting results, and to reconfirm our previous results, we conducted some more experiments in 1972 to determine the mating effectiveness of males irradiated with 10 kr as 2 days before or 2 days after adult emergence. Pooled results of the two experiments (1970 and 1972) are presented in this report.

Medflies used in the experiment were supplied by OIRSA laboratory in San Jose, Costa Rica, and were reared on a standard laboratory diet reported previously (8). Adults were fed on a mixture of sugar and protein hydrolyzate (testone S-150) and water. The flies were maintained in the laboratory at 25±3°C temperature and 75±5% relative humidity with natural light and dark regime (the lights were on in the laboratory between 7:00 AM and 4:30 PM only during working days). The sterile flies used in the experiment were irradiated with 10 kr in a pool-type Cobalt-60 irradiator with a dose rate of ca. 1300 r/m. All the flies used in the experiments were of uniform age since adult emergence was spread out between 5:00 and 9:00 AM. The adults were sexed out under the influence of CO_2 within 12 hr after emergence. The tests were carried out in 1 ft^3 wooden frame cages covered with plastic screen. The sterile and normal flies were put together in the cages 48 hr after adult emergence.

The mating competitiveness of sterile males irradiated

at 4 different stages (as pupae 48 or 24 hr prior to emergence and as adults 24 or 48 hr after emergence) was tested by caging them with normal males and normal females. The mating effectiveness of each type of male was tested at three different ratios: 1:1:1. 2:1:1 and 3:1:1 (sterile male:normal male:normal female). The tests included two controls to check the fertility of irradiated and non-irradiated males. Each cage had 50 and 25 normal females per ratio in 1970 and 1972 tests, respectively.

The egg collection started when adults were 5 days old and it was carried out over a 4-week period. Eggs were collected five times a week. Each sample consisted of 200-400 eggs. The experiment was repeated six times, each replication with different batches of pupae.

The percent egg viability of the mixed fly populations of sterile males (irradiated as pupae, 48 or 24 hr before emergence, or as adults 24 or 48 hr after emergence), normal males and normal females is summarized in Table 14. The results, in general, show slightly increased mating competitiveness of males irradiated as adults compared to those irradiated as pupae. This difference is more evident when comparison is made between males irradiated as adults and those irradiated as pupae, 48 hr before emergence. The males irradiated 24 or 48 hr after emergence were more effective in suppressing egg hatch than males irradiated 48 hr prior to eclosion.

Mating competitiveness of sterile males irradiated as 48 or 24 hr pre-emergent and 24 or 48 hr post-emergent was

Table 14. Mating competitiveness of medfly males irradiated with 10 kr as pupae (48 or 24 hr before emergence) or as adults (24 or 48 hr after emergence)

Adult ratios[a]	Female fertility (%) when caged with males irradiated			
	48 hr pre-emergant[b]	24 hr pre-emergant[c]	24 hr post-emergant[c]	48 hr post-emergant[c]
0:1:1	89.8 (23,287)[d]	91.6 (47,955)	89.6 (48,342)	90.4 (47,544)
1:0:1	0.4 (23,336)	0.8 (51,262)	0.8 (46,580)	0.4 (49,424)
1:1:1	72.7 (19,483)	62.0 (47,738)	60.8 (46,665)	58.4 (46,583)
2:1:1	67.7 (19,476)	49.2 (36,810)	45.8 (45,615)	44.2 (46,859)
3:1:1	53.5 (19,762)	41.9 (44,087)	42.0 (47,915)	43.5 (45,408)

[a] Treated males:untreated males:untreated females. Fifty and 25 females per ratio in 1970 and 1972 tests, respectively.
[b] Average % egg-hatch of 1972 experiment with 6 replicates.
[c] Average % egg-hatch of 1970 and 1972 experiments with 12 replicates.
[d] Figures in parenthesis are total number eggs examined.

calculated according to the method of Fried (1). Table 15 presents the mating competitiveness values (quantified) of each treatment. The data indicate that irradiation reduces the mating competitiveness in all the treatments. Competitiveness of males irradiated 1 or 2 days post-emergence is little different from that of males irradiated 1 day before emergence. While competitiveness of males irradiated 48 hr before emergence is reduced by ca. 50% as compared to those males irradiated 24 hr later, i.e. 24 hr before emergence. Mating competitiveness of males irradiated as adults increased from 1.6

Table 15. Values for the competitiveness[a] of male medflies irradiated with 10 kr as pupae (1-2 days before eclosion) or as adults (1-2 days after emergence) at three different ratios

Adult ratios[b]	Competitiveness values for males irradiated as			
	48 hr pre-emergant	24 hr pre-emergant	24 hr post-emergant	48 hr post-emergant
0:1:1	89.7[c]	91.6[c]	89.6[c]	90.4[c]
1:0:1	0.4[d]	0.8[d]	0.8[d]	0.4[d]
1:1:1	0.24	0.48	0.48	0.56
2:1:1	0.22	0.44	0.49	0.53
3:1:1	0.23	0.40	0.38	0.36

[a] Calculated from data in Table 14.
[b] Irradiated males:untreated males:untreated females.
[c] Percent egg-hatch of normal male x normal female cross.
[d] Percent egg-hatch of irradiated male x normal female cross.

to 2.4 times compared to that of males irradiated as pupae (48 hr before emergence). These results are in close agreement to those reported by Hooper (2), who found that competitiveness of medfly males irradiated as newly emerged adults (2-6 hr old) increased from 1.2 to 1.8 times over that of irradiated as pupae (2 days before eclosion). These results are also in agreement with that of Ohinata et al. (7) in that competitiveness of sterile males is increased by irradiating 2 day-old adults instead of irradiating pupae (2 days before eclosion). However, these authors reported 2.6 to 4-fold increased competitiveness of males irradiated as adults over that of those irradiated as pupae.

Sterilization of medfly males with 10 kr applied to pupae (2-1 day before eclosion) or to adults (1-2 days after emergence) reduces the mating competitiveness of treated males. In general, irradiation reduced the competitiveness (based on egg-hatch) of sterile males to approximately 1/4th (competitiveness ranged from 0.22 - 0.24) when treatment was given to the pupae (2 days before eclosion) and the mating competitiveness was reduced only to approximately 1/2 (ranging from 0.36 - 0.56), when irradiation was applied at later stages, i.e. to pupae, 24 hr before emergence or to adults, 24 or 48 hr after emergence. For field population suppression of medflies using sterile males, the earliest optimum stage for sterilization seems to be the pupal stage of 24 hr before adult emergence. Competitiveness of males irradiated 1 day before emergence was not different from those irradiated as 1 or 2 day old adults. On the other hand, males irradiated 48 hr before emergence were ca. 50% less competitive as compared to those irradiated 24 hr prior to emergence.

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

Adults of medfly can be sterilized by irradiating mature pupae (1-2 days before adult emergence) with 10 kr inducing more than 99.9% dominant lethal mutations in sperm of treated males. But males so treated do not equally compete in mating with normal males. Perhaps by lowering the sterilization dose from 10 kr (inducing almost 100% sterility in males) to sub-sterilization

doses (leaving up to 10.0% fertility in treated males), the sexual vigor of the irradiated males can be increased. Previously, some experiments were carried out with 5 kr, 7 kr, 9 kr and 11 kr dosages at a 5:1 ratio (treated:untreated adults). The results did not show any increase in the mating vigor (based on egg viability) of the males treated with low sterilization doses. Therefore, in cooperation with Dr. C. H. Hooper of Siebersdorf Laboratory of the IAEA, further experiments were carried out to compare the mating competitiveness of medfly males irradiated with gamma irradiation with various dosages. Results of the experiments carried out at Turrialba laboratory are presented here.

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

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

Irradiated and normal flies were caged together immediately after sexing. Perforated quart-sized polyethylene round freezer jars were used for oviposition. First egg collection

was made when flies were 5 days old and it was continued until adults were 3 weeks old. Eggs were collected 5 days per week. Individual egg sample consisted of 200-400 eggs.

Adults were confined in 1 ft^3 wooden frame cages covered with plastic screens. The experiment was replicated seven times. Each replicate was set on a different day with a different batch of pupae. All the tests were performed in the laboratory at 25$\pm$ 3°C temperature and 75$\pm$5% relative humidity. The summarized results of this experiment are presentedin Table 16.

The results of the 9:1:1 ratio showed no significant difference in the egg-hatch from the treatments using 5, 7, 9,or 11 kr treated males. This ratio test gave an average of 30.0, 25.8, 25.0 and 27.2% egg viability when sterile males were irradiated with 11, 9, 7 and 5 kr, respectively. Similarly, at a 19:1:1 ratio, no significant differen e in egg-hatch could be detected between treatments using 5 or 11 kr treated males. The mean egg-hatch of the females caged with sterile and normal males (at 19:1 ratio) were 19.9% and 17.7%, when sterile males were treated with 11 and 5 kr, respectively.

When male competitiveness was quantified according to Fried's method (1), it was found that male competitiveness tended to decrease slightly as the dose increased from 5 to 11 kr.

Based on the assumption that mating competitiveness of males treated with 5, 7, 9, or 11 kr is not affected, then the observed egg-hatch in Table 16 should decline with the increasing dose because inherent sterility of the male increases

Table 16. Mating competitiveness of medfly males irradiated at various dosages with gamma radiation at 24 hr before adult emergence

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

[a] Treated males:normal males:normal females.

[b] Figures in parenthesis are total eggs examined on which the percentages are based.

(male sterility increases from 89% at 5 kr to greater than 99% at 11 kr). However, the egg-hatch is reduced to approximately the same level in all the treatments. Thus some factor was nullifying the effect of increased sterility with increasing dose and leading to similar observed egg-hatch for 5, 7, 9, and 11 kr males. It is reasonable to assume that this factor was sexual competitiveness and it then follows that as the dose was increased, sexual competitiveness decreased.

d. Suppression of the reproductive potential of a wild strain mediterranean fruit fly by gamma irradiated males in caged coffee trees

At OIRSA laboratory in San Jose, Costa Rica, it has been noticed that irradiated laboratory strain medfly males do not equally compete in mating with untreated wild flies. Continuous rearing of medflies in the laboratory through several generations may alter the mating behavior of laboratory reared flies as compared to that of wild flies. In a field population-suppression experiment using sterile males, the released irradiated males must compete in mating with wild males to reduce the egg-hatch of normal females to an acceptable level. The present study was therefore conducted to determine the mating competitiveness of irradiated laboratory strain flies with untreated wild flies when simultaneously released in small outdoor cages more nearly approaching natural conditions.

This study was carried out during 1968 and 1969. Due to continuous nature of the work, the data from the 1968 test have been combined with that of the 1969 test, to present the

final results of this study in the present triennial report.

The tests were carried out in the field in four large screen cages (3.6 m x 3.6 m x 2.4 m), each erected over four fruiting coffee trees. These cages were put in place several weeks before the fly releases in order to avoid the presence of natural infestation on the coffee trees under test.

The mating competitiveness of sterile flies was evaluated at ratios of 20:1, 40:1 and 80:1 (irradiated:untreated) in 1968. In 1969, the 20:1 ratio was replaced by 120:1. Each test included one control with only normal flies. The initial release (affected in the beginning of September of each year) consisted of 20 normal wild flies (10 males and 10 females) per ratio in 1968 and 40 normal wild flies (20 males and 20 females) per ratio in the 1969 test. In subsequent releases, the number of adults was reduced to half. The flies were released over a 12-17 week period (one release per week).

The wild flies for releases were obtained as pupae from infested tropical almond (Terminalia catapa) fruits collected from the Puntarenas area (Puntarenas Province, Costa Rica). The irradiated flies were laboratory strain reared on bagasse in mass at the OIRSA laboratory in San Jose, Costa Rica. The number of sterile flies to be released in each treatment was calculated volumetrically (1000 pupae per 17 ml, and 90% adult emergence from the pupae). The sex ratio of irradiated flies was assumed 1:1. The adults which emerged from irradiated pupae were released without sexing, 12 to 30 hr after emergence.

All the normal flies used for releases were sexed out under CO_2 influence within 12 hr after emergence and males and females were kept in separate cages until released. This precaution was taken to avoid any matings of normal females with normal males before release. Since mating in medfly, like most other insects, depends upon physiological maturity of the adults, care was taken to release normal and sterile flies of the same age.

The first four releases of sterile flies in 1968 consisted of adults irradiated with 7 kr and the following 8 releases with 10 kr irradiated adults. In 1969, the first 8 releases of sterile flies consisted of adults irradiated with 10 kr and then the irradiation dose was reduced to 9 kr in the following 9 releases.

Food for adults in the cages was provided 5-6 times per week by spraying protein hydrolysate type-M plus sugar solution in water over the coffee foliage.

All mature coffee berries were picked up once or twice every week from each cage to prevent multiplication of the adult populations in the cages during the experimental period. From the total number of berries, samples were examined for the presence of larvae and unhatched eggs. The unhatched eggs were incubated in the laboratory for 72 hr on moist filter paper in order to determine egg viability. During the course of the experiment the amount of mature berries available diminished greatly. At the start, up to several kilograms of berries were harvested, from which a maximum of 250 berries per cage were

examined. During the last few weeks, however, quite often 20-50 berries were available for examination.

Weekly egg viability data from different treatments are presented in Tables 17 and 18 for 1968 and 1969, respectively.

The data indicate that in both years the egg oviposition was poor and erratic. The reason for this oviposition pattern of medfly females on coffee berries is not known. Table 19 (prepared from data in Tables 17 and 18) shows that irradiated (7-10 kr) laboratory strain flies do not compete equally in mating with normal wild flies. Comparisons between observed and expected percent egg-hatch (Table 19) indicate that sterile flies were approximately 1/6th to 1/5th sexually competitive. The observed egg-hatch in treatments receiving sterile and fertile flies in ratios of 20:1, 40:1 or 80:1 was ca. 5-6 times the expected egg-hatch. The expected percent egg-hatch in the last column of Table 19 was calculated on the basis of 98.5% egg-hatch in the control and on the assumption that matings of normal females with sterile males resulted in a 1.0% egg-hatch. At 7-10 kr, egg-hatch of approximately 1-2% can be expected when medfly males irradiated 1-2 days before eclosion are mated with normal females.

In the treatment receiving sterile and fertile flies in 120:1 ratio, the observed percent egg-hatch (1.0%) was less than the expected percent egg-hatch (1.8%). It seems therefore, that in field trials to control medfly with this method a ratio of 120:1 (sterile:normal) should be used. At this ratio of sterile to normal flies the reduced mating competitiveness of irradiated

Table 17. Suppression of reproductive potential of medfly (based on egg-hatchability) by weekly releases[a] of gamma sterilized adults (males and females) in various ratios, Turrialba, Costa Rica, 1968.

Adult ratios sterile:normal	Percent egg-hatch during 12 successive weeks[b]												Average % egg-hatch (weighted)
	1	2	3	4	5	6	7	8	9	10	11	12	
0 : 1[c]	100.0 (49)	97.0 (68)	100.0 (58)	100.0 (27)	100.0 (1)	100.0 (5)	100.0 (44)	95.2 (63)	97.8 (89)	95.6 (45)	100.0 (36)	100.0 (18)	98.2 (503)
20 : 1	14.3 (42)	45.9 (74)	18.5 (119)	10.5 (57)	- (0)	0.0 (11)	0.0 (36)	0.0 (12)	0.0 (15)	- (0)	100.0 (25)	100.0 (14)	26.4 (405)
40 : 1	1.2 (25)	20.3 (64)	25.4 (173)	31.6 (38)	100.0 (2)	- (d)	0.0 (7)	0.0 (6)	0.0 (7)	- (0)	- (0)	- (0)	22.9 (322)
80 : 1	1.9 (26)	15.9 (69)	20.2 (108)	15.0 (44)	- (0)	- (d)	0.0 (2)	7.1 (28)	0.0 (39)	- (0)	- (0)	- (0)	12.7 (316)

[a] In each treatment adults were released in a cage erected over 4 coffee trees bearing fruits. For the first 4 releases, sterile flies were irradiated with 7 kr and subsequently with 10 kr.

[b] Figures in parenthesis indicate total number of eggs examined.

[c] Initial release consisted of 20 flies (10 of each sex) per ratio. In subsequent releases the number of flies was reduced by 50%.

[d] Records were not taken.

Table 18. Suppression of reproductive potential of medfly (based on egg-hatchability) by weekly releases[a] of gamma sterilized adults (males and females) in various ratios, Turrialba, Costa Rica, 1969.

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

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

[b] Figures in parenthesis indicate total number of eggs examined.

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

[d] Records were not taken.

Table 19. Mating competitiveness[a] of laboratory reared irradiated (7-10 kr) medflies with untreated wild strain flies in small-cage tests in 2 experiments (1968-1969)

Fly population in cages sterile:normal	Mean % egg-hatch		Av. % egg-hatch for 2 tests	
	1968	1969	observed	expected[b]
0 : 1	98.2	98.7	98.5	-
20 : 1	26.4	-	26.4	5.6
40 : 1	22.9	19.0	21.0	3.4
80 : 1	12.7	13.2	13.0	2.2
120 : 1	-	1.0	1.0	1.8

[a] Prepared from data in Tables 17 and 18.
[b] Based on percent egg-hatch of normal male x normal female = 98.5%, and sterile male x normal female = 1.0%.

males seems to be compensated by their greater number.

e. Effect of gamma sterilization on mating competitiveness and the sexual maturity of the medfly males in the laboratory

Irradiation with 10 kr induces more that 99% sterility in the treated males. As pointed out earlier in this report, irradiated males do not equally compete for mates with normal males (based on egg viability data of normal females). It is suspected that perhaps irradiation delays the sexual maturity of the treated males. The present experiment was carried out with two objectives: 1) to determine in the laboratory the mating competitiveness of males sterilized with different dosages; 2) to find out if irradiation at sterilization and sub-sterilization levels has any effect on the rate of sexual maturity of the treated males.

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

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

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

On the day of emergence, mating pairs were not collected as on this day as soon as males and females were put together (between 3-4 PM) the cages were removed for overnight storage in dark at 25°C in temperature controlled cabinets. During the following three consecutive days, after the day of emergence, each day the mating pairs were collected from 7 AM to 4 PM. Every day, during the rest of the period when flies were not under mating observations (4 PM - 7 AM) cages were stored in

the dark (to prevent mating) at 25°C.

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

Table 20 presents the total number of males of each treatment mated during the first three consecutive days after emergence. The results indicate that the sterilization of the medfly males at pupal stage (24 hr prior to emergence) adversely affects the mating ability of the treated males. Mating competitiveness of the sterile males decreased with the increase of the radiation dose. Males irradiated with dosages of 6, 8 and 10 kr mated 84.9, 66.9 and 57.6%, respectively, as frequently as normal males.

Table 21 presents the hourly mating frequency data of males of each treatment (6, 8, 10 kr and normal) for the first four days of the adult life. No mating took place on the day of emergence and very few matings (maximum of 4 in normal and 6 kr) were recorded from one-day-old males. When males were 2 days old, in all treatments, mating started since early morning and continued until 4 PM with the peak mating frequency occurring between 9 AM and 1 PM. Three day old males started mating immediately at 7 AM when they were brought in the light from the temperature controlled cabinets where they were stored

Table 20. Effect of different sterilization doses on the mating competitiveness of the medfly males[a]

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

[a] Irradiated at pupal stage 24 hr prior to emergence.
[b] Based on total of 9 repetitions from 3 tests (each test with 3 repetitions). In each repetition, 250 virgin normal females were caged with 500 unmated males (125 males of each treatment)
[c] Matings were allowed 7 AM to 4 PM each day. During the rest of the time (4 PM to 7 AM) adults were kept in the dark in incubators at 25°C. No mating occured on day of emergence as males and females were put together between 3-4PM.
[d] Adults emerged between 5-10AM and no mating occurred on the day of emergence.

in the dark to prevent unobserved matings. In all the treatments by this time all the males were fully sexually matured as on this day the highest mating frequency was recorded during the first four hr of the observations, i.e. 7-11 AM.

The results indicate, therefore, that sterilization up to 10 kr does not delay the maturity rate of treated males. The mating frequency pattern of irradiated (6, 8 and 10 kr) males is similar to that of normal males. Normal as well as sterile males become sexually mature 2 days after emergence. On the third day of adult life, when, for the first time, the majority of males had reached sexual maturity, 41.2% normal male matings took place compared to 48.9% 10 kr sterile male matings on the same day. On this very day, during peak mating period, i.e. 9 AM to 1 PM, the hourly mating frequency of 10 kr

Table 21. Mating competitiveness data of normal and irradiated (6, 8 and 10 kr) medfly males simultaneously confined with normal females during 3-day period after emergence

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

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

[b] Ca. 12 hr old males.

[c] No mating pairs observed since females were added to males between 3-4 PM.

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

[e] Each day between 4 PM and 7 AM flies were kept in dark in incubators at 25°C.

sterile males varied 6.5% to 11.8% compared to 6.2 to 9.7% mating frequency of normal males recorded during the same period.

f. Effect of gamma irradiation on mating competitiveness and sexual maturity of the medfly males in small field cages

As we reported previously (section e of this report), 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. The previous laboratory experiment was repeated in the field in large cages (9'x9'x7') 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 AM - 3 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 8-8:30 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 AM - 4 PM), second day for 8 hr (7 AM - 3 PM) and the third day for 4 1/2 hr (7 AM - 11:30 AM). An attempt was not made to collect mating pairs before 7 AM. Preliminary observations made before actual tests, indicated that medflies do not mate until after 7 AM under our outdoor conditions. The roof of the cage was covered

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

Table 22 presents the total number of males of each treatment mated during a 3-day period. 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 22. Effect of different sterilization doses on the mating competitiveness of the medfly males[a]

Treatments	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'x9'x7') with 500 unmated males (125 males of each treatment).

Table 23 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 upto 10 kr, does not delay the rate of sexual maturity of treated males. Throughout the test period of three days, the hourly

Table 23. 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			
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'x9'x7') 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 parenthesis are total number of matings for which percentages are calculated.

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. 12. 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 slightly 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.

g. Effect of gamma sterilization on the mating duration of the medfly males

Sterile medfly males do not compete equally in mating with normal males (based on egg viability data of normal females caged together with normal males and sterile males). It is suspected that perhaps during matings sterile males transfer fewer sperm than normal males. The low volume of sperm transfer affected by sterile males may be the results of relatively shorter mating duration of sterile males as compared to that of normal males. The present experiment was designed to find out if irradiation of the medfly males at various sterilization levels affects the mating durations of the treated males.

Effects of three sterilization dosages (6, 8, and 10 kr) on the mating durations of the males was compared with untreated males. The adults used in the experiment were irradiated at

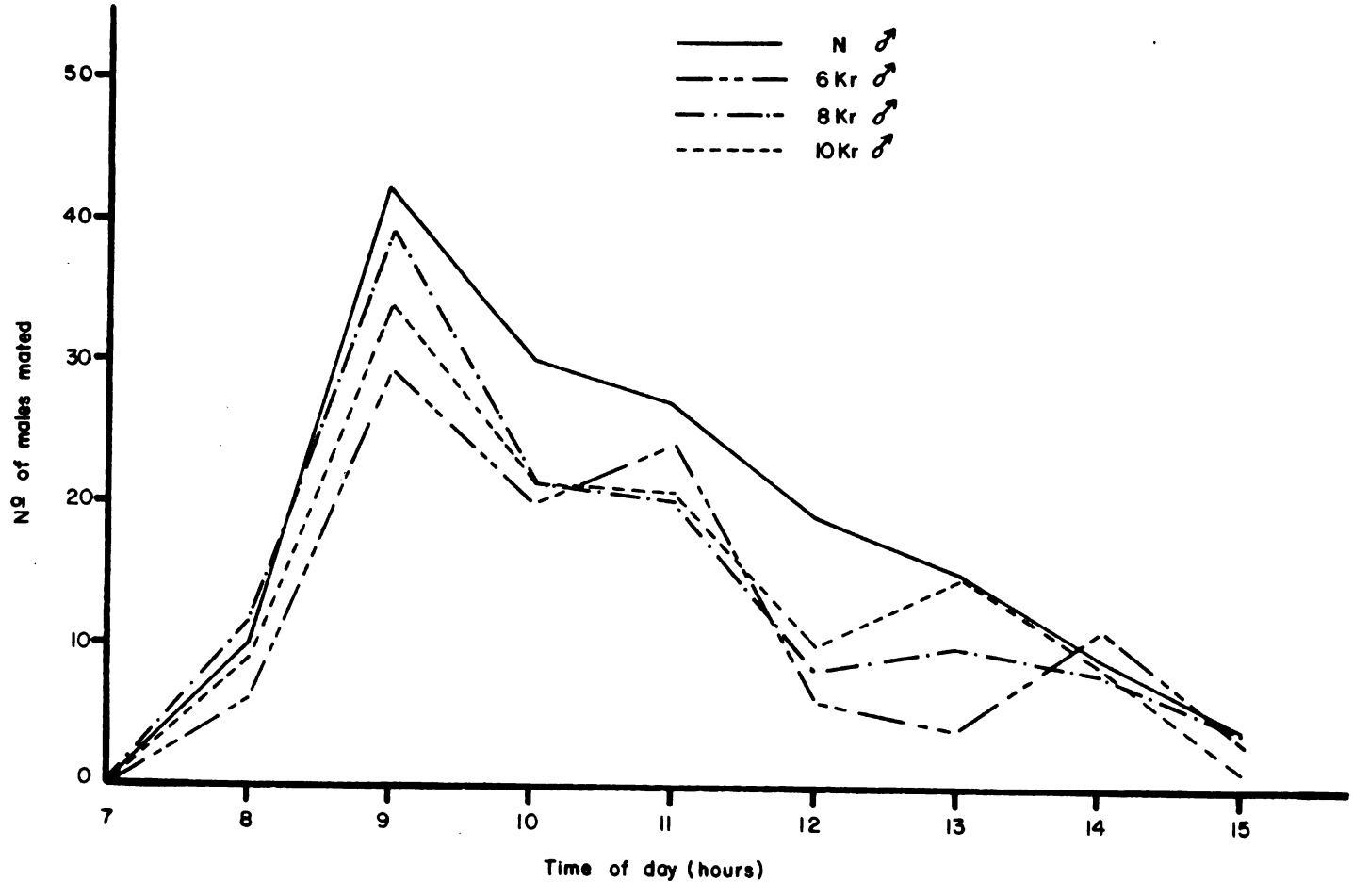


Fig.12 Mating frequency pattern of 3 day old normal and irradiated Medfly males

pupal stage (24 hr before emergence). The flies used for matings were sexed out within 12 hr after emergence.

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

Number of fly pairs set for mating varied 40-60 per treatment in different tests. The experiment was repeated on four different days, each day with a different batch of adults. The age of the flies used in all the tests varied from 3-6 days. Adults were not provided food or water while confined in mating jars. All the tests were carried out in the laboratory at temperatures ranging from 23-27°C and relative humidity varying from 70-80%.

Table 24 presents the average mating durations of the untreated and treated (6, 8, or 10 kr) males. The data indicate that sterilization of the medfly males upto 10 kr did not reduce significantly the mating duration of the treated male. Average mating duration of males irradiated with 10 kr was 140 minutes compared to 153 minutes of average mating duration of untreated males. Maximum mating duration found was 277 min (4 hr 37 min) in normal male while 10 kr males had a maximum mating duration

Table 24. Mating durations of medfly males irradiated with different doses as pupae (24 hr prior to emergence)

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

[a] Age of males used in the experiment varied 3-6 days.
[b] Figures in parenthesis are number of males on which mating durations are based.

of 230 min (3 hr 50 min). It appears, therefore, that under the present experimental conditions sterilization does not adversely affect the mating duration of the treated males upto 10 kr.

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

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

if sterile male matings transfer fewer sperm compared to normal male matings and whether receptivity of females is controlled by the amount of sperm received by the females from the first mating.

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

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

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

category 'very few' with rating of 1 hod only traces of sperm. The fifth category consisted of no sperm and was given zero rating.

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

Table 25 presents the summarized data from five experiments on the amount of sperm present in the spermathecae of the females re-mated or refused second mating by normal or sterile male during a 9-hr test period (7 AM - 4 PM). The results indicate that sterilization with 10 kr does not reduce the sperm transfer capacity of treated males, at least in the first mating. The sterile males, like the normal males, transfer a good amount of sperm in most of the matings. An equivalent amount of sperm is transferred by normal as well as sterile male. Sperm content ratins of females mated once to normal and irradiated males were 3.4 and 3.2, respectively.

The sperm content ratings of the females which accepted second matings were very similar to those which refused second mating. This indicates that receptivity of females was not controlled by the amount of sperm present in the spermathecae

Table 25. Sperm contents in the spermathecae of the medfly females remained receptive or non-receptive immediately after first mating with irradiated[a] (10 kr) or untreated males

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

[a] Treated with 10 kr as pupae, 24 hr before emergence
[b] Figures in parenthesis are number of females observed
[c] Include females which after first mating re-mated but in second mating the couples were forcibly separated soon after initiation of mating to prevent sperm transfer from second mating.

of the females. The sperm content ratings for the females re-mated were 2.9 (normal mating) and 3.3 (sterile mating) compared to ratings of 3.4 and 3.2 of those females which refused second mating with normal and sterile males, respectively. More experiments will be carried out to determine the cause of receptivity in medfly females.

i. Induction of visible medfly mutants by gamma radiation

In an effort to breed visible medfly mutants which can be used in sterile male release program, initially males were treated with ethyl methanesulfonate (EMS). But we were unable to get successful results. Therefore, since last year use of EMS was discontinued and gamma irradiation has been employed to obtain visible medfly mutants.

The test procedure is as follows: approximately 300, 2 or 3 day old unmated males were irradiated with 2500 r (inducing about 50% sterility in treated males). The adults were irradiated in a pool-type Cobalt-60 source at a dose rate of 1263 r/m. Three to four days after sterilization, treated males were allowed to mate in mass with untreated virgin females of the same age. One hundred mating pairs were removed and placed individually in cages for oviposition to obtain F_1 flies. As soon as mating couples separated, the males were killed in order to utilize the sperm from first mating only. F_1 and F_2 flies of a single pair were allowed sib matings in mass. F_3 adults were examined for visible mutants.

Because of longer duration of the tests and limited available assistance, so far we have been unable to obtain good visible mutants.

References

1. Fried, M., 1971. Determination of sterile-insect competitiveness. J. Econ. Entomol. 64:869-872.

2. Hooper, G.H.S., 1971. Competitiveness of gamma-sterilized males of Mediterranean fruit fly: effects of irradiating pupal or adult stage and of irradiating pupae in nitrogen. J. Econ. Entomol. 64:1364-1368.

3. Hooper, G.H.S. and Katiyar, K. P., 1971. Competitiveness of gamma-sterilized males of the Mediterranean fruit fly. J. Econ. Entomol. 64:1068-1071.

4. Katiyar, K. P. and Valerio, J., 1964. Effect of pupal irradiation on the sexual vigor of the male medfly. In The Application of Nuclear Energy to Agriculture. Annual Report 1964. Turrialba, Costa Rica, IICA. pp. 51-56.

5. Katiyar, K. P. and Valerio, J., 1964. Further studies on the possible use of sterile-male release technique in controlling or eradicating the Mediterranean fruit-fly, Ceratitis capitata Wied. from Central America. In Inter-American Symposium on the Peaceful Application of Nuclear Energy, 5th, Valparaiso, Chile, 1964. Washington, D. C. PAU. pp. 197-202.

6. Katiyar, K. P. and Ramirez, E., 1970. Sterilization of the Mediterranean fruit fly and its application to fly eradication. In The Application of Nuclear Energy to Agriculture. Annual Report 1970. Turrialba, Costa Rica, IICA. pp. 19-46.

7. Ohinata, K., Chambers, D. L., Fujimoto, M., Kashiwai S. and Miyabara, R., 1970. Sterilization of the Mediterranean fruit fly by irradiation: comparative mating effectiveness of treated pupae and adults. J. Econ. Entomol. 64: 781-784.

8. Peleg, B. A. and Rhode, R. H., 1970. New larval medium and improved pupal recovery method for the Mediterranean fruit fly in Costa Rica. J. Econ. Entomol. 63:1319-1321.

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

(K. P. Katiyar, E. Ramirez and J. A. Reyes)

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 non-chemical 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 present report summarizes the research carried out under this project during July 1969 through June 1972.

a. Effects of sterilization on fertility, fecundity, and longevity of the coffee leaf miner adults.

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 (3). 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 and 8 AM. Full grown larvae left the mines and pupated on these leaves. The twigs were removed at 5 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 hr. 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.

Radiation effects on adult fertility

The results of gamma irradiation on fertility of the male coffee leaf miner (treated as late stage pupae or as newly emerged moths) are presented in Table 26 and Fig. 13. The results indicate that there is no difference in radiation sensitivity of males irradiated either as pupae or as adults.

The percent egg-hatch from females mated to males irradiated during pupal or adult stage is similar at every radiation level tested (upto 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 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

Table 26. Fertility of male coffee leaf miner, Leucoptera coffeella (Guerin-Meneville) 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

coffee leaf miner irradiated either as late-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 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

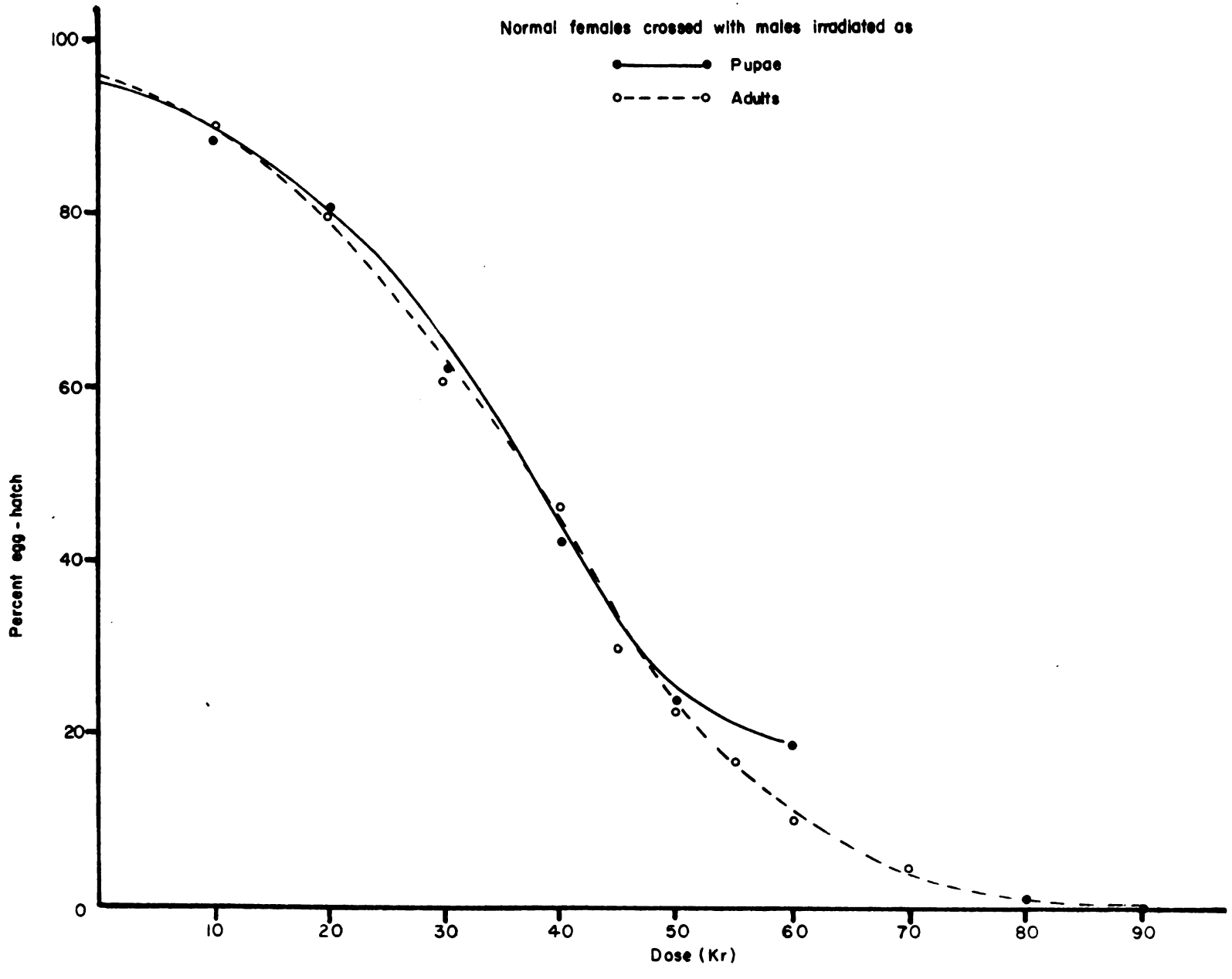


Fig.13 Effects of gamma irradiation on fertility of coffee leaf miner males

(21-14 hr before emergence) or as adults (15-21 hr after emergence) are presented in Table 27 and Fig. 14. The dose-response curve for induced sterility in females also seems to be sigmoidal (Fig. 13). 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. 14). Percent egg-hatch (Table 27) of pupal irradiation is comparable to that of adult irradiation at every dose level tested (1-40 kr).

Table 27. Fertility[a] of female coffee leaf miner, Leucoptera coffeella (Guerin-Meneville) 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

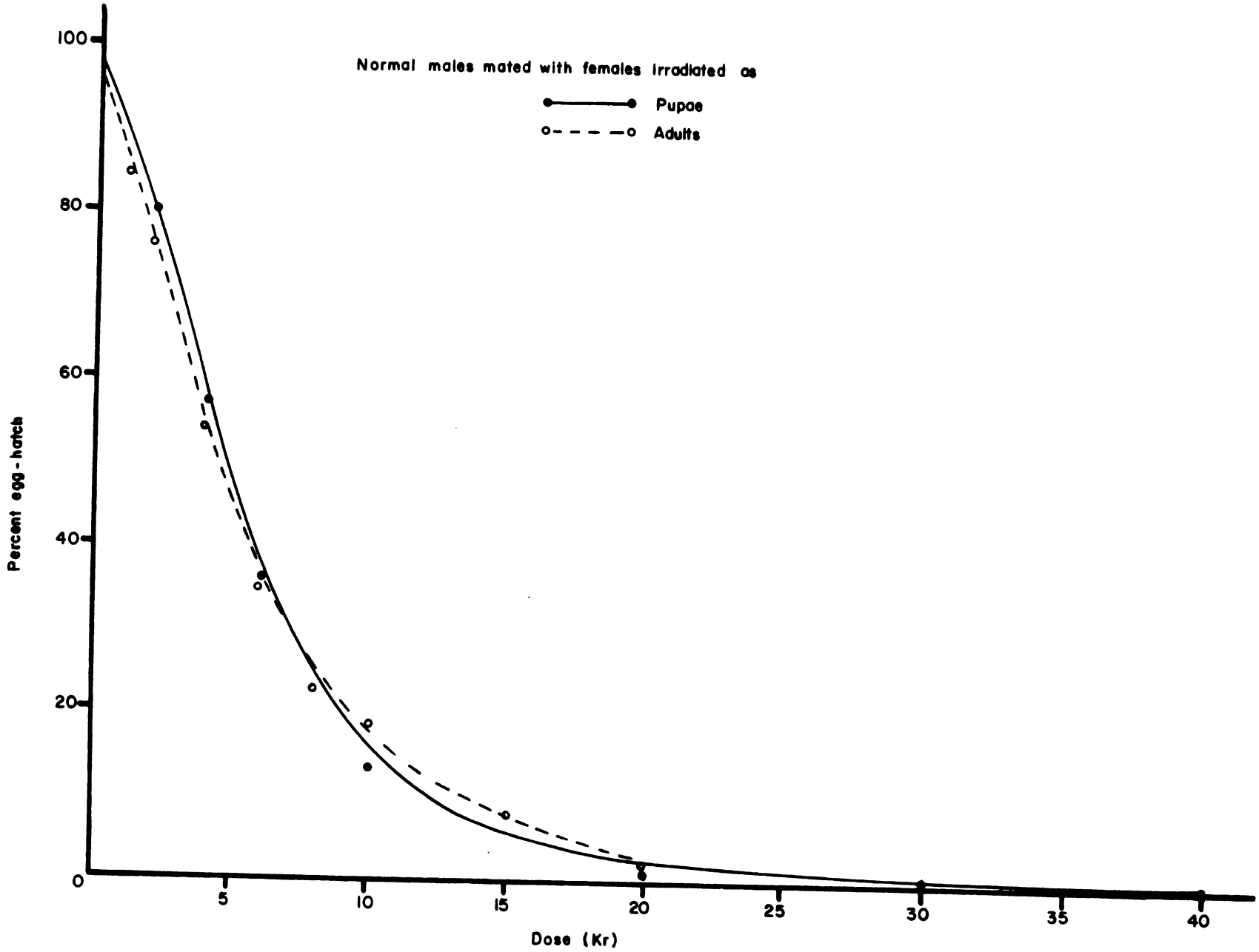


Fig.14 Effects of gamma irradiation on fertility of the coffee leaf miner females

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 induced very little sterility in males (Table 26: 88.8 ±1.42 hatch in pupal irradiation and 90.0±0.82% hatch in adult radiation) and a high degree of sterility (Table 27: 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 28 and Fig. 15 present the fecundity of untreated females 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

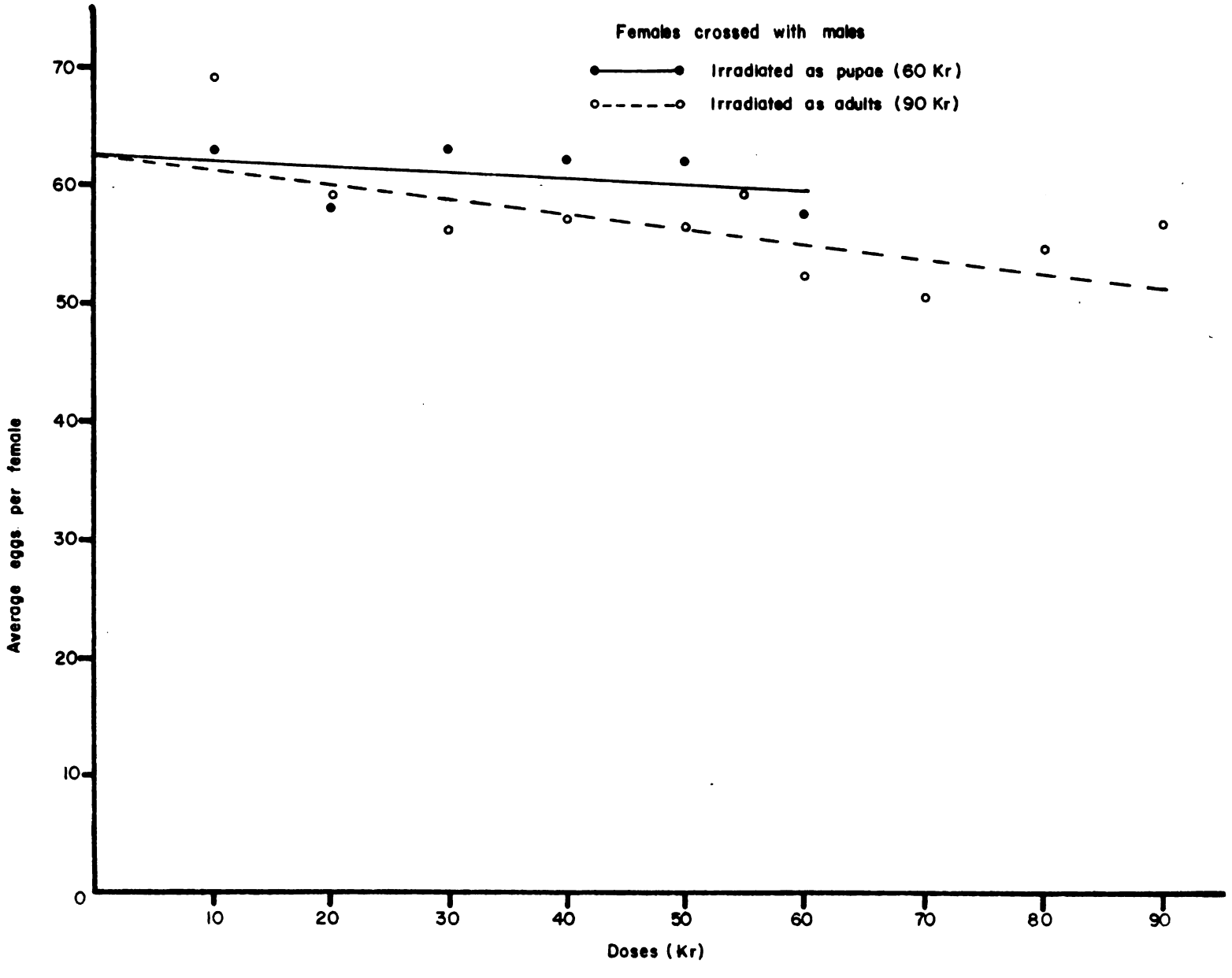


Fig. 15 Fecundity of normal coffee leaf miner females crossed with irradiated males

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 29 and Fig. 16. The results suggest that an increase in irradiation dose caused a decrease in oviposition capacity of the treated females.

Table 28. Fecundity of untreated female coffee leaf miner, Leucoptera coffeella (Guerin-Meneville) 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

Table 29. Fecundity of female coffee leaf miner, Leucoptera coffeella (Guerin-Meneville) irradiated as pupae or as adult and crossed with untreated males

Dose in kr	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

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

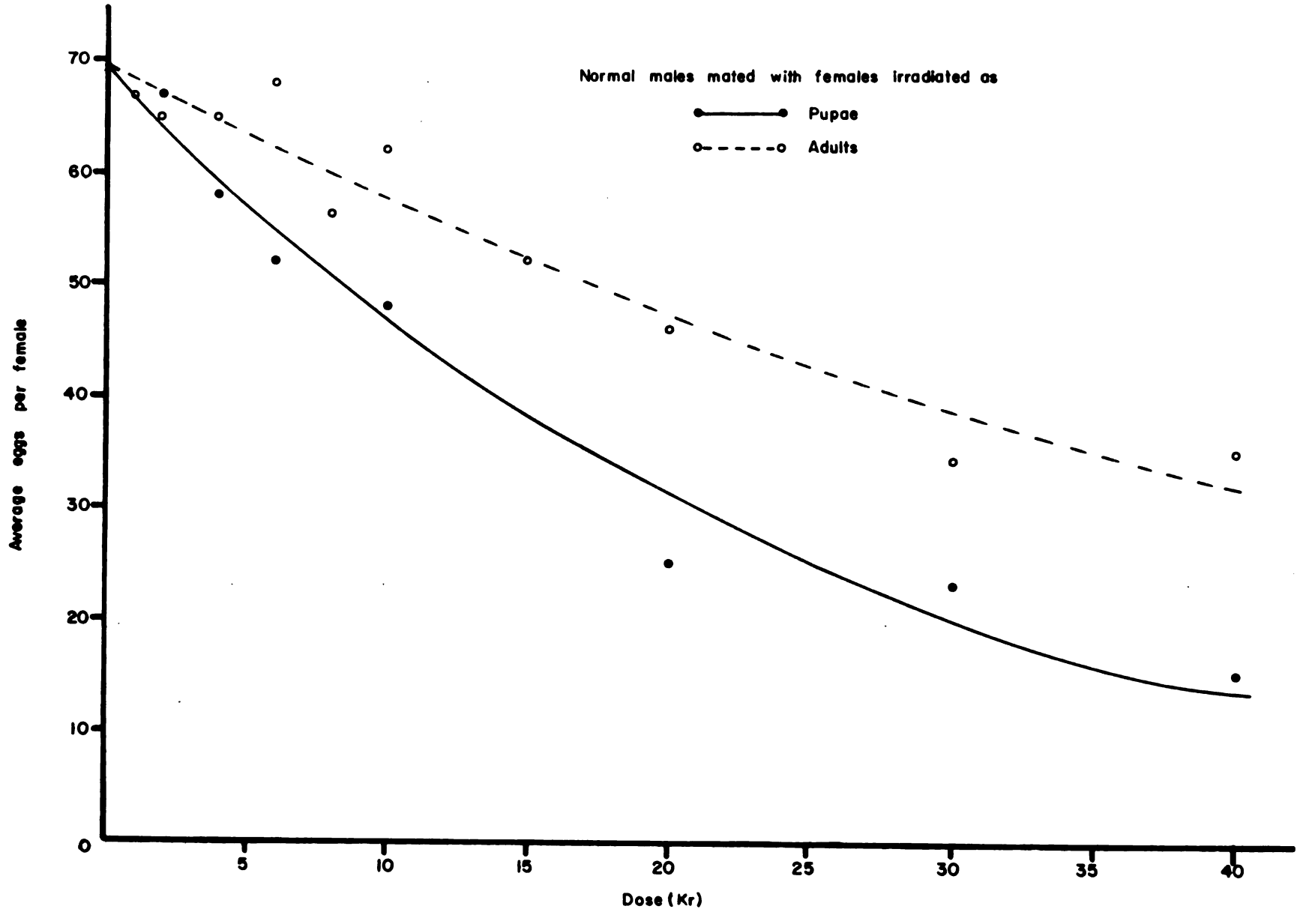


Fig. 16 Fecundity of irradiated coffee leaf miner females

Table 30. Longevity of male coffee leaf miner, Leucoptera coffeella (Guerin-Meneville) irradiated as pupae or as adults and crossed with untreated females

Doses (kr)	$T_{50} \pm S\,d$ (in days[a]) after irradiation Pupae[b]	Adults[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

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 Figs. 17, 18, 19 and 20. Tables 30 and 31 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 life span of irradiated males was significantly shortened (at 5% level) at all the doses tested (Table 30). 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

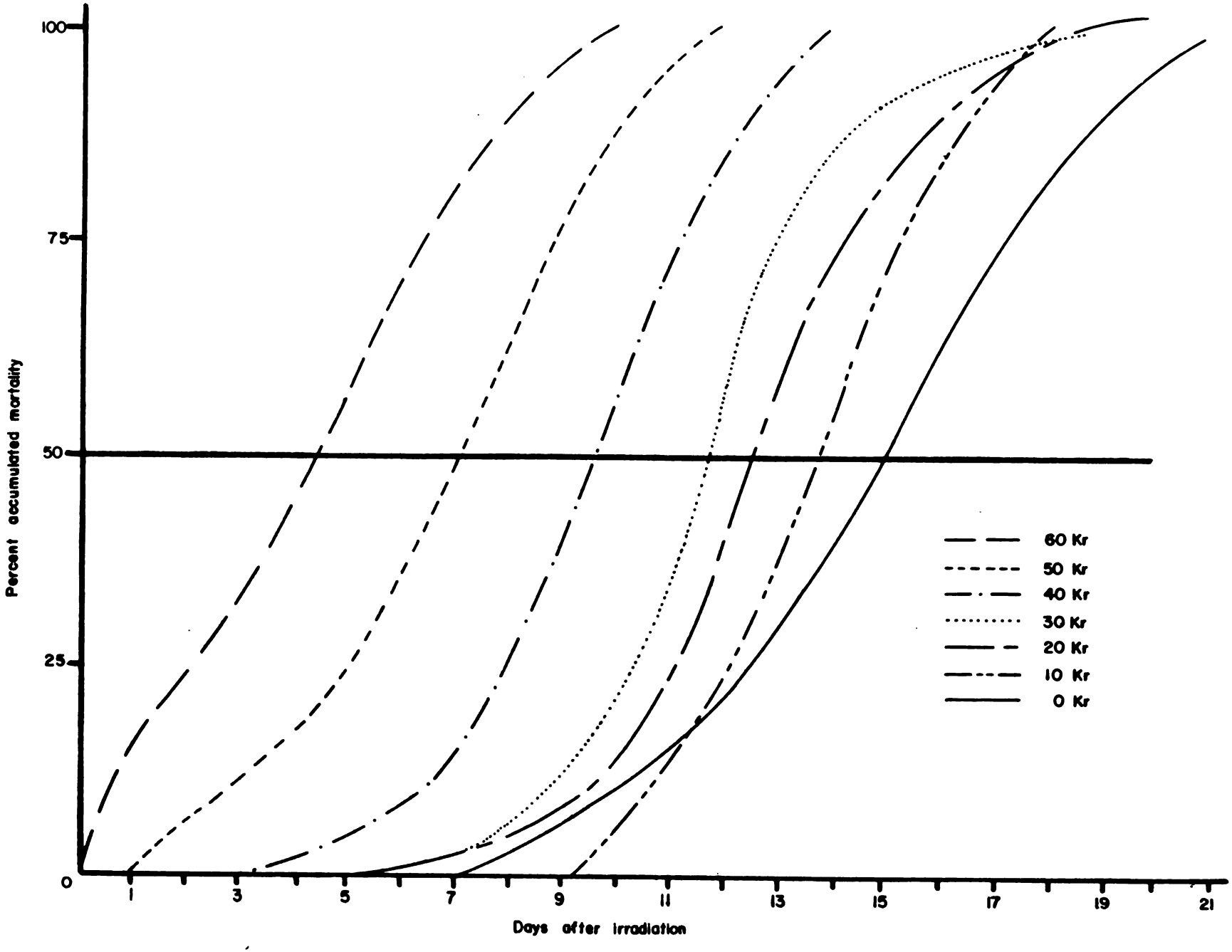


Fig. 17 Accumulated mortality rates as a function of time in coffee leaf miner males irradiated as pupae

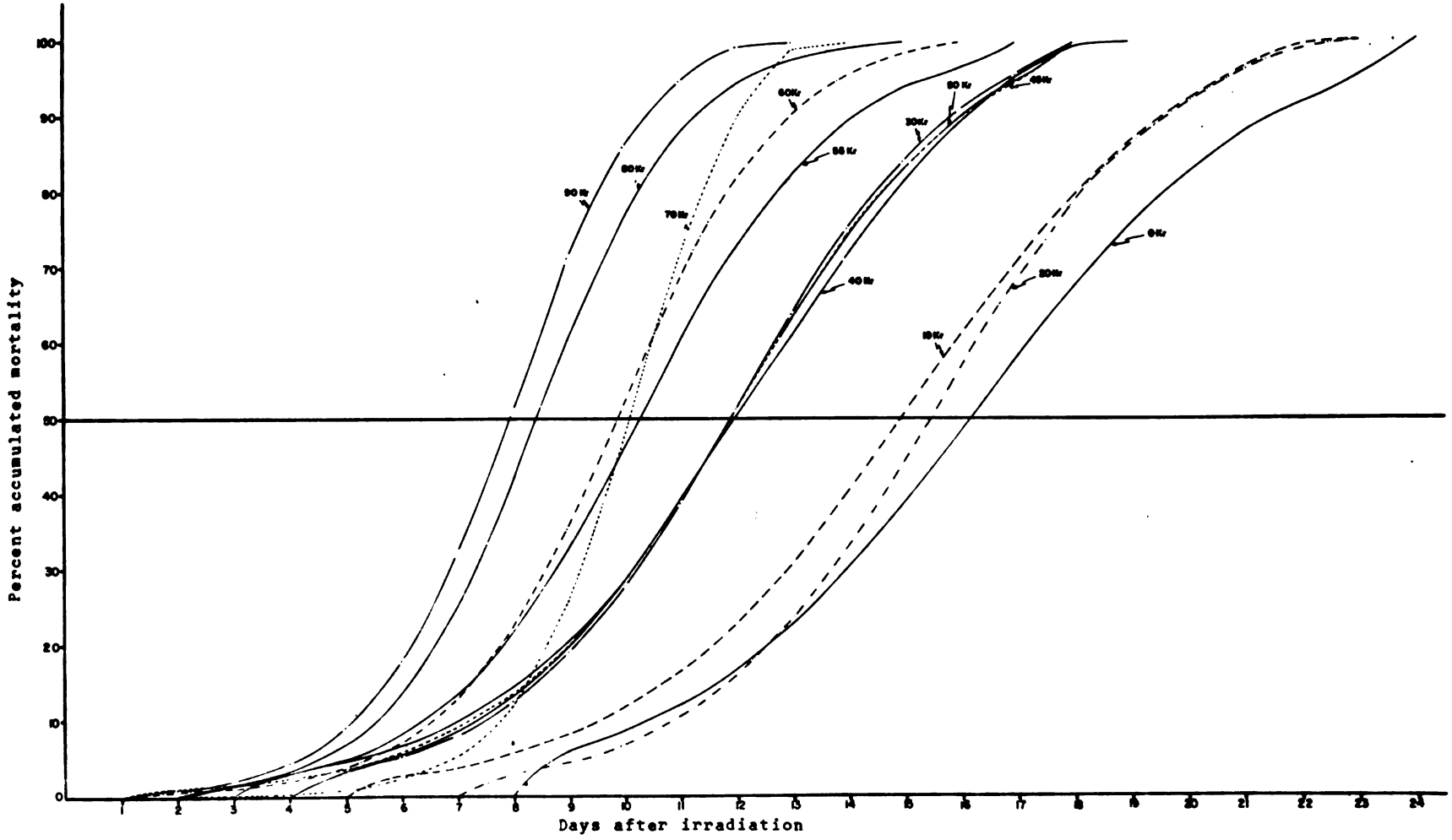


Fig. 18 Accumulated mortality rates as a function of time in coffee leaf miner males irradiated as adults

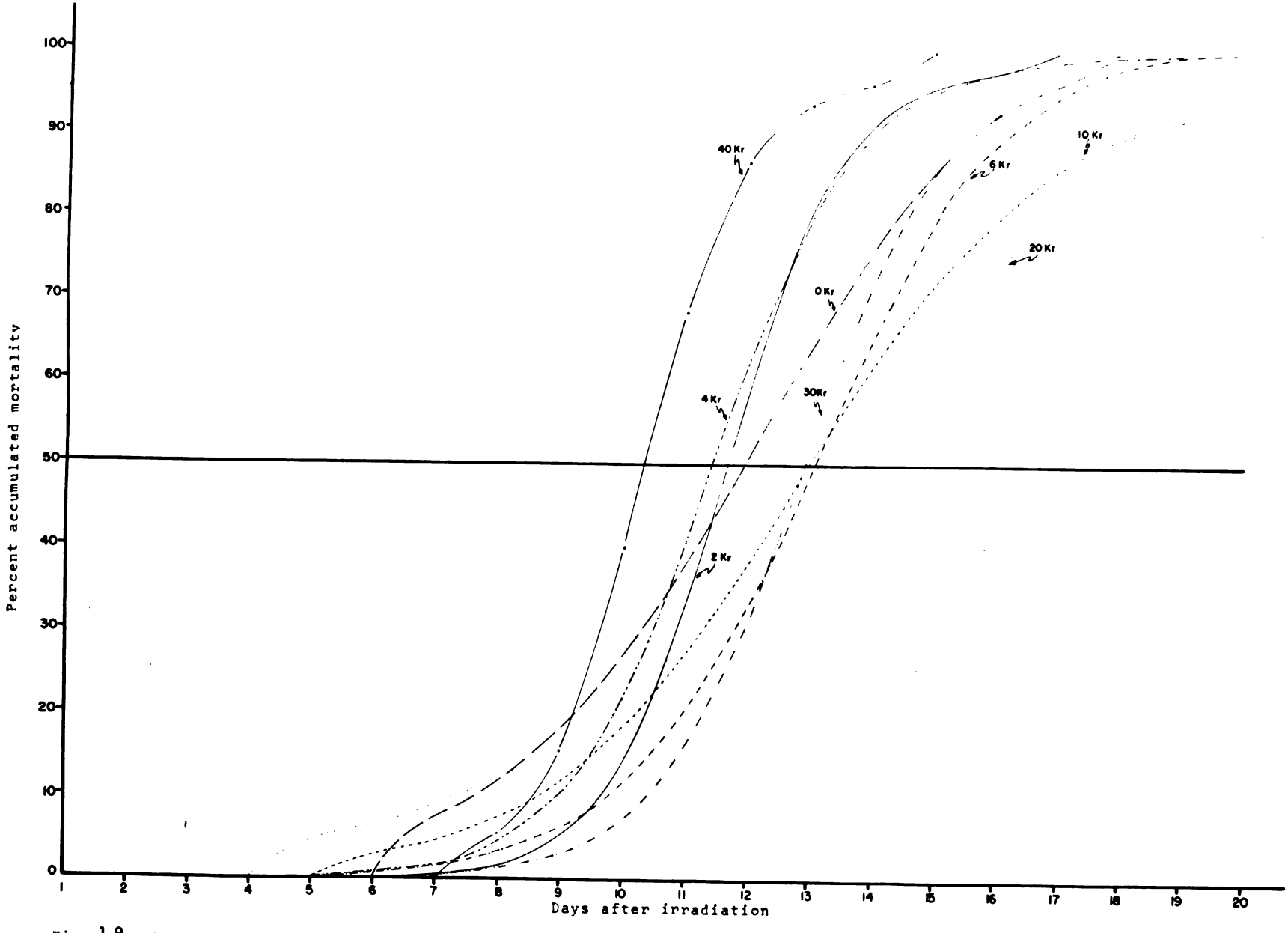


Fig. 19 Accumulated mortality rates as a function of time in coffee leaf miner females irradiated as pupae

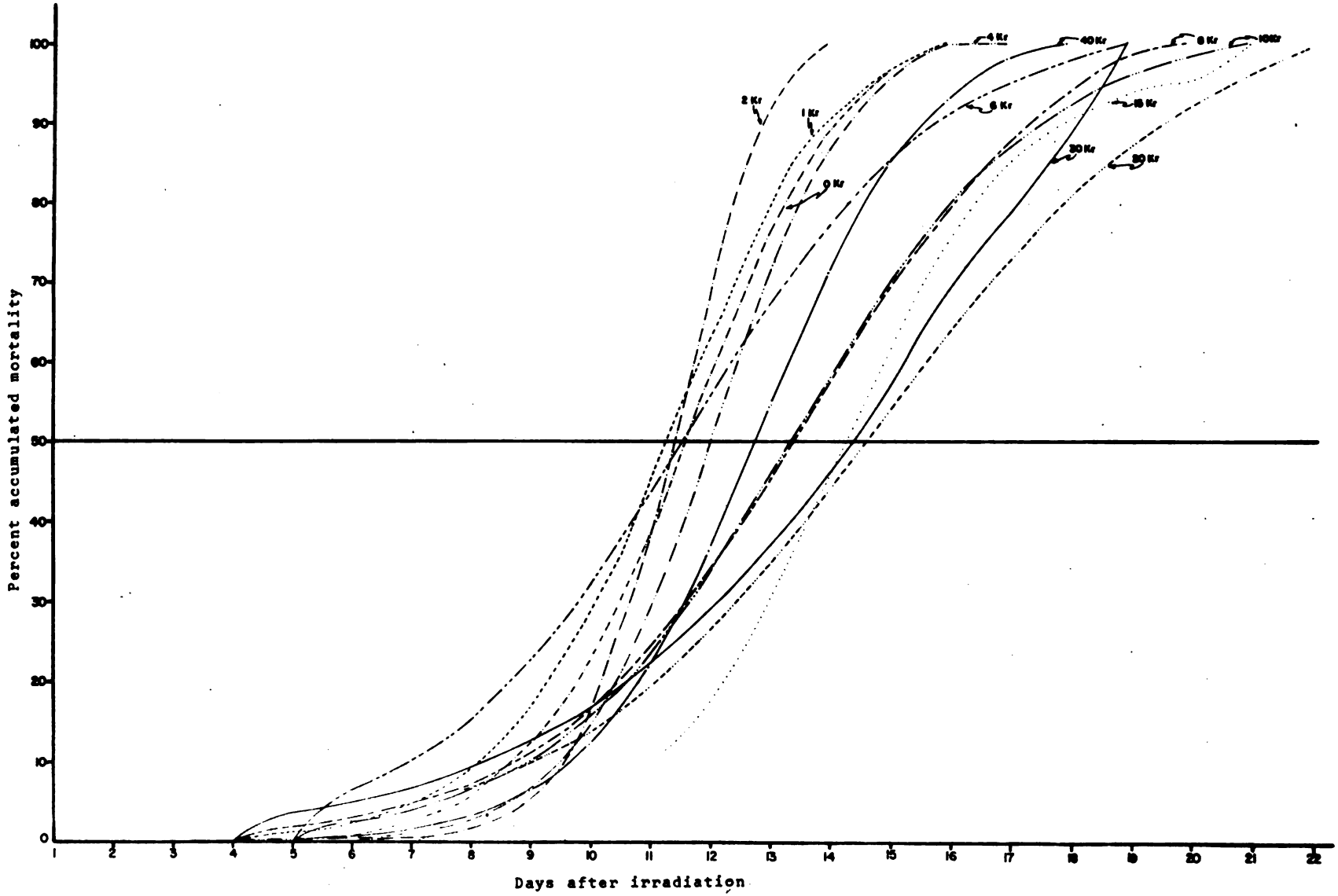


Fig. 20 Accumulated mortality rates as a function of time in coffee leaf miner females irradiated as adults

Table 31. Longevity of female coffee leaf miner, Leucoptera coffeella (Guerin-Meneville) irradiated as pupae or as adult and crossed with untreated males

Dose in kr	$T_{50} \pm$ 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

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 31). Life span 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) (1) and Lespeyresia (=Carpocapsa) pomonella (L) (8).

b. Effect of sterilization on the mating vigor of the treated males

In insect control by the sterile male technique it is vary 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.

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 26).

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 socred as inseminated. Each surviving male

was provided with five fresh virgin females for another 24 hr period. This procedure was repeated until all 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.

Table 32 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 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.

c. Competitiveness of gamma-sterilized males of the coffee leaf miner, irradiated as adults

We had reported earlier that a dose of 90 kr applied to newly emerged moths, induces more that 99% sterility in the treated males. However, this irradiation adversely affects the

Table 32. 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

sexual vigor of treated males. Insemination capacity of irradiated males was reduced to only 1/7th that of untreated males. The present experiment was carried out to evaluate the mating competitiveness (based on egg-hatch suppression of normal females) of 90 kr irradiated males at a dose rate of ca. 1250 r/m.

Irradiation effect on the mating competitiveness of sterile males was studied at 3 different ratios: 1:1:1, 2:1:1 and 3:1:1 (sterile male:normal male:normal female). The tests included two controls to check the fertility of irradiated and non-irradiated males. Each cage had 10 females per ratio.

Daily egg collection from each cage was made for eight consecutive days following crosses (adults were paired immediately after irradiation). The eggs were incubated 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 experiment was replicated five times, each replication with different batches of insects. All the tests were carried out in the laboratory at 25±3°C temperature and 75±5% relative humidity.

Table 33 presents the egg-hatch obtained when male coffee leaf miner moths irradiated as newly emerged adults were combined with untreated males and females at 1:1:1, 2:1:1 and 3:1:1 ratios. The results show that irradiation drastically reduced the mating competitiveness of treated males. Irradiated males were effective in suppressing egg-hatch of normal moths only 9.0 to 11.7%.

Table 33. Mating competitiveness (based on egg-hatch) of coffee leaf miner males irradiated with 90 kr, as newly emerged moths

Adult ratios[a]	% egg-hatch[b] in different replications					Average % egg-hatch
	1	2	3	4	5	
0:1:1	93.4 (321)	85.8 (402)	83.2 (719)	91.4 (290)	86.1 (381)	88.0
1:0:1	0.0 (478)	0.5 (215)	0.0 (493)	0.0 (146)	0.3 (331)	0.2
1:1:1	77.1 (441)	86.2 (406)	82.0 (483)	77.0 (366)	71.6 (190)	78.8
2:1:1	82.2 (465)	76.4 (444)	77.0 (478)	75.3 (376)	83.6 (226)	79.0
3:1:1	55.4 (388)	81.0 (289)	71.2 (396)	85.4 (403)	88.5 (165)	76.3

[a] Treated males:normal males:normal females. 10 females per ratio.
[b] Figures in parenthesis are total number of eggs examined on which percentages are based.

The maximum egg-hatch reduction of 11.7% (88.0% in check to 76.3% in treatment) was found at a ratio of 3:1:1. The reason for drastically reduced mating competitiveness of irradiated males is not known. We have reported earlier that newly emerged moths irradiated with 90 kr when caged alone with normal females, mate and transfer sperm. On an average, five females were inseminated by each irradiated male during his life period.

It appears, therefore, that perhaps reduction in mating competitiveness (based on egg-hatch when normal and treated males are competing for mates) of irradiated males is due to adverse effect of high sterilization dose (90 kr) on mating ability and sperm activity of treated males.

d. Inherited sterility in the progeny of irradiated male coffee leaf miner: effects on fertility, fecundity, longevity, larval and pupal mortality and adult sex ratio of F_1 moths

Like other Lepidoptera, the coffee leaf miner is resistant to gamma irradiation when sterility is used as a criterion to measure the sensitivity. As reported earlier (Table 26) 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 32).

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. (7), the cabbage looper, Trichoplusia ni (Hübner) (4), the sugar cane borer, Diatraea saccharalis (Fab.) (9), the tobacco budworm, Heliothis virescens (F.) (6) 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 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 (3).

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 $26\pm3°C$ and relative humidity of $75\pm5\%$. 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.

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 34. 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 35 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

Table 34. Fertility of F_1 coffee leaf miner adults obtained from crossing normal females with irradiated males

Dose to $P_{1\sigma}$ [a] (kr)	% egg-hatch $P_{1\sigma} \times N_{\varphi}$	% egg-hatch [b] ± S d		
		$F_{1\sigma} \times N_{\varphi}$ [c]	$F_{1\varphi} \times N_{\sigma}$ [c]	$F_{1\sigma} \times F_{1\varphi}$ [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)
45	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 parenthesis 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.

approximately 40% in all the treatments and by about 75% in F_1 females resulting from parent males treated with 60 kr.

Table 36 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 recued longevity of F_1 moths of both sexes. Maximum reduction (approximately 17% in males and 25% in females) in life span 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 37 indicate that post-

Table 35. 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 36. 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.

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 37. 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.

Sex ratio of F_1 progeny

As reported previously considerable mortality of F_1 progeny occurs in the larval stage (Table 37). We did not determine the sex of the dead larvae and pupae in Table 37. 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 38 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.) (7), the tobacco budworm, Heliothis virescens (F.) (6), navel orangeworm, Paramyelois transitella (Walker)(2) and the cabbage looper, Trichoplusia ni (Hübner)(5).

Table 38. Ratio of F_1 males:F_1females coffee leaf miner when P_1 males were treated with 0, 30, 45 or 60 kr gamma irradiation

Dosis to $P_1\sigma$ [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.

e. Further studies on induced F_1 sterility among progeny of male coffee leaf miner treated with gamma irradiation

Since, in our previous experiments, 30 kr (lowest dose studied) given to parent male, produced more than 99% sterility in F_1 progeny. Studies, therefore, were further carried out to determine the minimum possible irradiation dose applied to males necessary for obtaining F_1 progeny with 5-10% residual fertility. In the present experiment, sterilization doses of 25, 20 and 15 kr were tested.

Fertility of F_1 progenies derived from parent males (P_1) irradiated with 15, 20 or 25 kr are presented in Table 39. The preliminary results indicate that the inherited F_1 sterility can be induced in coffee leaf miner males at very low sub-sterilization doses (20 or 25 kr) given to the parent male. When the parent males were treated with 20 or 25 kr, egg-hatch of crosses between F_1 male and normal female, were 0.0 and 0.5%, respectively. The results from previous tests show that fertility of P_1 males irradiated with 20 kr was 80.1%. Irradiation of parent

Table 39. Fertility of F_1 coffee leaf miner adults obtained from crossing normal females with irradiated males

Dose to $P_1♂$ [a] (kr)	Percent egg-hatch[b]		
	$F_1♂ \times N♀$	$F_1♀ \times N♂$	$F_1♂ \times F_1♀$
0	85.7 (721)	---	---
15	33.4 (610)	33.8 (654)	21.2 (85)
20	0.0 (347)	12.2 (701)	3.8 (160)
25	0.5 (370)	---	---

[a] P_1 male (parent) irradiated as newly emerged adult
[b] Number in parenthesis are total eggs on which percentages are based.

male with 15 kr produces sub-sterile progeny (33.4% fertility in F_1 males and 33.8% fertility in F_1 females).

Thus 20 kr appears to be the practical lowest dose for obtaining completely sterile F_1 males and sub-sterile (12.2% fertility) F_1 female coffee leaf miner moths. However, more experiments will be carried out to confirm these results.

f. Mating competitiveness of F_1 males

A dose of 90 kr (applied to newly emerged adults) is required to induce more than 99% sterility in male coffee leaf miner (Table 26). This sterilization dose seriously reduces the insemination ability of treated males (Table 32). 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 34). 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.

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 40. It seems that 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

Table 40. 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

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.

g. Further studies on mating competitiveness of F_1 males obtained from partially sterilized. P_1 males.

Since reasonably high sterility can be induced in F_1 progenies arising from parent males receiving less than 30 kr (Table 39). Reduction in sterilization dose of parent male might further increase the mating competitiveness of resulting F_1 moths. The present experiment was carried out to determine

the mating competitiveness of F_1 males obtained from parent males treated with very low sterilization doses (15, 20 or 25 kr). The preliminary results of this study are presented in Table 41.

Table 41. Mating competitiveness (based on egg-hatch) of F_1 male coffee leaf miner when P_1 males received 15, 20 or 25 kr of gamma irradiation

Adult ratio[a] $F_1♂ : N♂ : N♀$	% egg viability[b] of mixed population of N♂, N♀ and $F_1♂$ when P_1 males were treated with		
	15 kr	20 kr	25 kr
0 : 1 : 1	96.8 (124)	96.8 (124)	84.4 (616)
1 : 0 : 1	27.1 (199)	0.0 (197)	0.5 (370)
1 : 1 : 1	84.4 (122)	88.1 (412)	79.3 (600)
2 : 1 : 1	75.2 (113)	78.5 (326)	83.9 (409)
3 : 1 : 1	62.7 (416)	58.8 (306)	69.7 (518)

[a] Ten females per ratio
[b] Number in parenthesis are total eggs on which percent egg-hatch is based.

Results in general indicate that F_1 coffee leaf miner males do not compete satisfactorily with normal moths for mates. The maximum egg-hatch suppression of 38.0% (96.8% in check to 58.8% in treatment) was found in the 3:1:1 ratio of F_1 males obtained from P_1 males irradiated with 20 kr. Next year we plant to carry out detailed experiments to evaluate the potential use of inherited F_1 sterility in coffee leaf miner control.

References

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

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

3. Katiyar, K. P. and Ferrer, F., 1968. 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, IAEA. pp. 165-175.

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

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

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

7. Proverbs, M. D. and Newton, J. R. Some effects of gamma radiation on the reproductive potential of the Codling moth, Carpocapsa pomonella (L.) (Lepidoptera:Olethreutidae). Con. Entomol. 92:1162-1170. 1962.

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

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

3. Biology and Sterilization of the Shootborer, Hypsipyla grandella Zeller (Lepidoptera:Phycitidae) (K. P. Katiyar)

Due to unforseen circumstances, we were unable to start this project at the beginning of last year. However, during

the past year, considerable progress has been made in the rearing of this insect on an artificial diet in the laboratory. We have modified the basic corn earworm, Heliothis zea, diet for rearing H. grandella. Larvae reared individually in 1 oz glass jars on this diet grow normally and produce normal sized pupae (average weight for pupae being ca. 210 mg for males and 250 mg for females). Larval mortality due to contamination of the diet by bacteria, fungi, etc., is less than 10%.

The ingredients of the modified Heliothis zea diet used for rearing H. grandella larvae are:

Carrot powder	50	g
Acemite	75	g
Soybean powder	35	g
Testone S-150	20	g
Sugar	80	g
Agar	10	g
Alfacel	15	g
Salts Wesson	5	g
Vitamin mixture (NBC)	10	g
Methyl-p-hydroxybenzoate	1	g
Sorbic acid	0.5	g
Acetic acid (25%)	10	ml
KOH (4M)	5	ml
Formaldehyde (10%)	5	ml
Aureomycine (1000 mg/120 ml H_2O)	5.5	ml
Water	650	ml

With improvements in the rearing and oviposition technique, work will be initiated next year on the effect of gamma irradiation on fertility, fecundity, sexual competitiveness and longevity of this insect.

C. RADIOBIOLOGY IN INSECT PATHOLOGY

1. Pathological Control of Insect Pests

a. Susceptibility of *Hypsipyla grandella* Zeller to the fungus *Metarrhizium anisopliae* (Metch.)

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. 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.

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 nycellium pierces out of their bodies through the spiracles and prolegs. This growth soon covers the cadaver, and later on takes on a green color, characteristics of the spores of this fungus. The whole larva becomes a mass of spores which disintegrates with the slightest touch.

Under conditions of the experiment 50% of the larvae were killed by the fungus (Table 42). 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. 23) with a range of four to nine days. Natural mortality usually extended over the entire larval period.

Table 42. 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

Total mortality is shown in Fig. 24. The mortality of the control larvae was 13% and reflects causes other than the fungus under study. The cummulative 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, B. bassiana being more effective that B. tenella but less than M. anisopliae.

References

1. Berrios, F. and Hidalgo-Salvatierra, O., 1971. Estudios sobre el barrenador de las Meliáceas, Hypsipyla grandella Zeller. VI. Suscepbitilidad de la larva al hongo Metarrhizium anisopliae. Turrialba 21:231-232.

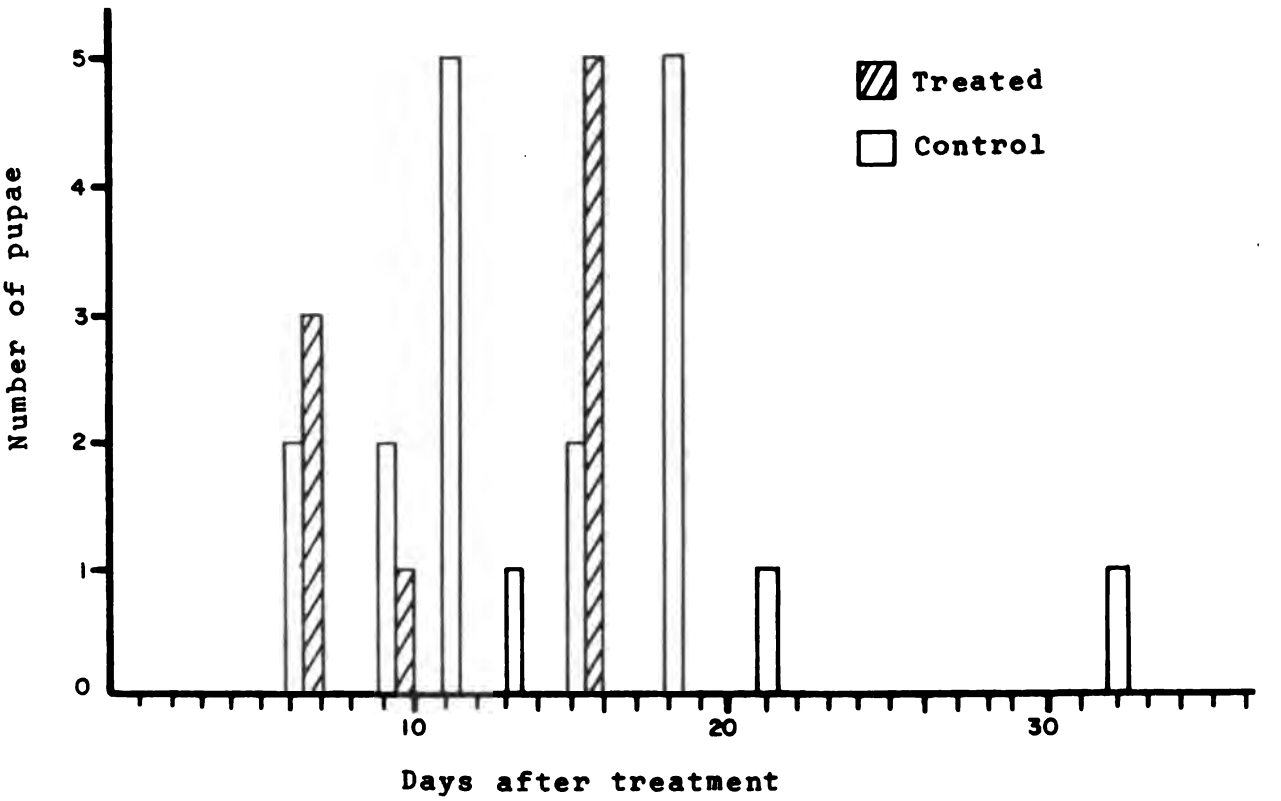


Fig. 21. Pupation of Hypsipyla grandella Zeller larvae exposed to spores of Metarrhizium anisopliae at a concentration of 1.2×10^7 viable spores/ml.

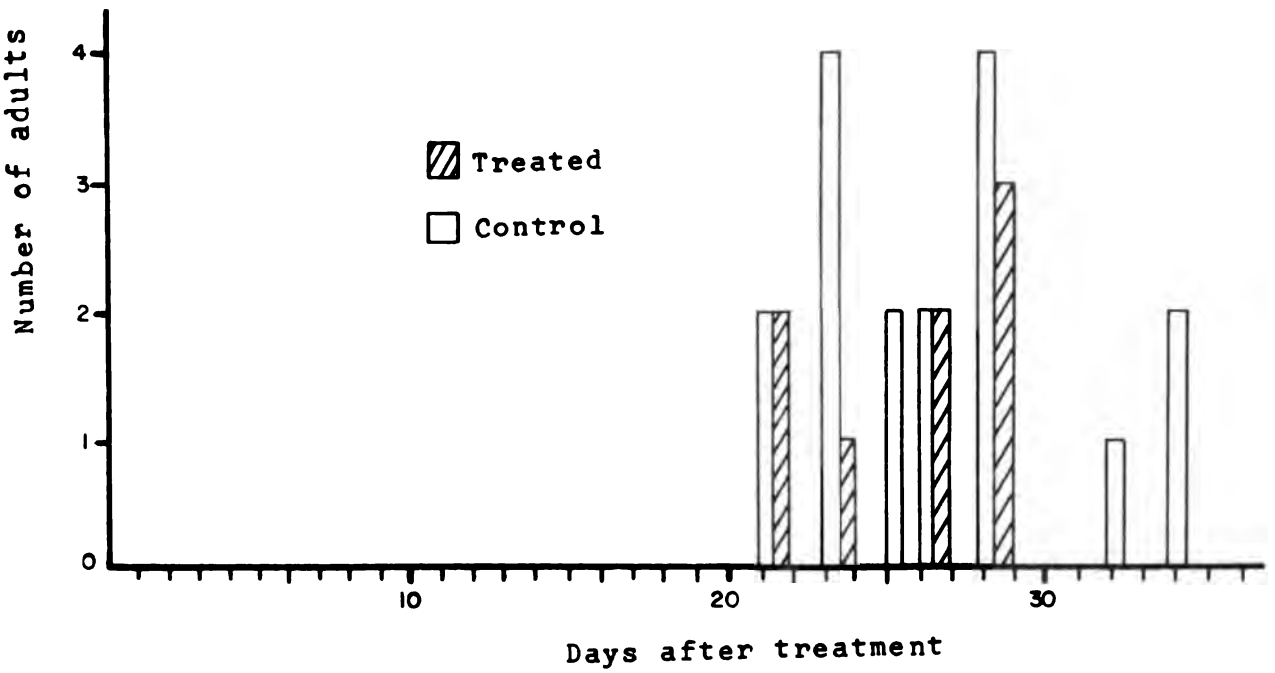


Fig. 22. Emergence of Hypsipyla grandella Zeller adults after the larvae were exposed to spores of Metarrhizium anisopliae at a concentration of 1.2×10^7 viable spores/ml.

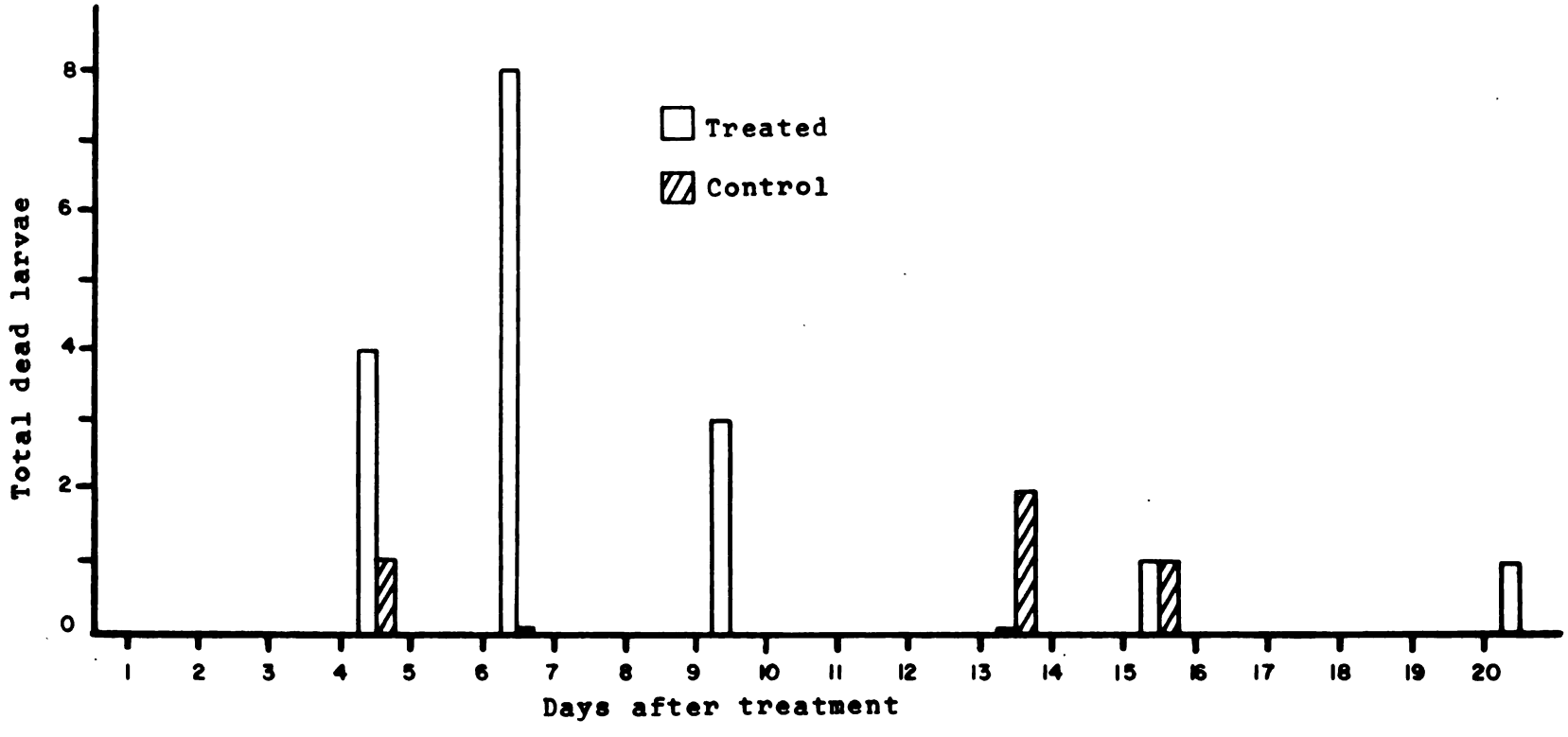


Fig. 23. Mortality distribution of Hypsipyla grandella Zeller larvae exposed to spores of Metarrhizium anisopliae at a concentration of 1.2×10^7 viable spores/ml.

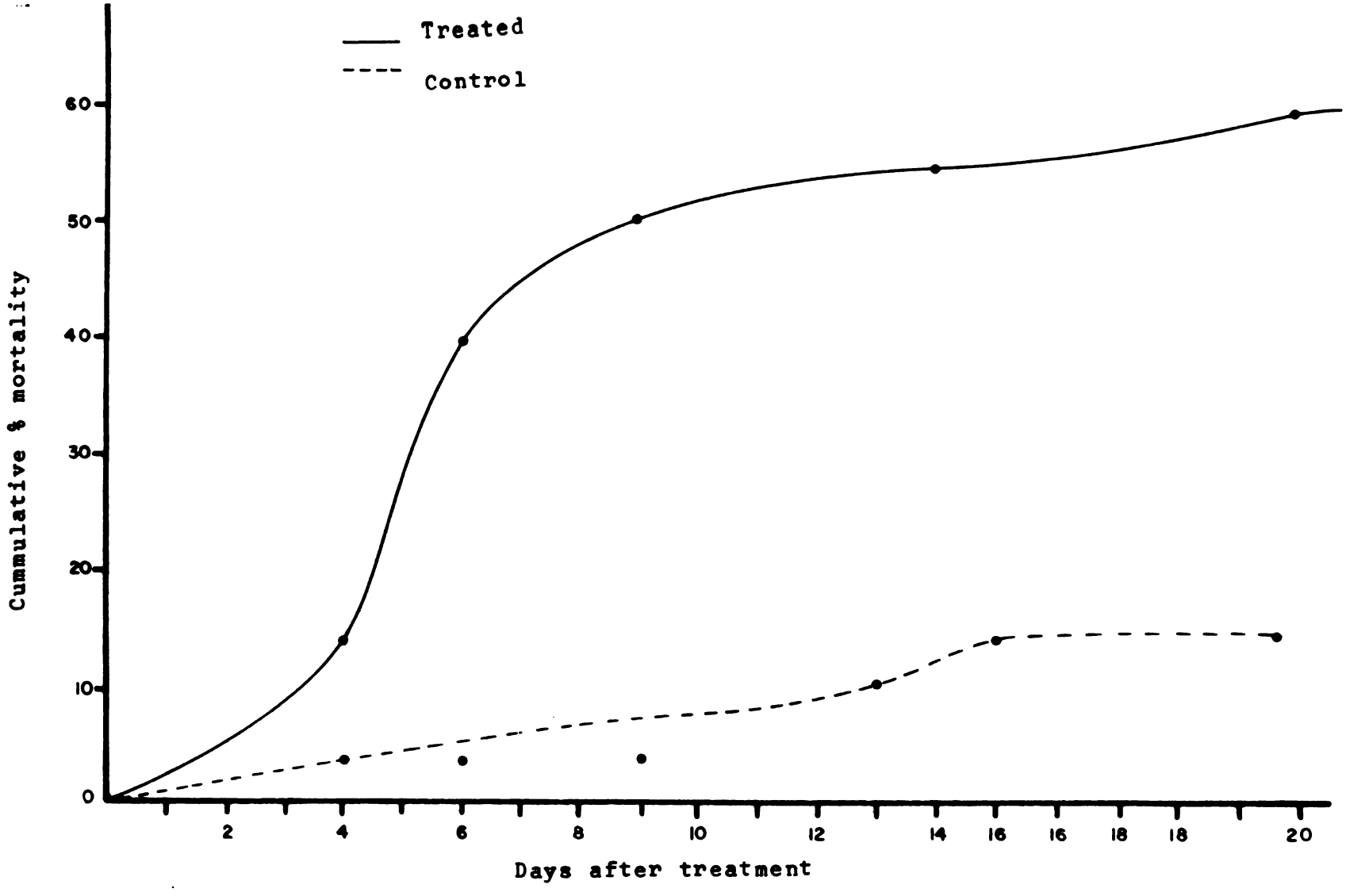


Fig. 24. Cummulative percent mortality of Hypsipyla grandella Zeller larvae exposed to spores of Metarrhizium anisopliae at a concentration of 1.2×10^7 viable spores /ml.

2. Burton, R. L., 1967. Mass rearing the fall armyworm in the laboratory. U.S.D.A., ARS 33-117, 12 p.

3. Burton, R. L., 1969. Mass rearing the corn earworm in the laboratory. U.S.D.A, ARS 33-134, 8 p.

4. Entwistle, P. F., 1968. The current situation on shoot, fruit and collar borers of the Meliaceae. 9th British Commonwealth Forestry Conference.

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

6. HIdalgo-Salvatierra, O., 1971. Estudios sobre el barrenador de las Meliáceas Hypsipyla grandella Zeller. VII. Determinación del sexo en pupas. Tu rialba 21:221.

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

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

9. Rao, V. P. and Bennet, F. D., 1969. Possibilities of biological control of Hypsipyla spp. Commonwealth Inst. of Biological Contro. Technical Bulletin No. 12:61-81.

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

b. Susceptibility of Hypsipyla grandella to the fungi Beauveria bassiana and Beauveria tenella

Previous to this study it has been published that H. grandella is susceptible to Cordyceps sp. (4) and Metarrhizium anisopliae (1). The purpose of this investigation was to determine if H. grandella was also susceptible to the fungi Beauveria bassiana and Beauveria tenella.

Slant cultures of B. bassiana and B. tenella were kindly supplied by G. M. Thomas, Insect Pathology Laboratory, University of California, Berkeley.

B. bassiana was grown in Sabouraud Dextrose Agar plus yeast extract (SDAY) and incubated under laboratory conditions. B. tenella was grown in the same agar but incubated at 28°C. Previous experiments have shown that these were the best conditions for sporulation.

The spores were harvested by adding six ml of sterile water plus two drops of a Triton X-100 suspension to each tube, agitating and filtering through fine mesh cloth. Filtration was repeated until microscopic observation revealed the absence of mycelium.

H. grandella was reared as described previously (1). For the test we used 17 to 20 day old larvae.

The larvae were inoculated by bathing them in a spore suspension for one minute. With B. bassiana we used 72 larvae, and B. tenella 126 larvae. These were inoculated in groups of nine and immediately drained and placed in petri dishes with nine divisions containing synthetic diet. Half of the larvae were bathed with sterile water and served as control.

Results with B. bassiana

Pupae and dead larvae were separated and placed in different containers on a moist filter paper in order to monitor the frequency of mortality, pupation and adult emergence. All dead larvae showing decoloration, rigidity, and later on was covered by the white mycelium of the fungus showing typical sporulation, was considered killed by the fungus.

Table 43 shows the greatest mortality 8 days after

Table 43. Frequency of mortality, pupation and adult emergence of *H. grandella* after treatment with *B. bassiana* spores at a concentration of 1.4×10^6 colony forming conidia/ml. C = control; T = treated.

Days after inoculation	Number of dead larvae		Number of pupae		Number of adults	
	C	T	C	T	C	T
3		2	1	0		
5		1	0	0		
6		0	0	5		
8		4	3	0		
10	1	1	2	7		
13	1	1	11	2		
15	2	0	1	5	1	0
17	1		1	0	0	0
19	0		1	1	0	0
21			1	0	0	2
24			0	0	0	2
26			1	0	5	4
28			0	1	5	5
31			0	0	9	1
33			0	0	0	3
35			1	0	0	0
38			0	0		

inoculation. It also shows earlier pupation and earlier adult emergence in the treated group than in the control. However, percentage adult emergence, Table 44, was about the same in both groups, showing that larvae that remained alive was able to pupate and emerge normally.

Cumulative mortality of treated and control group can be seen in Fig. 25. Of the final mortality in the treated group only 13.9% was due to the fungus, as shown in Table 44.

Results with *B. tenella*

Pupae and dead larvae were separated and placed in different containers on moist filter paper. Larvae killed by the

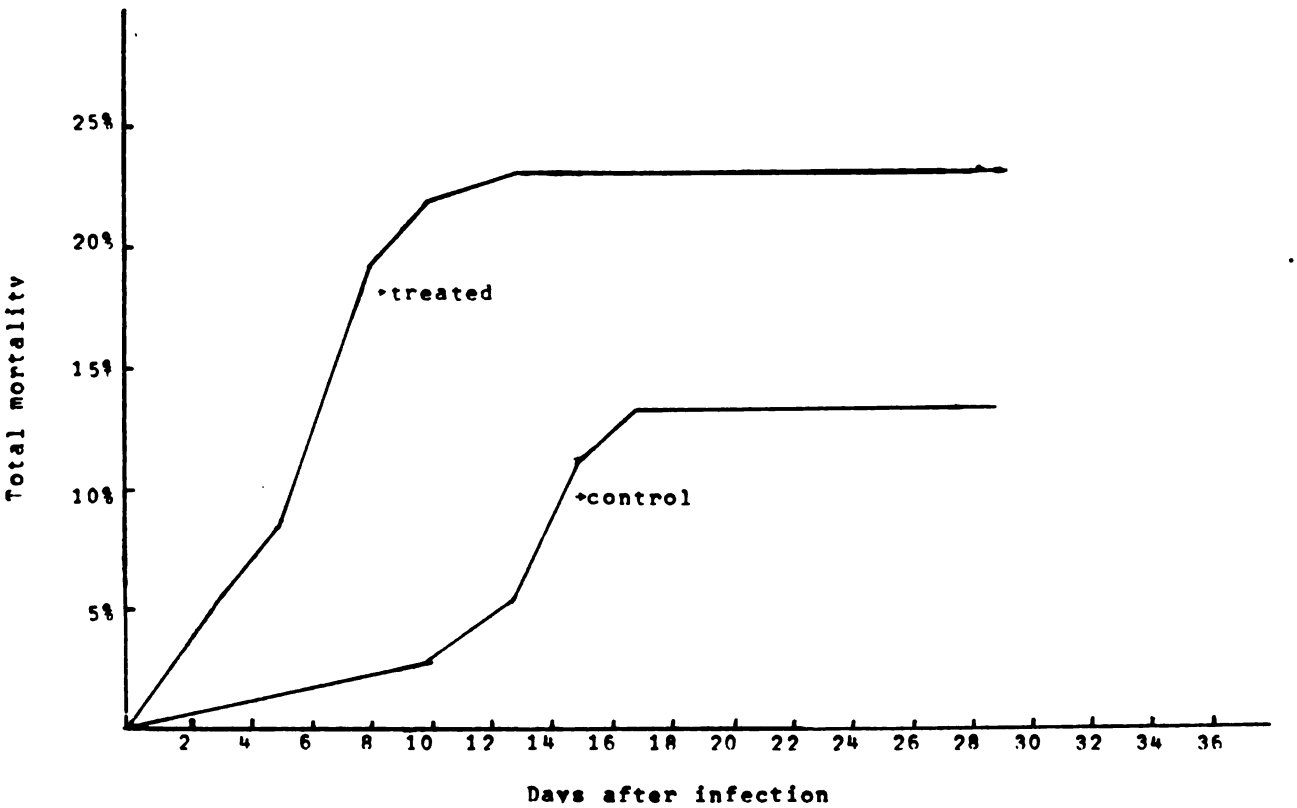


Fig. 25. Percent total mortality of Hypsipyla grandella larvae bathed with a conidial suspension of Beauveria bassiana at a concentration of 1.2×10^6 viable spores per ml.

Table 44. Total mortality, pupation and adult emergence of H. grandella after treatment with B. bassiana spores at a concentration of 1.4×10^6 colony forming conidia/ml

Initial no. of larvae	Total dead	Larvae killed by B. bassiana	Total pupae	Total adults	% adult emergence	Lost larvae
Control						
36	5	0	28	20	72	3
Treated						
36	9	5	22	17	77	5

fungus were identified because their bodies were mummified and covered with the cream white sporulating mycelia, see Fig. 26.

Frequency of mortality, pupation and adult emergence can be seen in Table 45. It shows greatest mortality ten days after inoculation. Pupation and emergence occurred at the same time in the treated group as in the control group. The percentage emergence was also similar, as shown in Table 46.

Table 45. Frequency of mortality, pupation and adult emergence of H. grandella after treatment with B. tenella spores at a concentration of 2.9×10^6 colony forming conidia/ml. C = control; T = treated

Days after inoculation	Number of dead larvae		Number of pupae		Number of adults	
	C	T	C	T	C	T
8	0	2	0	1		
10	1	5	2	3		
13	0	1	19	14		
15	2	0	5	12		
17	3	0	9	7		
20	0	0	8	1		
22	1	0	0	0		
24					3	4
27					10	7
29					1	7
31					5	2
35					1	0

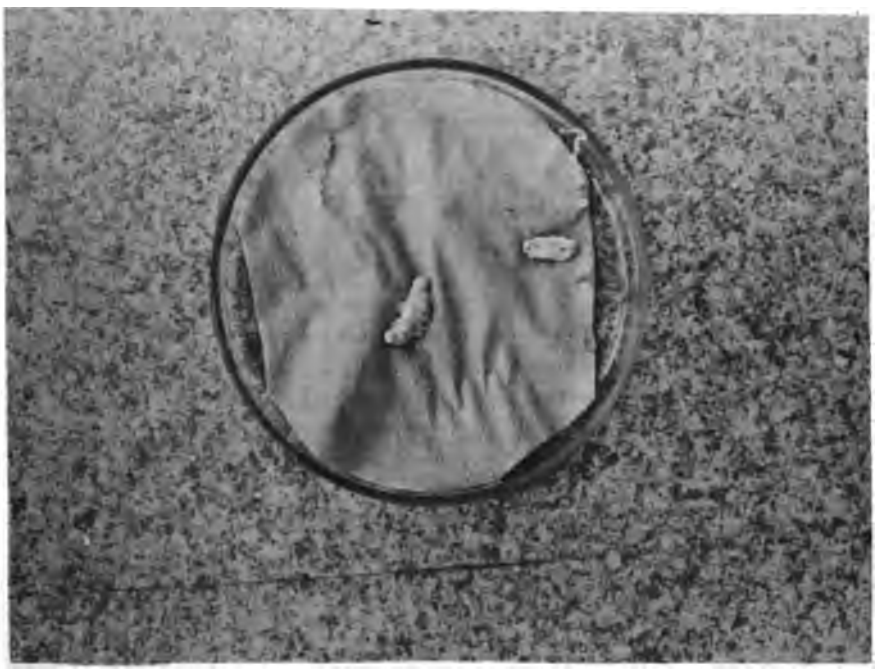


Fig. 26. Larvae of the shootborer Hypsipyla grandella Zeller dead of infection by the fungus Beauveria tenella (Delacroix)

Table 46. Total mortality, pupation and adult emergence of H. grandella after treatment with B. tenella spores at a concentration of 2.9×10^6 colony forming conidia/ml

Initial no. of larvae	Total dead	Larvae killed by B. tenella	Total pupae	Total adults	% adult emergence	Lost larvae
Control						
363	7	0	43	24	56	13
Treated						
63	8	8	38	20	53	17

Cumulative mortality is seen in Fig. 27. On the final mortality, 12.7 percent was due to B. tenella, as shown in Table 46.

It has been shown that H. grandella is susceptible to the fungi B. bassiana and B. tenella. Mortality starts three days after inoculation with B. bassiana and eight days after inoculation with B. tenella. A similar result was found by Paschke (3) working with B. bassiana and Oulema melanopa, and by Kandasamy (2), working with B. tenella and H. robusta. Mortality was higher with B. bassiana than with B. tenella, even though it was used at a lower concentration. This results seem to indicate that B. bassiana may serve better as a pathogenic control agent than B. tenella although confirmation should await until the LD_{50} is determined for both fungi using only test larvae of the same instar.

References

1. Berrios, F. and Hidalgo-Salvatierra, O. Estudios sobre el barrenador Hypsipyla grandella Zeller. VI. Susceptibilidad de la larva al hongo Metarrhizium anisopliae (Metch.). Turrialba 21(2):214-219. 1971.

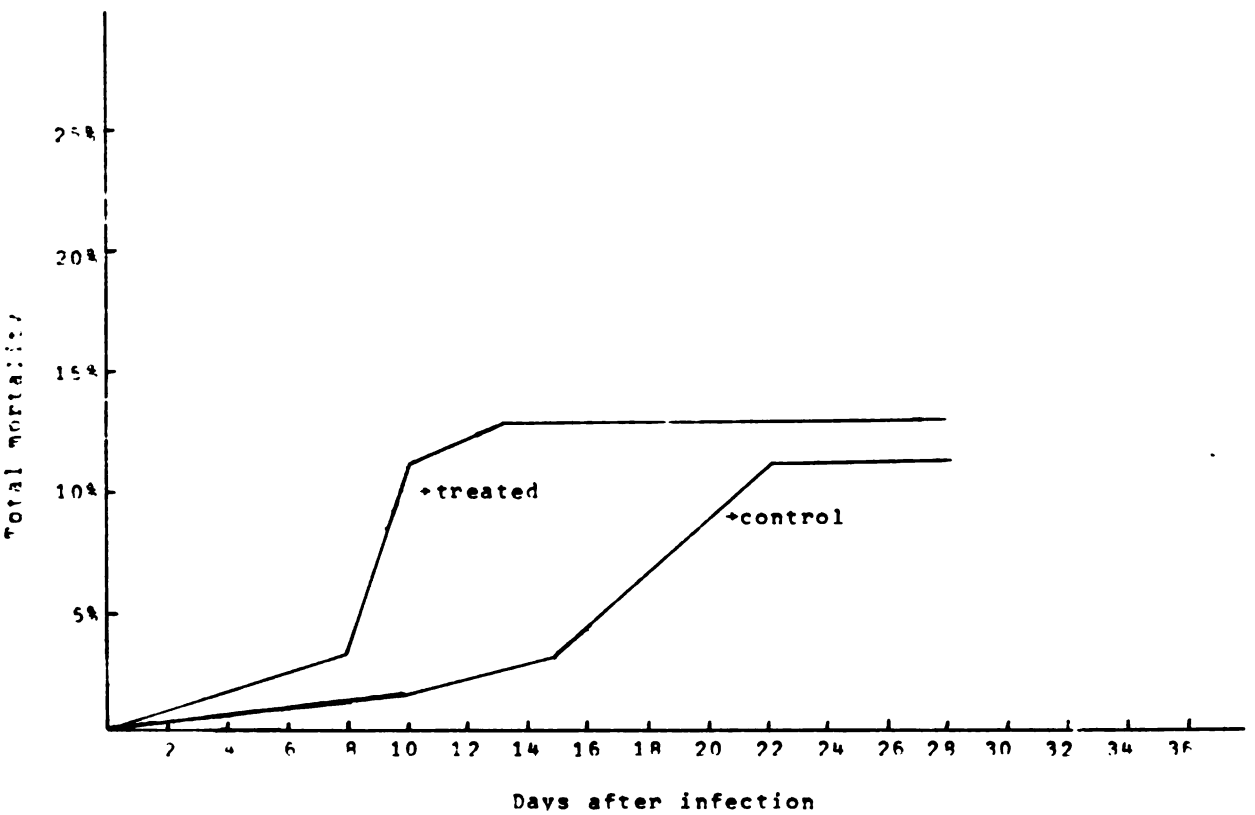


Fig. 27. Percent total mortality of Hypsipyla grandella Zeller larvae bathed with a conidial suspension of Beauveria tenella at a concentration of 1.2×10^6 viable spores/ml.

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

3. Paschke, J. D. Infection of the cereal leaf beetle Oulema melanopa (Linnaeus) by Beauveria bassiana (Bal.) Vuill. J. Inv. Pathol. 97(1):101-102. 1965.

4. Rao, V. P. and Bennet, F. D. Possibilities of biological control of the Meliaceous shoot borer Hypsipyla spp. (Lepidoptera:Phycitidae). London, Commonwealth Inst. of Biol. Control. Technical Bulletin no. 12:16-81. 1969.

c. Trichogramma sp., an egg parasite of Hypsipyla grandella Zeller

H. grandella is considered the greatest detriment to the establishment of plantations of valuable meliaceous tree species in Latin America. So far, no chemical or cultural treatment proved to be practical to keep the damage caused by this insect, below the threshold of economic importance.

The possibility of biological control has been suggested by Rao and Bennet (1). Although Trichogramma has been reported in their list as an egg parasite of Hypsipyla robusta Moore in the Old World, no publication has yet indicated, to the best of our knowledge, the existence of this genus as an egg parasite of H. grandella in Latin America.

Trichogramma sp. has been found recently, in investigations carried out at the Research and Training Center of the Institute of Agricultural Sciences, in Turrialba, Costa Rica, parasitizing eggs of H. grandella Zeller. It apparently has a preference for freshly laid eggs. After parasitation, the H. grandella eggs change color from red to black in two to four days. From the time of complete color change to emergence

of the parasites, a lapse of five to six days was observed, indicating that the egg-larval cycle would be approximately between seven to ten days. The parasite adults live from two to four days, in the absence or presence of H. grandella eggs. Observations made during the rainy season on the natural parasitation in the Turrialba area, indicate that 10-40 percent of the H. grandella eggs in a Cedrella plantations were parasitized in this period.

The parasitized Hypsipyla eggs show compartamentalization; little bumps on the egg wall indicate the presence of several parasites. Two to four minute wasps emerge generally from two emerging holes in the egg. Some of the adult characteristics are: three tarsal segments, elbowed antennae with hairs, some pubescence on the wings, red eyes and three oceli. We have been successful in rearing this Trichogramma sp. on eggs of the Mediterranean flour moth Anagasta kuehniella. An indication of host specificity was shown by the fact that single, freshly laid eggs of Spodoptera sp. were not parasitized by this Trichogramma sp.

Recent investigations from outside workers, seem to indicate that we probably have three species, Trichogramma pretiosum, T. semifumatum and T. near pretiosum and a genus Trichogrammatoidea. As far as we have been able to determine T. pretiosum and T. semifumatum have not been described previously as existing in Costa Rica. Also, there are no records of introduction of Trichogramma to Costa Rica. It is difficult to say at this moment that these are native parasites because

we know of some people in Nicaragua who have been importing Trichogramma from the United States.

Reference

1. Rao, V. P. and Bennet, F. D., 1969. Possibilities of biological control of the Meliaceous shoot borers Hypsipyla spp. (Lepidoptera:Phycitidae). Commonwealth Inst. of Biological Control, Technical Bulletin no. 12:61-81.

d. Sexing pupae of Hypsipyla grandella Zeller

In 1969 Maddox (1) published the characteristics used to sex pupae of the borer Vogtia malloi Pastrana. In this report we describe similar characteristics observed on the Meliaceae shoot borer, Hypsipyla grandella Zeller.

Fig. 28 shows that the female genital opening runs from the caudal margin of the 7th segment across the 8th segment cutting its caudal margin and extending a small distance inside the 9th segment.

In the male the genital structure is found in the 9th segment and shows a characteristics pair of rounded pads. Thus, the genital opening and the anal opening are in closer proximity in the male than in the female.

The 8th segment is recognized by the presence of the last spiracle. The caudal margin of segments 8 and 9 are barely discernible becuase of lack of pigmentation.

Reference

1. Maddox, D. M. Sex determination of pupae of Vogtia malloi (Lepidoptera:Phycitidae). Annals of the Entomol. Soc. of Amer. 62(5):1212-1213. 1969.

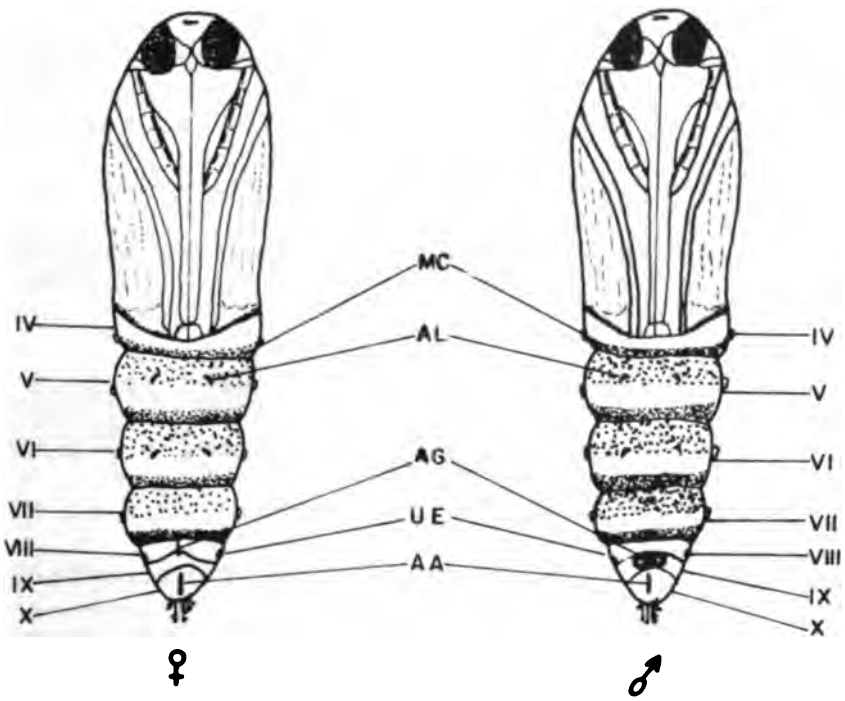


Fig. 28. Pupae of Hypsipyla grandella Zeller showing the ventral characteristics of the female (left) and the male (right): AL, alveoli; AA, anal opening; AG, genital opening; MC, caudal margin; UE, last spiracle. Roman numerals mark the different abdominal segments.

e. Susceptibility of H. grandella Zeller to Bacillus thuringiensis var. thuringiensis and Bacillus thuringiensis var. entomocidus

The use of bacterial pathogens of insects as microbial insecticides has been extensively reviewed by Angus (1, 2), Jaques (3), Heimpel (4) and Rogoff (5). Especifically B. thuringiensis has been tested in many different insect species, however, to the best of our knowledge, no one has tested it against the Meliaceae shootborer H. grandella Zeller. This is a preliminary report on the susceptibility of H. grandella

to B. thuringiensis.

B. thuringiensis was kindly supplied by G. Thomas, Insect Pathology Laboratory, University of California, Berkeley, and maintained in nutrient agar slants.

H. grandella eggs were obtained from the mass rearing laboratory of this Institute.

The composition of the insect diet used in this experiment was: cracked soybeans, 53 g; granulated agar, 6.6 g; Brewer's yeast, 33 g; wheat germ, 53 g; ascorbic acid, 3.5 g; Met-p-hydroxy benzoate, 2.1 g; sorbic acid, 1.1 g; formaldehyde 10% solution, 12 ml; Kanamicine, 1 capsule; NBCo Vitamin fortification mixture, 13 g; corn cob grits, 53 g; water, 670 ml.

Treatment A (control) consisted of 50 g of diet plus 2 g of 2% agar solution plus 50 ml sterile water. Treatment B, 50 g of diet plus 2 g of 2% agar solution plus 50 ml of bacterial overnight culture in nutrient broth. Treatments C, D, and E were 1/10, 1/100 and 1/1000 dilutions of B, respectively. Each treatment was tested against 50 first instar larvae in small, tightly closed, individual plastic boxes.

The mortality induced by the contamination of the diet is shown in Table 47. It is clear that H. grandella first instar larvae are susceptible to B. thuringiensis var. thuringiensis and var. entomocidus. As expected, highest concentration of bacteria gave the highest mortality. Microscopic examination of blackish dead larvae revealed the presence of the crystalliferous bacteria. A more detailed investigation is under was to determine the LD_{50} of preparations of different varieties

Table 47. Mortality of Hypsipyla grandella first instar larvae fed on synthetic diet contaminated with Bacillus thuringiensis

Bacteria	Treatment	Dead larvae	Lost larvae
B. thuringiensis var. thuringiensis	B	48	2
	C	24	5
	D	28	17
	E	0	11
B. thuringiensis var. entomocidus	B	40	10
	C	17	11
	D	5	5
	E	7	1
Control	A	4	0

of B. thuringiensis to determine the most pathogenic strain against H. grandella Zeller.

References

1. Angus, T. A., 1965. Bacterial pathogens of insects as microbial insecticides. Bacteriological Reviews 29 (3):364-372.

2. Angus, T. A., 1968. The use of Bacillus thuringiensis as a microbial insecticide. World Rev. of Pest Control 7(1):11-26. 1968.

3. Jaques, R. P. Insect control by bacterial pathogens. Annals of the Entomol. Soc. of Quebec 9:17-29. 1964.

4. Heimpel, A. M. A critical review of Bacillus thuringiensis var. thuringiensis Berliner and other crystalliferous bacteria. Annual Rev. of Entomol. 12:287-329. 1967.

5. Rogoff, M. N. Crystal forming bacteria as insect pathogens. Advances in Applied Microbiology 8:291-313. 1966.

f. Growth of H. grandella Zeller reared on a synthetic diet

A knowledge of the life cycle of H. grandella under our experimental conditions is extremely important if we want

to select that stage of the insect which is most suitable for our experiments.

Ramírez (2) in his preliminary studies of natural populations was able to detect, from a small number of observations, six instars of the insect.

Working with larvae reared on synthetic diet Grijpma (1) determined the larval and pupal periods of H. grandella but did not determine the number and duration of the different stadia.

It is the purpose of this study to follow a group of individually reared larvae, fed on synthetic diet, through their complete life cycle, to determine the number of instars through which they pass, their growth rate, and head capsule size distribution.

The composition of the diet has been described previously (see section e).

Individual rearing cells were made of formica strips with a slit, and two cover slides acting as viewing windows, Fig. 29. Each larva was given a small but enough amount of diet according to its size, and was changed daily during observation and measurement. As the larva was growing, the number of formica strips was increased to allow freedom of movement. The cells were kept inside a covered plastic box (26x19x10 cm) on paper towel on top of a 2.5 cm layer of moist sand to keep the diet from drying out. This arrangement simulated in a way the natural environment of the larvae, and facilitated observation at the time of ecdysis and localization of the head capsule.

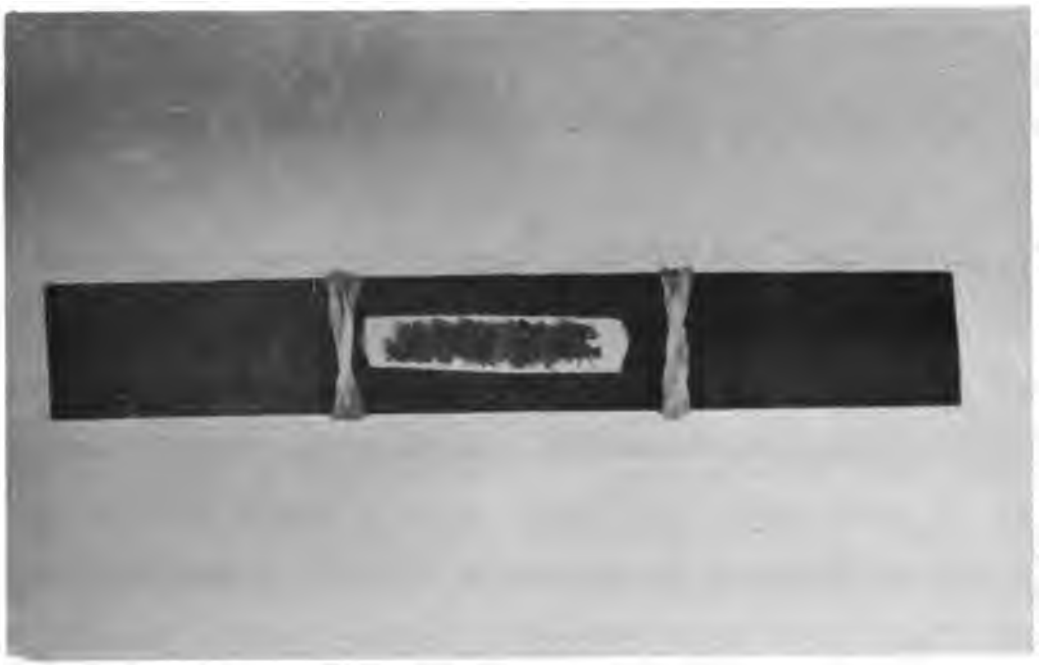


Fig. 29. Rearing cell made of strips of formica 17x2.5 cm with a 5x1 cm slit. Two cover slides held by rubber bands completed the cell and acted as viewing windows.

To measure a larva it was first immobilized with ether. Whenever possible a calibrated micrometer and the low magnification objective (2.5x) of a Zeiss research microscope were used.

Larval growth

The experiment started with 50 newly hatched larvae but only 31 were able to complete the cycle from eclosion to adult emergence. The main problem was escaping of the very young larvae.

All larvae were examined daily. Table 48 gives the lapse of time from eclosion to ecdysis, pupation and adult emergence. We calculate that 45% of the larvae had 7 instars with a larval period of 28.7 $\pm$ 2.4 days and 52% had 6 instars with a larval period of 26.6 $\pm$ 2.8 days. A t-test showed that these means are significantly different at the 5% level ($P \leq 0.05$) but not at the 2% level ($P \leq 0.02$). Maybe with a bigger population sample it should be possible to determine if this difference is real or not. One larva went through 5 instars with a period of 33 days.

The length of time of each stadium is given in Table 49. This data indicates that the last stadium of a larva is the longest and the most variable. After the fifth ecdysis the population was divided into two groups, those with six instars and those with seven instars.

Length of the larvae at the time of ecdysis is shown in Table 50. The growth rate of each larva was fitted with the logistic function

$$L = \frac{B_o}{1 + B_1 e^{-B_2 t}}$$

Table 48. Days from eclosion to ecdysis, pupation and adult emergence

Larva No.	Eclosion	1st.ecd.	2nd.ecd.	3rd.ecd.	4th.ecd.	5th.ecd.	6th.ecd.	Pupae	Adults
1	0	3	8	15	19	27	--	30	--
2	0	3	7	10	13	18	21	29	43
3	0	3	6	10	13	17	26	28	38
4	0	3	7	11	14	17	--	26	--
5	0	3	7	10	13	17	23	30	42
6	0	3	6	9	12	17	20	26	36
7	0	3	7	10	13	17	--	26	31
8	0	3	7	11	16	18	23	29	41
9	0	3	7	10	13	18	--	31	--
10	0	3	7	10	13	17	23	27	38
11	0	3	7	9	12	15	21	31	43
12	0	3	9	12	15	18	23	26	44
13	0	3	7	11	14	18	--	27	38
14	0	3	7	9	12	15	--	26	36
15	0	3	9	17	21	26	--	33	44
16	0	3	7	10	15	18	--	26	36
17	0	3	7	9	12	15	--	23	35
18	0	3	7	9	11	14	--	22	35
19	0	3	6	10	14	16	20	31	43
20	0	3	6	9	12	16	19	30	42
21	0	3	6	9	14	18	--	28	42
22	0	3	7	10	14	17	--	26	42
23	0	3	7	10	14	19	24	32	43
24	0	3	7	11	14	18	--	25	36
25	0	3	6	9	12	16	--	26	36
26	0	3	6	10	14	18	--	25	37
27	0	3	7	10	13	16	19	26	40
28	0	3	6	9	13	17	22	25	--
29	0	3	8	12	19	--	--	33	43
30	0	3	6	10	13	17	--	25	36
31	0	3	6	9	12	17	22	32	--

Table 49. Average length, in days, and standard deviation of the six or seven different stadia of Hypsipyla grandella Zeller. Roman numerals indicate ecdysis number

Instar	Stadium	$\bar{x}$	s
1	Eclosion - I	3.0	0.0
2	I - II	3.9	0.8
3	II - III	3.5	1.3
4	III - IV	3.3	0.9
5	IV - V	3.9	1.2
6	V - Pupation	8.5	2.2
6	V - VI	4.9	1.5
7	VI - Pupation	6.9	2.9

and the parameters calculated using an IBM-1130-8K research computer. From these data a theoretical growth curve was drawn in Fig. 30. We can see that older larvae are more variable in size than younger larvae. If we compare the observed average length with the theoretical average length at the time of ecdysis, Table 51, we find perfect agreement, indicating that the logistic function describes well the growth rate of H. grandella larvae.

Head capsule size

The head capsules were measured very accurately to one hundredth of a millimeter on the same day of ecdysis. The average width and average length are shown in Tables 52 and 53. As we can see from the standard deviation, and the distributions shown in Figs. 31 and 32, the variability in head capsule size is greater in the older instars than in the younger ones. This points out that it is difficult to determine the number of instars from the head capsule measurements alone of a number of head capsules collected at random in the field.

Table 50. Length (in mm) of Hypsipyla grandella Zeller larvae at the time of ecdysis

Larva	Eclosion	1st. ecd.	2nd. ecd.	3rd. ecd.	4th. ecd.	5th. ecd.	6th. ecd.
1	1.68	2.38	4.1	8.2	9.5	10.5	--
2	1.66	2.43	5.1	9.0	10.5	16.0	21.0
3	1.68	2.45	5.0	8.0	10.5	17.0	20.0
4	1.67	2.50	4.1	7.6	11.5	15.0	--
5	1.68	2.47	4.2	5.8	9.1	13.0	20.0
6	1.68	2.48	5.0	9.2	12.0	19.0	22.0
7	1.68	2.49	4.0	7.8	11.4	16.3	--
8	1.68	2.43	3.2	8.2	11.5	13.8	20.0
9	1.67	2.51	5.0	9.0	12.5	18.0	--
10	1.68	2.49	5.0	7.0	11.2	16.5	22.0
11	1.68	2.47	5.2	7.3	10.4	18.5	26.4
12	1.66	2.47	5.0	9.0	12.5	15.5	23.0
13	1.68	2.45	3.5	7.2	9.2	13.5	--
14	1.69	2.47	5.0	6.8	10.3	17.0	--
15	1.67	2.35	3.8	9.1	12.1	16.0	--
16	1.66	2.50	4.2	7.0	12.0	16.0	--
17	1.68	2.50	5.1	7.0	10.0	14.0	--
18	1.68	2.35	4.4	12.0	14.0	20.0	--
19	1.67	2.44	3.8	6.8	15.0	17.0	18.0
20	1.69	2.50	5.0	7.0	10.2	19.0	20.0
21	1.68	2.47	5.0	6.8	8.5	16.0	--
22	1.66	2.41	4.0	9.0	11.0	17.0	--
23	1.68	2.49	4.9	6.2	9.0	13.8	15.0
24	1.65	2.41	5.0	7.5	10.3	15.5	--
25	1.67	2.49	5.1	6.0	11.0	16.0	--
26	1.81	2.38	4.9	8.0	11.2	13.1	--
27	1.82	2.41	7.3	7.5	12.1	13.1	17.0
28	1.68	2.47	5.0	7.0	11.4	16.5	24.0
29	1.64	2.35	4.0	6.1	9.8	--	--
30	1.66	2.38	4.1	6.4	9.3	13.2	--
31	1.68	2.45	4.2	5.0	8.9	12.0	16.0

If we plot the average head capsule width or length, against the average time of ecdysis, Figs. 33 and 34, we find a good linear correlation. In both cases we calculated an average Dyar's factor of 1.5. There was no correlation however, between head capsule size and larval size. Fig. 35 shows a series of head capsules collected during the experiment.

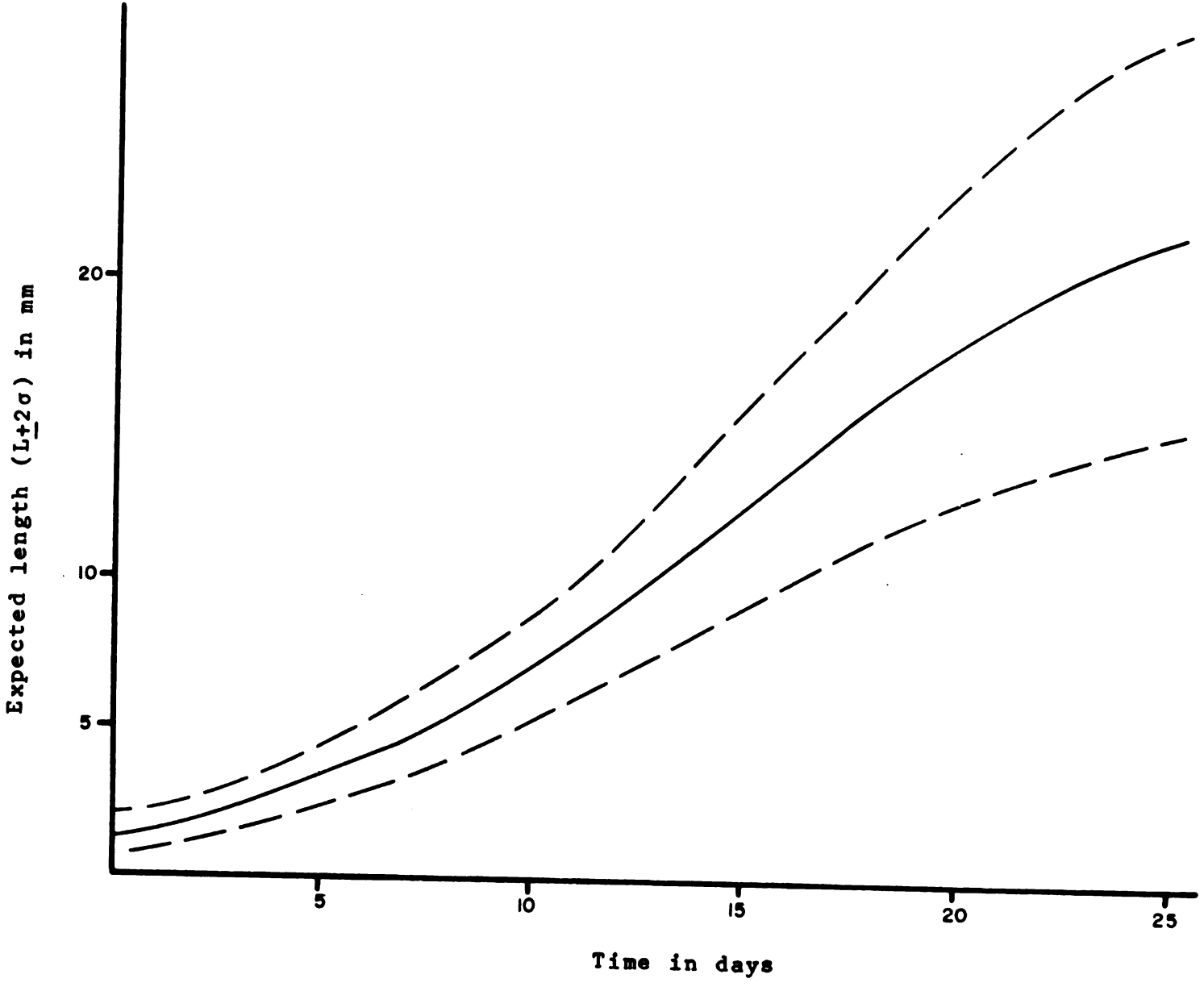


Fig. 30. Theoretical growth rate and 96.4% confidence band of Hypsipyla grandella larvae

Table 51. Observed ($\bar{X}$) and theoretical ($\bar{L}$) average length (in mm) of Hypsipyla grandella larvae at the time of ecdysis

Ecdysis No.	$\bar{X}$	$\bar{L}$
I	2.4	2.4
II	4.6	4.6
III	7.6	7.4
IV	10.9	11.0
V	15.6	15.4
VI	20.3	20.3

Table 52. Average width and standard deviation of Hypsipyla grandella head capsules immediately after ecdysis

Ecdysis No.	$\bar{X}$ (mm)	s
I	0.28	0.01
II	0.44	0.03
III	0.73	0.06
IV	1.05	0.12
V	1.73	0.14
VI	2.18	0.16

Table 53. Average length and standard deviation of Hypsipyla grandella head capsules immediately after ecdysis

Ecdysis No.	$\bar{X}$ (mm)	s
II	0.37	0.03
III	0.63	0.07
IV	0.91	0.09
V	1.42	0.17
VI	1.80	0.15

References

1. Grijpma, P. Studies on the shootborer Hypsipyla grandella Zeller. V. Observations on a rearing technique and on host selection behavior of adults in captivity. Turrialba 21(2):202-213. 1971.

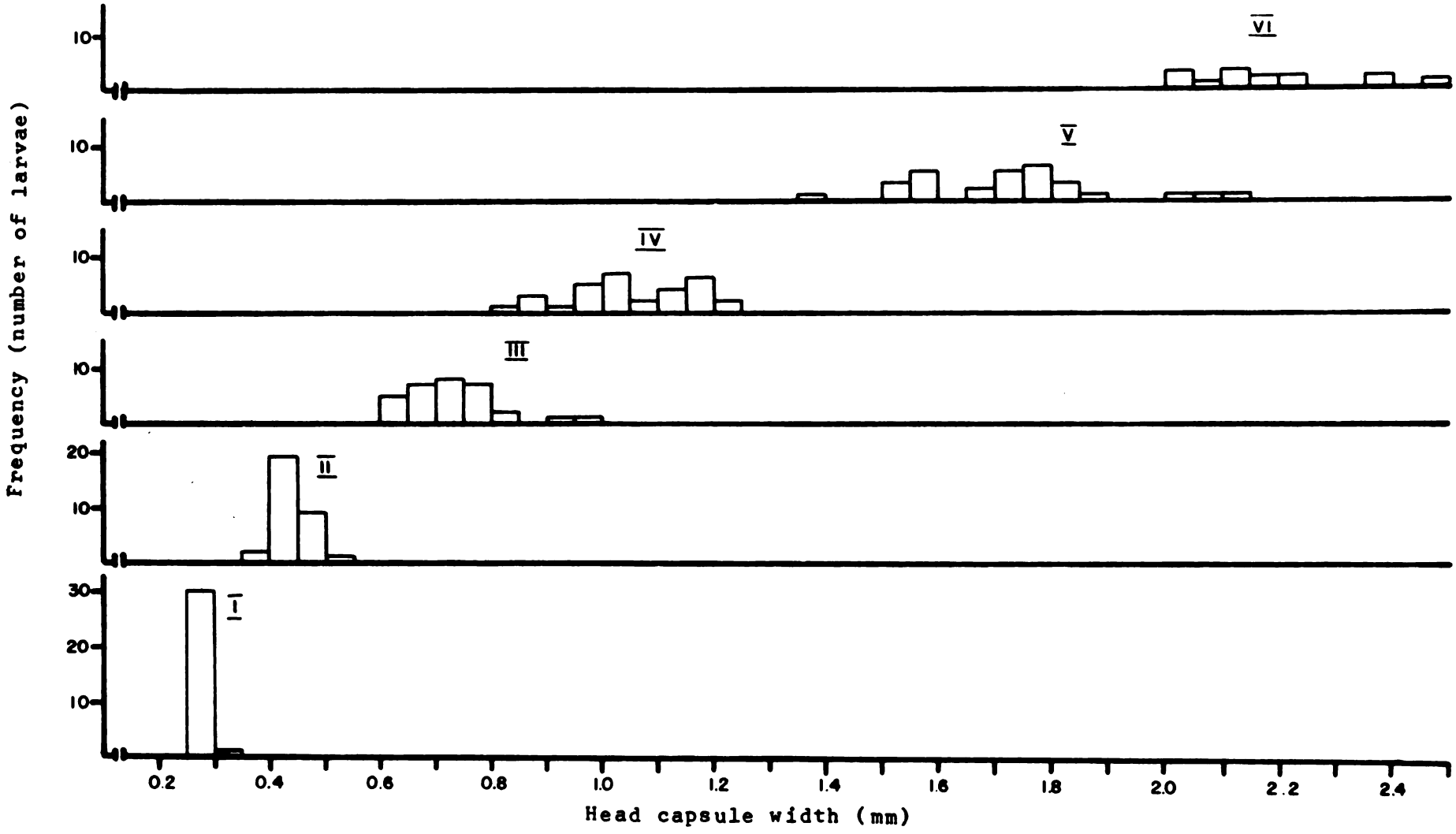


Fig. 31. Hypsipyla grandella head capsule width distribution immediately after the indicated ecdysis number.

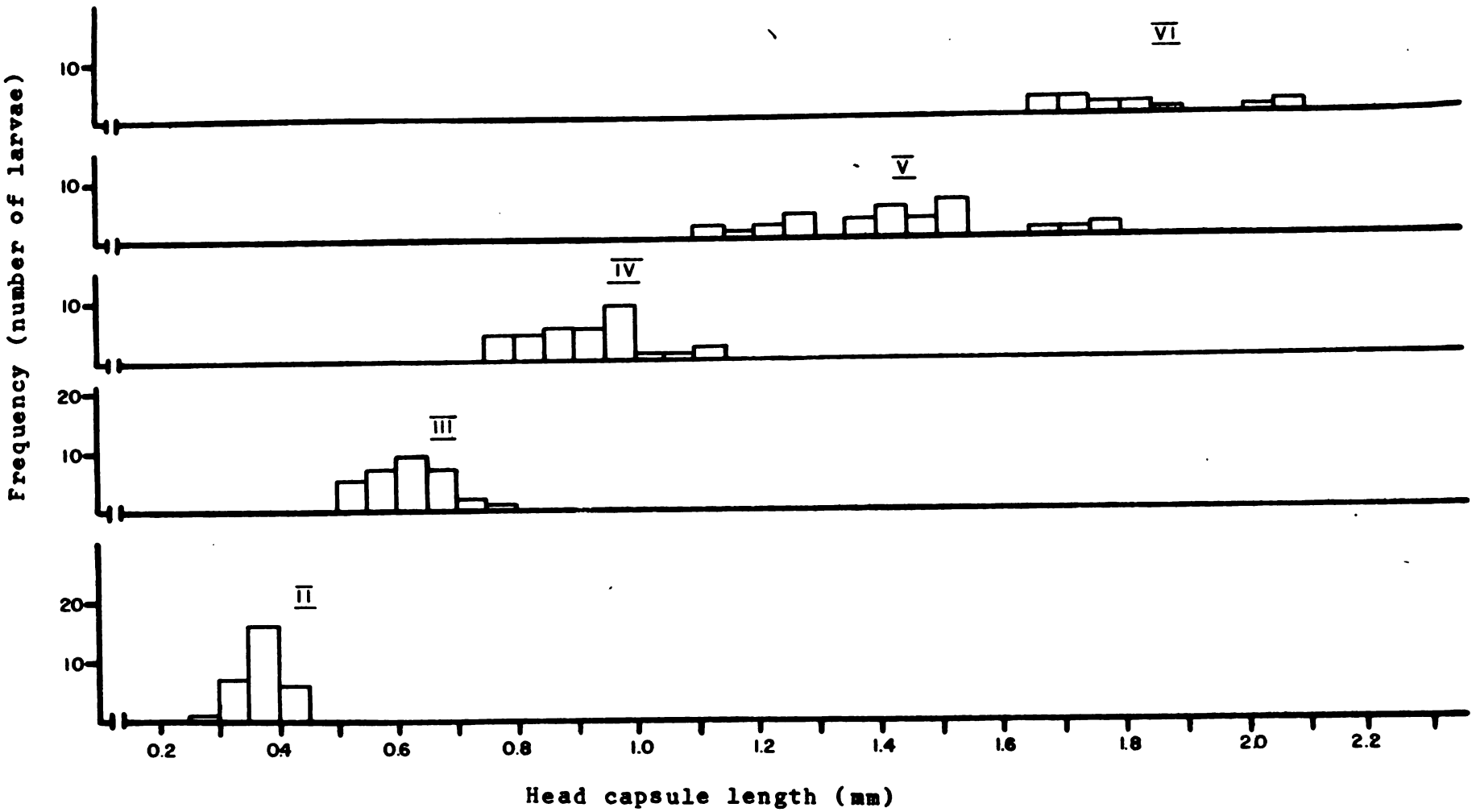


Fig. 32. Hypsipyla grandella head capsule length distribution immediately after the indicated ecdysis number

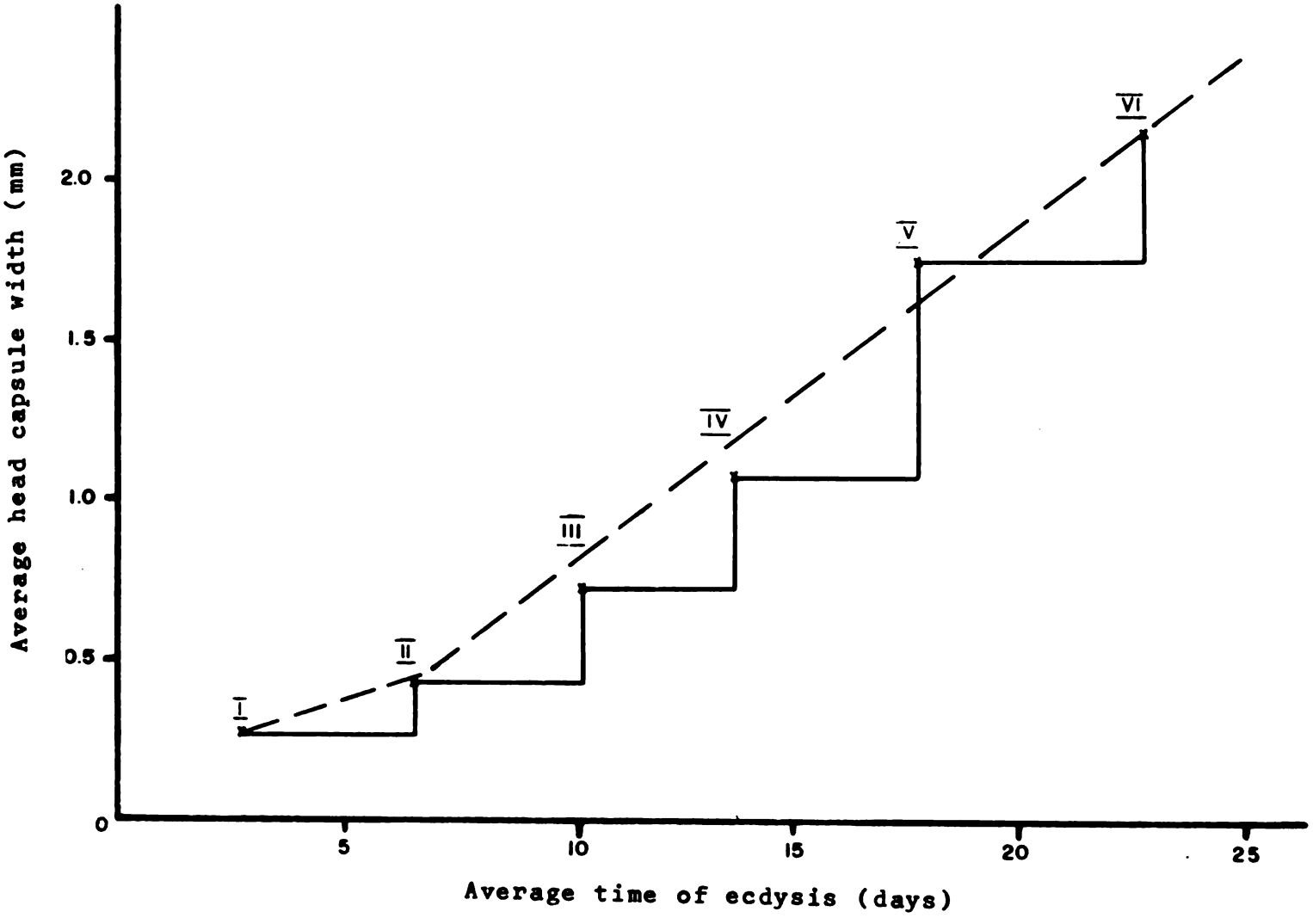


Fig. 33. Correlation between average time of ecdysis and average head capsule width.

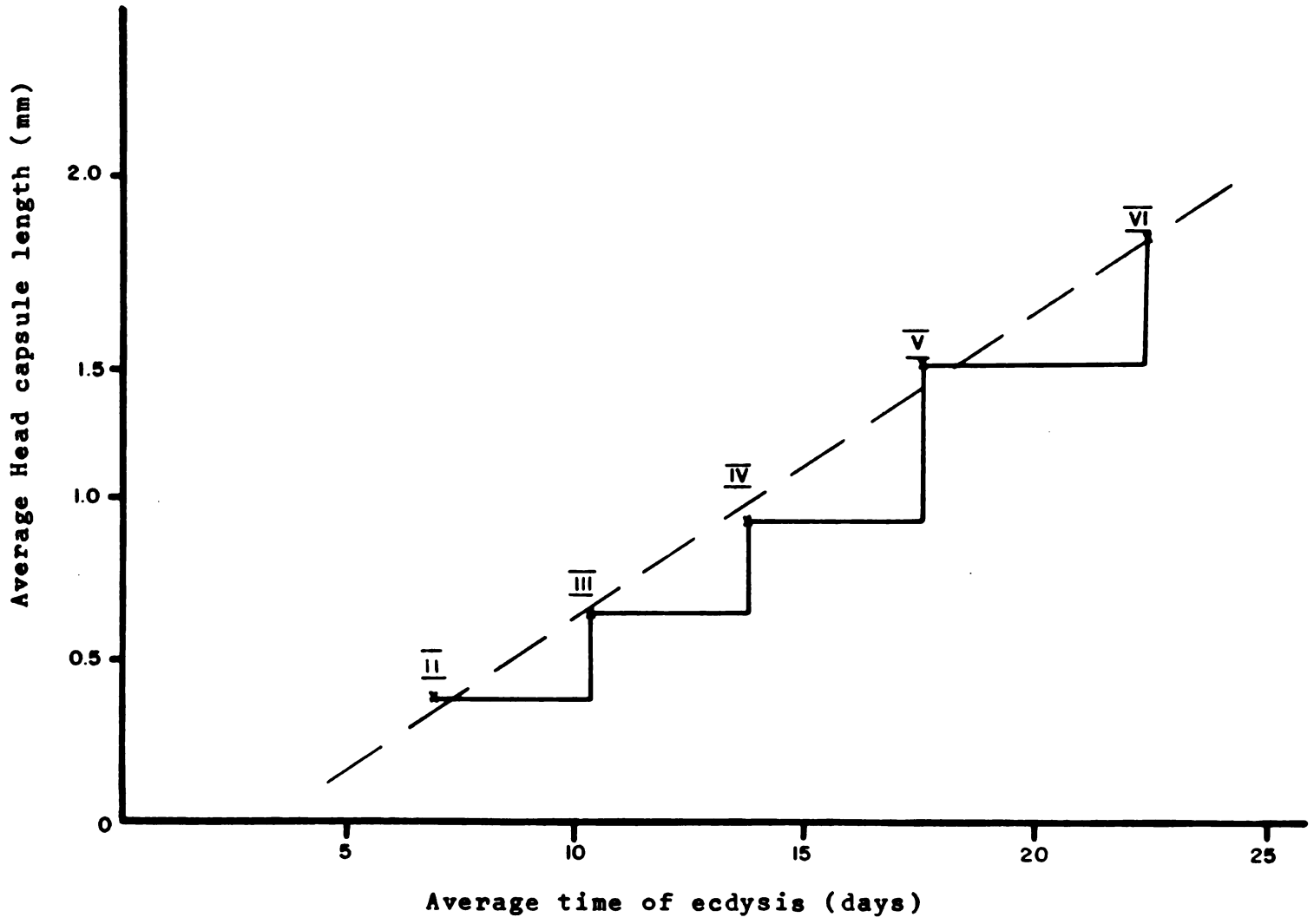


Fig. 34. Correlation between average time of ecdysis and average head capsule length.

Fig. 35. Head capsules of six of the seven instars of Hypsipyla grandella Zeller. The last one remains inside the pupal case and was always broken.

2. Ramirez, J. Investigación preliminar sobre biología, ecología y control de Hypsipyla grandella Zeller. Boletín del Instituto Forestal Latinoamericano de Investigación y Capacitación (Venezuela) 16:5-77. 1964

g. Determination of the LD_{50} of Metarrhizium anisopliae on fifth instar larvae of Hypsipyla grandella Zeller

In a previous experiment (1) it was found that H. grandella is susceptible to the fungus Metarrhizium anisopliae, It is the purpose of this investigation to determine the instar more susceptible to the pathogen and to quantify the effect in terms of the LD_{50}.

Larvae were supplied by the mass rearing laboratory of this Institute. The different instars were separated by the size of the head capsule (2).

The fungus was grown on Sabouraud Dextrose Agar plus yeast extract, and the spores collected as a powder.

To prepare a spore suspension a known weight was added to 50 cc of sterile water plus one drop of sterile Triton X-100, and dispersed for 2 minutes with a Sorval Omnimixer. The concentration of total spores was determined with a hemocytometer, and of viable spores by dilution and plating.

Inoculation was performed in small groups either by spraying or submerging the larvae for one minute in the spore suspension. Mortality was calculated dividing the number of larvae killed by the fungus by the number of live larvae in the control.

In the determination of the most susceptible instar we used 63 larvae of each stadium and inoculated them by spraying

with a suspension of 9.2×10^7 viable spores/ml. Stadia 6th and 7th were grouped together because not all larvae completed the seven stages. An equal number of larvae of each stadium was used as control. The results are shown in Fig. 36. It can be seen that the 5th instar gives the higher mortality, 30%, followed by the VI - VII instar with 17.8%, and then the IV and the III instars with 7.0% and 5.3%, respectively.

We tried to induce the disease in I and II instar larvae in several independent trials but were unsuccessful except for one larva of the 2nd instar which was attacked in one trial, Fig. 37. We can say therefore, that the first instar is resistant but the second instar shows a very small degree of susceptibility.

To determine the LD_{50} of M. anisopliae we inoculated by immersion and used only 5th instar larvae. The results are tabulated in Table 54. We can see that mortality is a function of the concentration of spores in the inoculum. A plot of percent mortality on a probabilistic scale or probit units on a linear scale versus concentration of spores on a logarithmic scale, Fig. 38., gives a linear function from which we can calculate not only LD_{50} but also other indexes. Table 55 gives the values of 5.4×10^6, 3.6×10^7 and 2.7×10^8 for LD_{25}, LD_{50} and LD_{75}, respectively, plus their fiducial limits at the 95% level.

If we compare these results with the results shown in Fig. 36 we can deduce that inoculation by immersion gives a higher mortality than inoculation by spraying at the same concentration of spores.

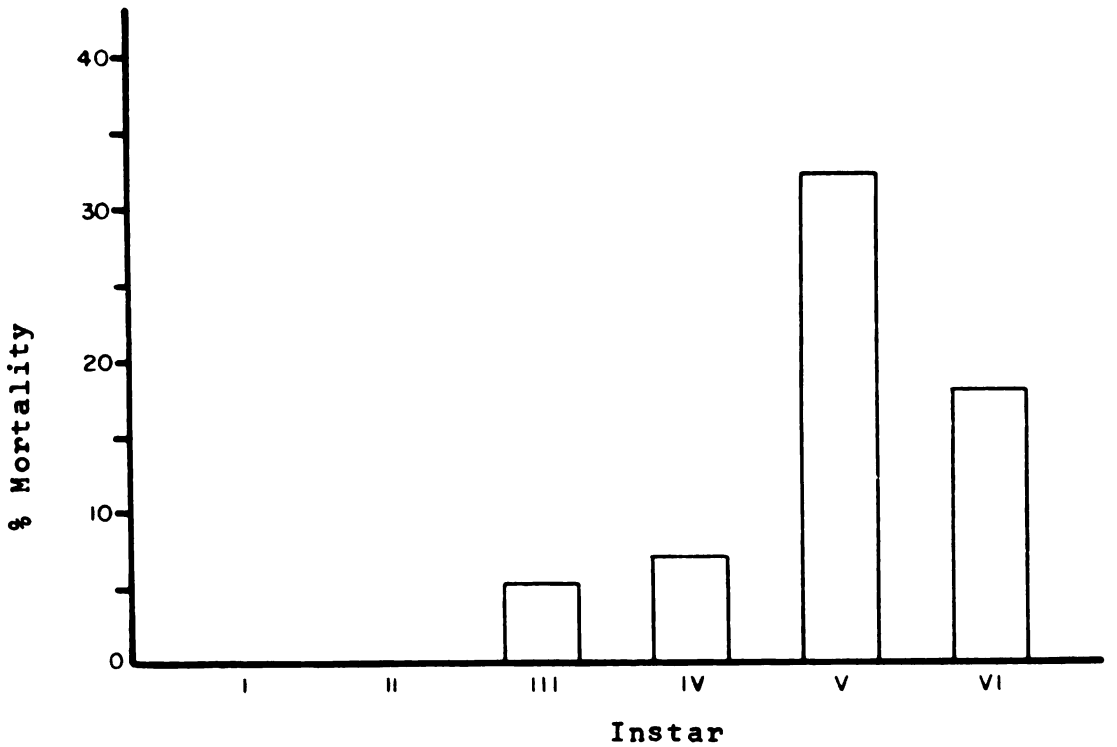


Fig. 36. Susceptibility of the different instars of Hypsipyla grandella sprayed with a spore suspension of 9.2×10^7 viable spores per ml.

Fig. 37. Different instars of Hypsipyla grandella covered with spores of Metarrhizium anisopliae

Table 54. Mortality of 5th instar Hypsipyla grandella larvae 15 days after immersion in a suspension of Metarrhizium anisopliae. No. of larvae per treatment = 99

Viable spores per ml	Total dead larvae	No. of larvae killed by fungus	Lost larvae	Live larvae	Live pupae	% Mortality	Probit
0	1	0	3	68	27	0	-
0.39×10^7	25	25	2	59	13	18.7	4.43
0.89×10^7	22	22	10	53	14	23.4	4.27
1.59×10^7	41	40	6	24	28	42.6	4.81
1.59×10^8	56	56	1	22	20	59.6	5.24
3.19×10^8	78	78	2	4	15	82.8	5.95

Table 55. Concentration of Metarrhizium anisopliae viable spores/ml required to induce 25, 50 and 75 percent mortality of 5th instar larvae of H. grandella when these are bathed in the suspension during one minute

% mortality	Viable spores/ml	95% fiducial limits	
		higher	lower
25	5.4×10^6	7.2×10^6	2.9×10^6
50	3.6×10^7	7.5×10^7	2.3×10^7
75	2.7×10^8	7.7×10^8	2.0×10^8

References

1. Berrios, F. and Hidalgo-Salvatierra, O. Estudios sobre el barrenador Hypsipyla grandella Zeller. VI. Susceptibilidad de la larva al hongo Metarrhizium anisopliae (Metch.) Turrialba 21(2):214-219. 1971.

2. Hidalgo-Salvatierra, O. and Berrios, F. Growth of Hypsipyla grandella Zeller on synthetic diet. To be submitted to Turrialba.

h. Susceptibility of Dermatobia hominis Linn to Bacillus thuringiensis thuringiensis

This was a preliminary experiment carried out as a laboratory project for the course "Principles of Biochemistry".

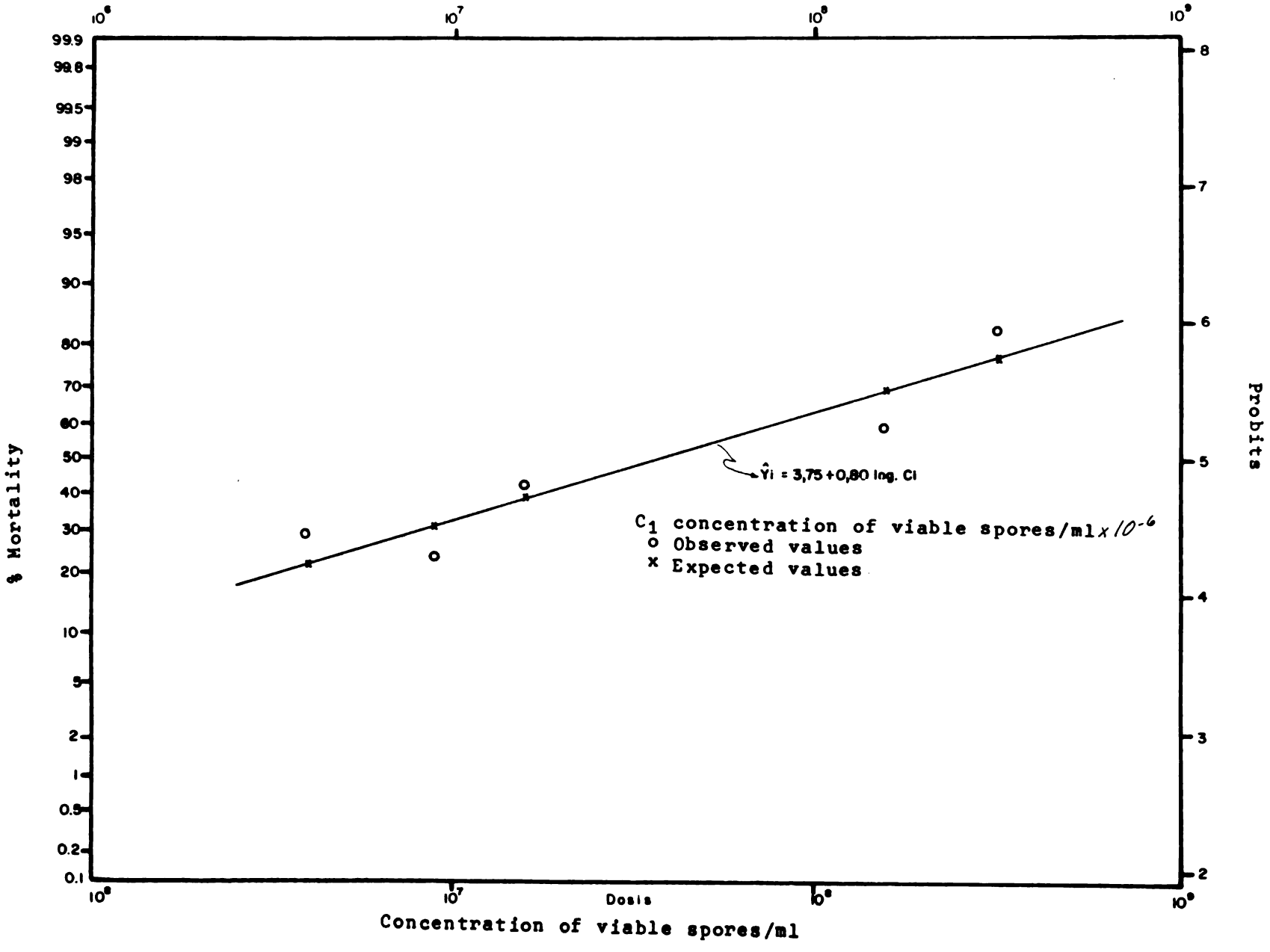


Fig. 38. Survival of 5th instar Hypsipyla grandella larvae bathed for one minute with different concentrations of spores of Metarrhizium anisopliae

What motivated this experiment was the positive results that have been published in the control of some dipterous species like Haematobia irritans, Musca autumalis, Musca domestica and Stomoxys calcitrans, using preparations from Bacillus thuringiensis. One of the insect toxic compounds produced by this bacteria is commonly known as 'fly toxin'.

Dermatobia hominis, better known as 'torsalo', is a pest of importance to man, cattle and other animals from Mexico down to Central and South America. Larvae of this fly bore into the skin of cattle where they encyst. Partial control can be achieved using systemic toxic compounds. However, the use of conventional chemical insecticides is limited by economic and, most important, health factors. Meat containing insecticides is not fit for human consumption. So our objective was to run preliminary experiments that may lead to an evaluation of preparations from B. thuringiensis in the control of this pest.

Materials:

1. A pure overnight culture of B. thuringiensis grown in nutrient broth.
2. Two pregnant Santa Gertrudis cows infested with torsalo cysts of all sizes. Cow No. G143 weighing 461 kg and No. G114 weighing 419 kg.

One area containing from 10 to 15 very small cysts was marked in each cow for periodic observations. One cow was given a subcutaneous injection on the side of the neck of 2.5 ml of pure overnight culture for every 100 kg of weight every other day. The other cow received half that dose in the same

way. Both cows were injected six times. Observations were made daily:

Date	Cow No.	T°	Dose	Time
Oct.20	G143	105F	11.25 ml	2:45 PM
	G114	103F	5.5	3:00 PM

Observations: The areas were marked. Many ticks were observed around the base of the tail in both cows.

Oct.21	G143	101F		1:15 PM
	G114	101F		1:35 PM

Observations: Temperature seemed normal. In the morning the cows presented small swelling at site of injection but it decreased by mid-afternoon.

Oct.22	G143	102.6F	11.25 ml	2:00 PM
	G114	103.5F	5.5	2:30 PM

Observations: One live larva was extracted from cow G143. The amount of ticks looked the same.

Oct.23	G143	101.2F		8:30 AM
	G114	101.3F		9:00 AM

Observations: The swelling was very small.

Oct.24	G143	103.8F	11.25 ml	4:30 PM
	G114	104.1F	5.5	5:00 PM

Observations: Had a hard time getting the cows into the corral. A live larva was extracted from cow G143. We marked 9 ticks on the left side of the base of the tail of cow G143, and 12 ticks on cow G114.

Oct.26	G143	102.9F	11.25 ml	4:30 PM
	G114	102.5F	5.5	5:00 PM

Observations: The ticks previously marked disappeared in both cows.

Oct.28	G143	103 F	11.25 ml	4:30 PM
	G114	103.2F	5.5	5:00 PM

Observations: Two dead larvae were extracted from cow G114. Two areas with ticks were marked on cow G143: Five ticks on the right hind leg and four on the right side of the base of the tail

Oct.29	G143	101.8F		4:30 PM
	G114	102.1F		5:00 PM

Observations: Not recorded.

Oct. 30	G143	102.2F	11.25 ml	4:30 PM
	G114	103.2F	5.5	5:00 PM

Observations: On cow G143 only one tick remains on the tail, and the ticks on the leg were dry.

Nov. 5

Observations:
1. The area with small cysts marked at the beginning of the experiment does not show any development in both cows.
2. A dead larva was extracted from cow G114.
3. Both cows looked free of ticks. The few remaining ones were dry.

Nov.25

Observations:
1. The cows are still free of ticks.
2. They look in perfect health.
3. The small cysts did not develop.

It is hard to arrive at any meaningful conclusion from this experiment. However, there are some indications that demand further experimentation, like the effect on the ticks and the effect on the small torsalo larvae.

The pure culture of bacillus injected subcutaneously

affected neither the health of the cows nor the health of the baby born calves.

The effect on small larvae will be studied by placing several larvae on several animals, with the aid of a brush. The site of penetration of each larva will be marked and its development followed in the animals treated with the preparation from B. thuringiensis and in the controls.

If it turns out that the toxin inhibits or kills the small torsalo larva, then a whole area of experimentation will be open. Radioisotopes will have to be used to determine the optimal concentration of toxin in the blood of the animal; to determine its biological half-life, to determine the metabolism of the toxin, etc.

An ideal situation that might keep an animal free of torsalo will be to maintain a certain level of toxin in the blood of the animal. This may be achieved by implanting, under the skin of the animal, a capsule containing the toxin that will dissolve slowly, thus maintaining the required blood level. Could this be possible? I don't know.

2. Radiation Biology and Mutagenesis of Insect Pathogens

a. Survival of Metarrhizium anisopliae spores after ultraviolet or gamma rays irradiation

The amount of literature on the radiobiology and photobiology of spores of fungi like Aspergillus, Penicillum, Neurospora, Rhizopus, Ustilago, is relatively greater than that of spores of entomogenous fungi like Metarrhizium anisopliae.

It is known that radiation can induce changes in

color, morphology, nutritional requirements, enzymatic activities, pathogenicity, resistance or sensitivity to chemical treatments or physical treatments like the radiation itself. Very few of these investigations, however, have been carried out with our test organism. It is our purpose to apply the principles of radiation induced mutagenesis in the search for mutants which can be used to our advantage in the control of insect pests. We would like to induce and select marked mutants that show a higher pathogenicity index and resistance to weather conditions, especially sunlight radiation.

M. anisopliae was grown on Sabouraud Dextrose Agar plus yeast extract (SDAY) slants. The spores were harvested as powder 30 days after inoculation, and kept this way in baby food flasks under laboratory conditions.

The spore suspension was prepared by adding 50 cc of sterile water plus one drop of Triton X-100 to 0.5 g of spores, and dispersing for two minutes in a Sorvall omni-mixer. The conidia are single uninucleate cells, cylindrical (2.3 to 3.7 mm wide and 5.0 to 7.5 mm long), and green color.

The UV source was a GE 15 W germicidal lamp with an intensity at the point of irradiation of 17.5 ergs/mm^2/sec, measured with a Black Ray ultraviolet light meter.

The gamma source was the Co-60 well of this Institute with a dose rate of 1.2 kr/min, measured by ferrous sulfate dosimetry.

For UV irradiation we used a volume of 10 cc in a petri

dish constantly agitated by means of an Eberbach rotator at the mark of 180.

For gamma irradiation we used 35 cc in 20 x 2.5 cm test tubes.

The plates were inoculated immediately after irradiation, incubated at 30°C, and the colonies counted 3 to 4 days after plating.

Survival of spores of different age after ultraviolet irradiation is shown in Fig. 39. During the first cycle of inactivation, (6300 ergs/mm^2), all the spores, age 35 days to 120 days, show a shoulder characteristic of systems with radiation damage repair mechanism. The pigment might confer some degree of protection but this has to be investigated further working with a colorless mutant.

Upon continued irradiation the cells show an exponential decay, and at doses above 14700 ergs/mm^2 we see differences between younger spores and older spores, the older ones being more resistant than the younger ones.

Survival of spores of different age after Co-60 gamma irradiation is shown in Fig. 40. Spores of 1 to 1.5 months of age after inoculation show a multitarget response with an LD_{50} of 60 kr. Older spores show a higher sensitivity to gamma rays and a single hit kinetics with an LD_{50} of 36 kr for 2 to 2.5 month old spores and 26 kr for 3.5 to 4 month old spores.

The UV sensitivity of M. anisopliae is similar to that one of Neurospora crassa (3), and Beauveria bassiana (5), but it seems to be somewhat more resistant than Aspergillus nidulans (1, 4).

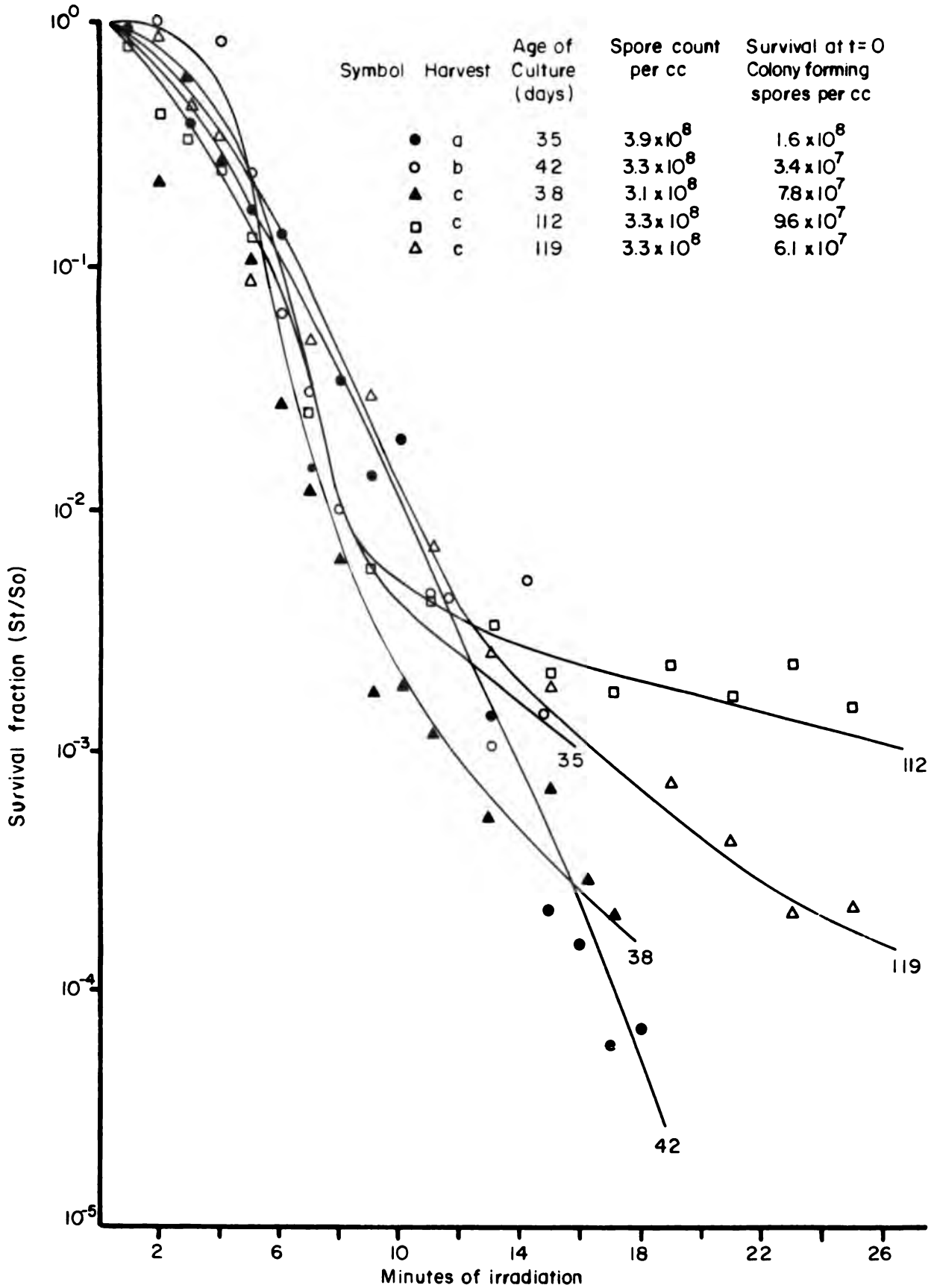


Fig. 39. Survival of Metarrhizium anisopliae spores after UV-254 irradiation. I_o = 17.5 ergs/mm^2/sec.

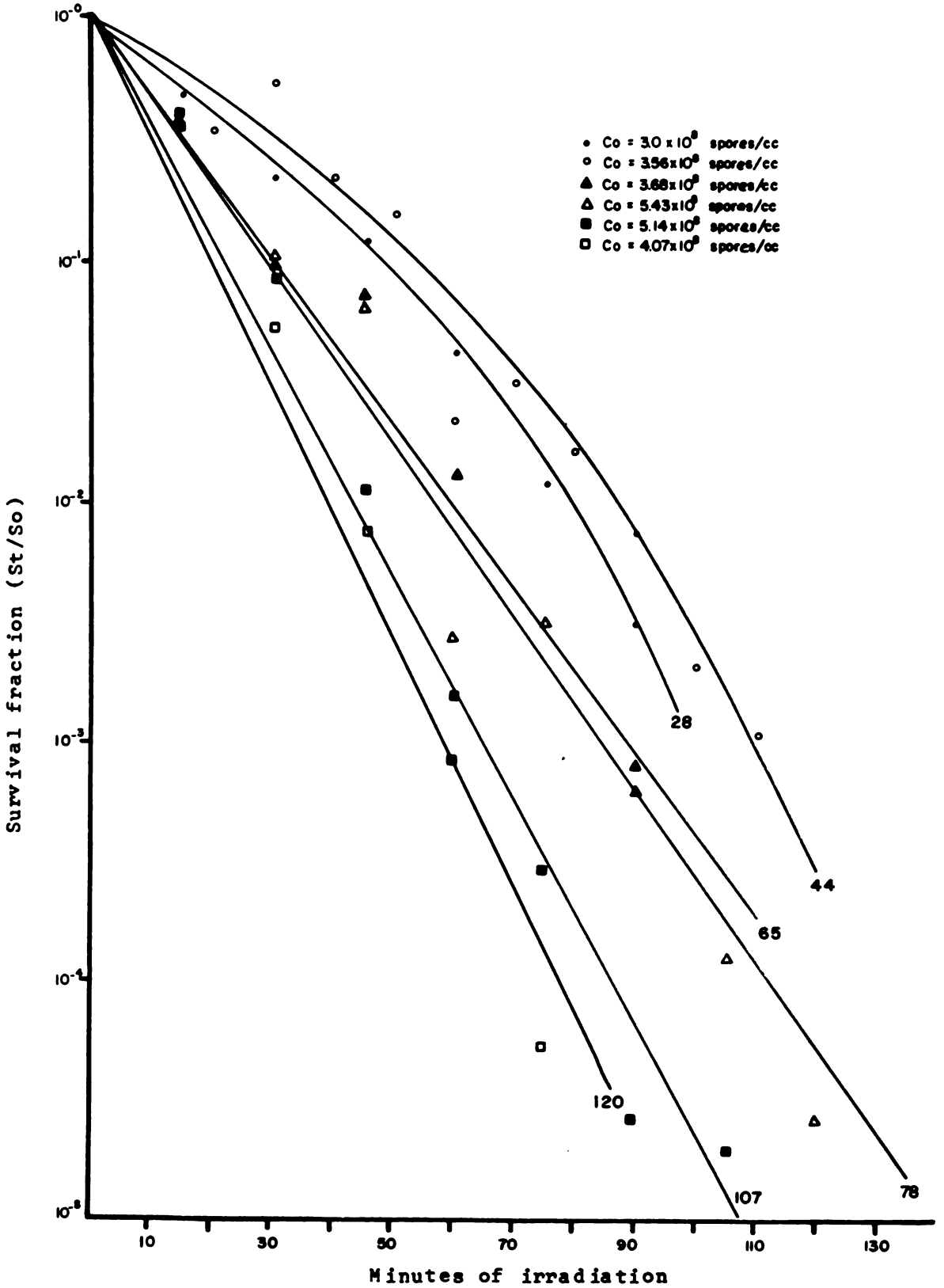


Fig. 40. Survival of Metarrhizium anisopliae spores after Co-60 gamma irradiation ($\overline{I_o}$= 1.2 kilorads/min). Numbers after each line refer to the age of the spores in days.

The response to gamma-irradiation seems to be similar to Penicillum expansum (2), and more resistant than B. bassiana (5).

So far we have isolated 20 gamma-radiation induced mutants. We have spore color mutants (yellow, brown, light green, gray), morphological mutants, mycelium color mutants, mutants with very few spores, mutants whose spores change color with age, and so on. We are beginning to work on the characterization of some of these isolates. We know for instance, that mutant FB-7-3 and FB-9-1 are pathogenic to Hypsipyla grandella. In SDAY slants FB-7-3 has yellow spores and conserves the color on the deceased larva. However, FB-9-1 is reddish brown on the agar but sporulates green on the larva, like the wild type. We plan to characterize further these two mutants during the coming years.

References

1. Arlett, C. F. The influence of the cytoplasm on mutation in Aspergillus nidulans. Mutation Res. 3:410-419. 1966.

2. Chou, T. W. et al. Effects of gamma radiation on Penicillum expansum L. I. Some factors influencing the sensitivity of the fungus. Radiation Bot. 10:511-516. 1970.

3. Gampel, Z. and Toha, J. Aging and repair in Neurospora crassa studied by ultraviolet irradiation. Radiation Research 40(3):525-533. 1969.

4. Lanier, W. B., Tuveson, R. W. and Lennox, J. E. A radiation sensitivie mutant of Aspergillus nidulans. Mutation Research 5:23-31. 1968.

5. Levitin, M. M. et al. Genetics and selection of Beauveria bassiana (Bals) Vuill. I. Lethal and mutagenic effects of U.V. and X-rays. Nuclear Sci. Abstracts 25(12): Ab. No. 26703. 1971.

b. Comparative survival of several varieties of Bacillus thuringiensis after ultraviolet irradiation

As we have said before, our general objective is to do research in radiation biology, but using a system or systems of importance in the field of agriculture. One of these systems is the crystalliferous bacteria Bacillus thuringiensis, commonly used for the control of insect pests.

It is a well known fact that the germicidal action of sunlight radiation is due mostly to its ultraviolet radiation components. Spores of fungal pathogens and spores and crystals of bacterial pathogens are more resistant to radiation inactivation than vegetative cells, however, they still are inactivated by long exposures to sunlight, especially in the tropics.

To begin with we are aiming our work at the characterization of vegetative cells and spores of several strains of B. thuringiensis with respect to their sensitivity to ultraviolet radiation of 254 and 360 mm wavelength, Co-60 gamma rays, and with respect to their pathogenicity toward our test insect, the Meliaceae shootborer Hypsipyla grandella Zeller.

Next step would be to select mutants with higher pathogenicity and higher resistance to radiation.

So far we have determined the ultraviolet radiation survival curves of 8 varieties, having different pathogenicity indexes (1, 2) supplied by Dr. T. A. Angus of the Insect Pathology Research Institute, Ontario; by G. Thomas, Insect Pathology Laboratory, University of California, Berkeley; by Dr. H. Dulmage, USDA-ARS, Brownsville, Texas, and some of our UV-radiation induced mutants.

Table 56 gives their survival after low and high UV doses.

Table 56. Survival of B. thuringiensis and B. finitimus vegetative cells after low and high UV doses.

Variety	Isolate	UV_{254} dose in ergs/mm^2	
		700	1750
		Survival	
sotto		$2x10^{-1}$	$3x10^{-2}$
entomocidus		$1x10^{-2}$	$2x10^{-3}$
kurstaki	HD-1	$1x10^{-2}$	$1x10^{-4}$
alesti		$1x10^{-3}$	$1x10^{-5}$
thuringiensis	IICA-13-1-1-5	$4x10^{-1}$	$2x10^{-2}$
thuringiensis	IICA-13-1-1-4	$2x10^{-1}$	$6x10^{-3}$
thuringiensis		$3x10^{-1}$	$5x10^{-4}$
finitimus		$2x10^{-1}$	$3x10^{-2}$

We can see that among the most pathogenic strains like sotto and kurstaki we have a difference in radiation sensitivity, the former being 100 to 1000 times more resistant to UV radiation.

It is clear also that we can induce radiation resistance without difficulty as shown by isolate IICA-13-1-1-5; of course, we do not know yet if pathogenicity was conserved or lost. It seems reasonable therefore that we should try to increase the radiation resistance of kurstaki strain and the pathogenicity of sotto strain.

References

1. Angus, T. A. The use of Bacillus thuringiensis as a microbial inseciticde. World Rev. of Pest Control 7(1):11-26. 1968.

2. Dulmage, H. T. Production of δ-Endotoxin by eighteen isolates of Bacillus thuringiensis; serotype 3, in 3 fermentation media. J. Inv. Pathol. 18:353-358. 1971.

D. SOIL CHEMISTRY

Research Accomplishments

The agricultural production in tropical regions depends on good soil management practices and a better understanding of its basic properties. It is important to develop information on chemical and mineralogical compositions and reactions that occur in the soils of these areas to permit: a) estimation of lime and fertilizer needs; b) to allow assessment of the feasibility and requirement for agricultural development, and c) to provide background information for effective progress of field experimentation.

Thus, in order to gain information on tropical soils, our research in the past two years pursued the following objectives: a) soil acidity and exchange properties, b) phosphate chemistry, and c) trace elements. Herein we report some of the accomplishments on the research carried out during that period.

1. Soil Acidity and Exchange Properties

a. Characterization of aluminum in Andosols (K. Igue and R. Fuentes)

A reliable method to characterize Al was needed in order to understand the acidic properties of these soils and the effect of liming.

In order to gain information on these problems, the predominantly volcanic ash soils were studied. Aluminum was extracted with conventional buffered (3, 4) and unbuffered salt solutions (2, 27). The successive extraction method of Skeen

and Sumner (5) was also used in order to characterize exchangeable Al.

Large amounts of Al can be extracted from these soils by N NH_4OAc mainly at low pH's (Fig. 41). Table 57 gives the values of exchangeable acidity extracted with N KCl according to the procedure of Lin and Coleman (2). These values are lower than those obtained by N NH_4OAc. Although buffered N NH_4OAc extracted high amounts of Al from Andosols, relatively small amounts are extracted by N KCl. This seems to indicate that gibbsite-like material, allophane, or organically complexed Al were extracted.

Fig. 42 shows the cumulative values of Al extracted by unbuffered salts for different soils. By extrapolation the linear portion of the curve back to zero extraction we obtain 'exchangeable' Al, as indicated by Skeen and Sumner (5). As the soils become more weathered, higher values for exchangeable Al are obtained as indicated on Table 58.

Fig. 43 shows how the exchangeable Al, as affected by liming, can be influenced by successive extractions. As we can see, when 4 meq/100 g of lime was applied, almost negligible amounts of Al are extracted which indicates that almost all exchangeable Al originally present was eliminated by liming. The much higher values for Al extracted by N NH_4OAc pH 4.8 are also included.

The release of Al in acid solutions was studied by first eliminating the organic matter with H_2O_2 treatment. The results indicated that upon destruction of organic matter an increase

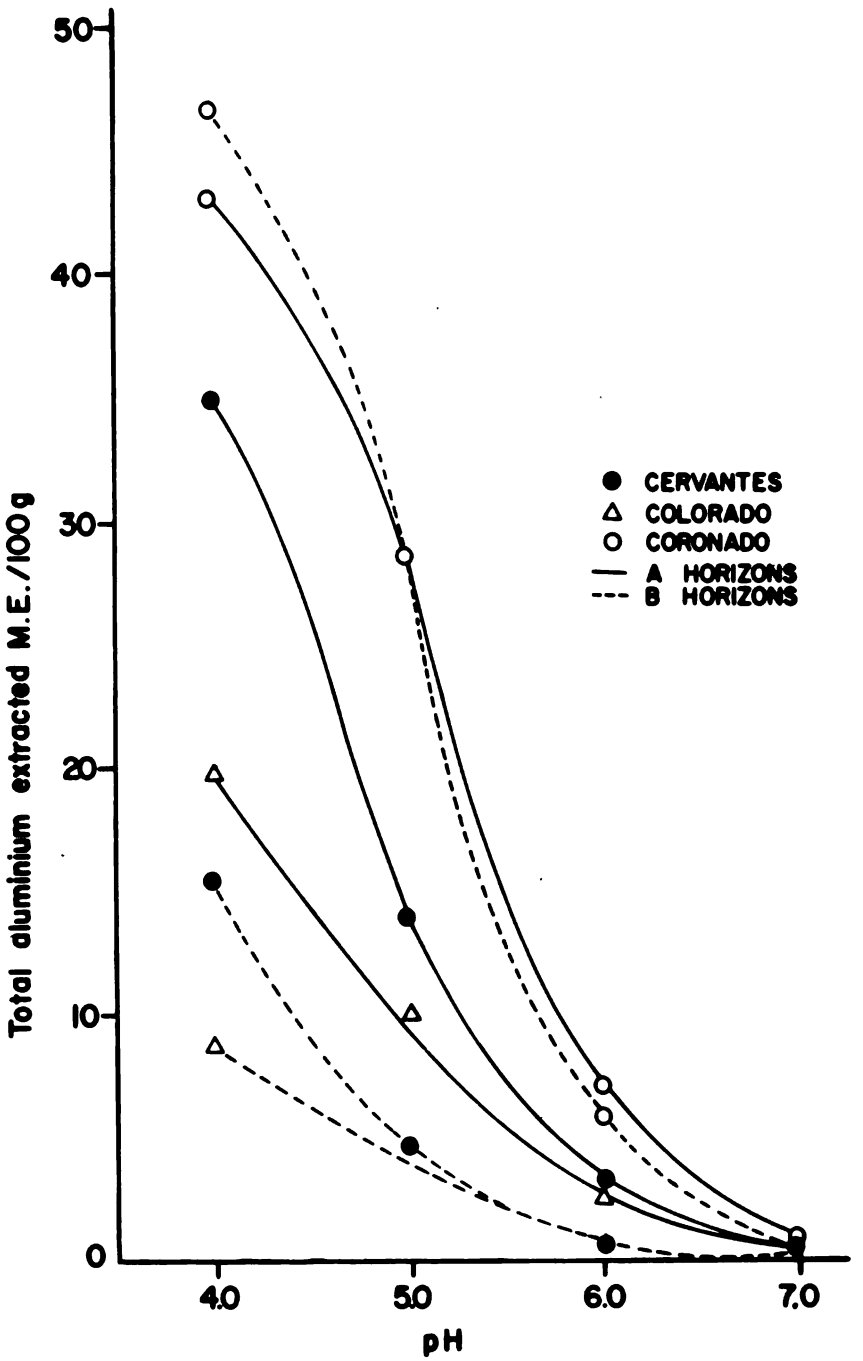


Fig. 41. Extractable Al from volcanic soils by the method of NH_4OAc buffered at different pH's.

Table 57. Amounts of H^+ and Al^{+++} displaced by 250 ml of unbuffered salts from volcanic ash soils of Costa Rica

Soil	Horizon	Extracting sol. N	H^+	Al^{+++}	Total
			meq/100 g		
Cervantes	A	KCl	0.23	0.52	0.75
	B	KCl	0.53	0.30	0.83
Birrisito	A	KCl	1.25	2.45	3.70
		NaCl	0.78	1.01	1.80
		$CaCl_2$	1.62	3.49	5.11
	B	KCl	0.53	0.38	0.90
Colorado	A	KCl	1.90	4.43	6.33
		NaCl	0.82	3.65	4.45
		$CaCl_2$	3.31	2.46	6.67
	B	KCl	1.16	3.95	5.11
San Isidro	A	KCl	2.72	6.15	8.87
	B	KCl	1.02	1.00	2.02

Table 58. Exchangeable and non-exchangeable Al determined by successive extractions with N NH_4Cl and N KCl

Soil series	Horizon	N NH_4Cl			N KCl		
		Total	Exch.	Non-exch*	Total	Exch.	Non-exch*
		Al^{+++} meq/100 g					
Birrisito	A	2.73	1.22	0.101	3.18	1.25	0.129
	B	0.87	0.25	0.041	1.79	1.25	0.036
Colorado	A	3.89	2.34	0.103	4.74	2.65	0.139
	B	3.51	2.72	0.053	4.12	3.40	0.048
San Isidro	A	6.96	4.48	0.165	6.35	4.30	0.137
	B	1.24	0.70	0.035	1.15	0.75	0.027

* Aluminum 'dissolved' per extraction.

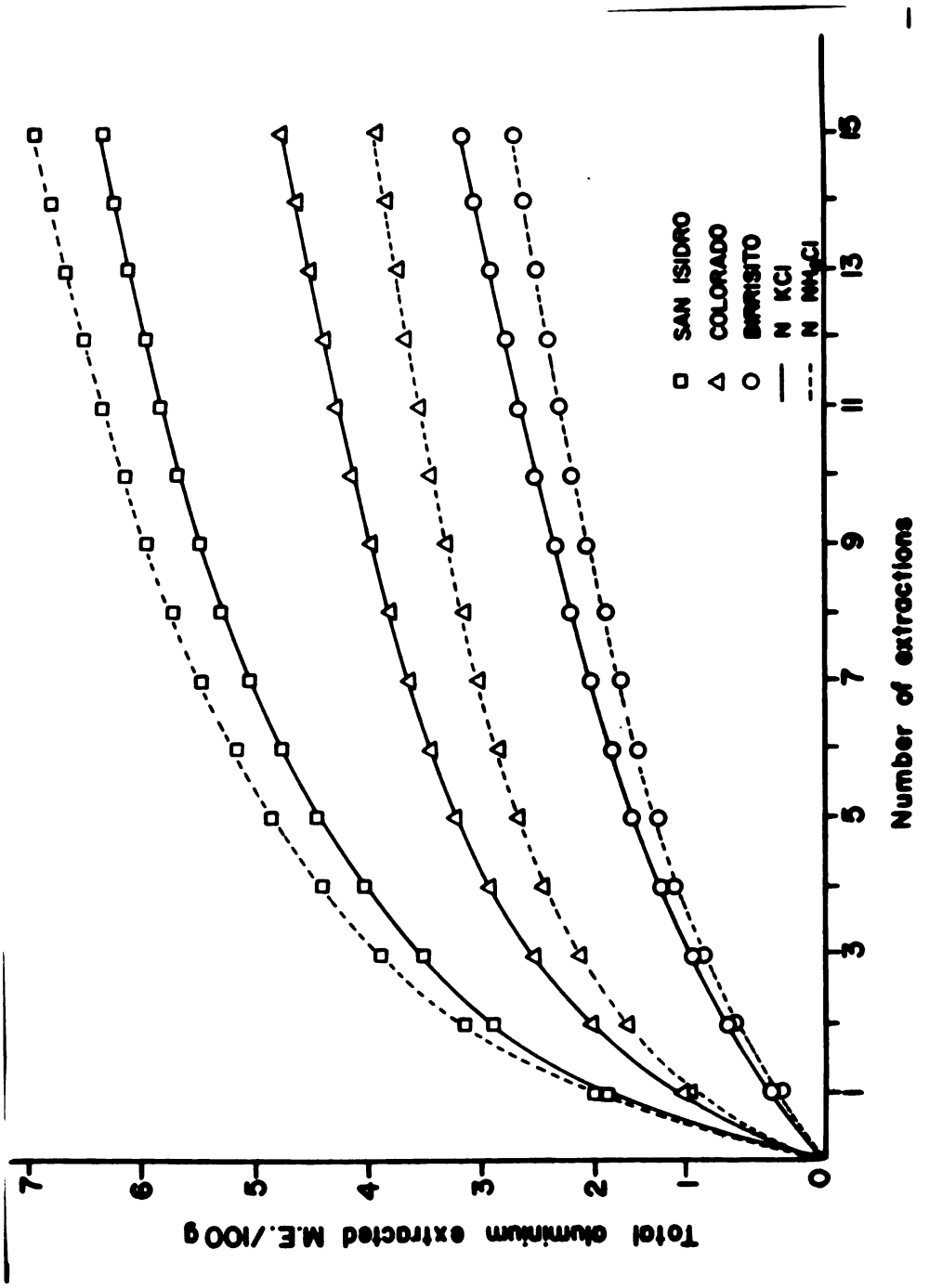


Fig. 42. Cumulative values of Al extracted by N KCl

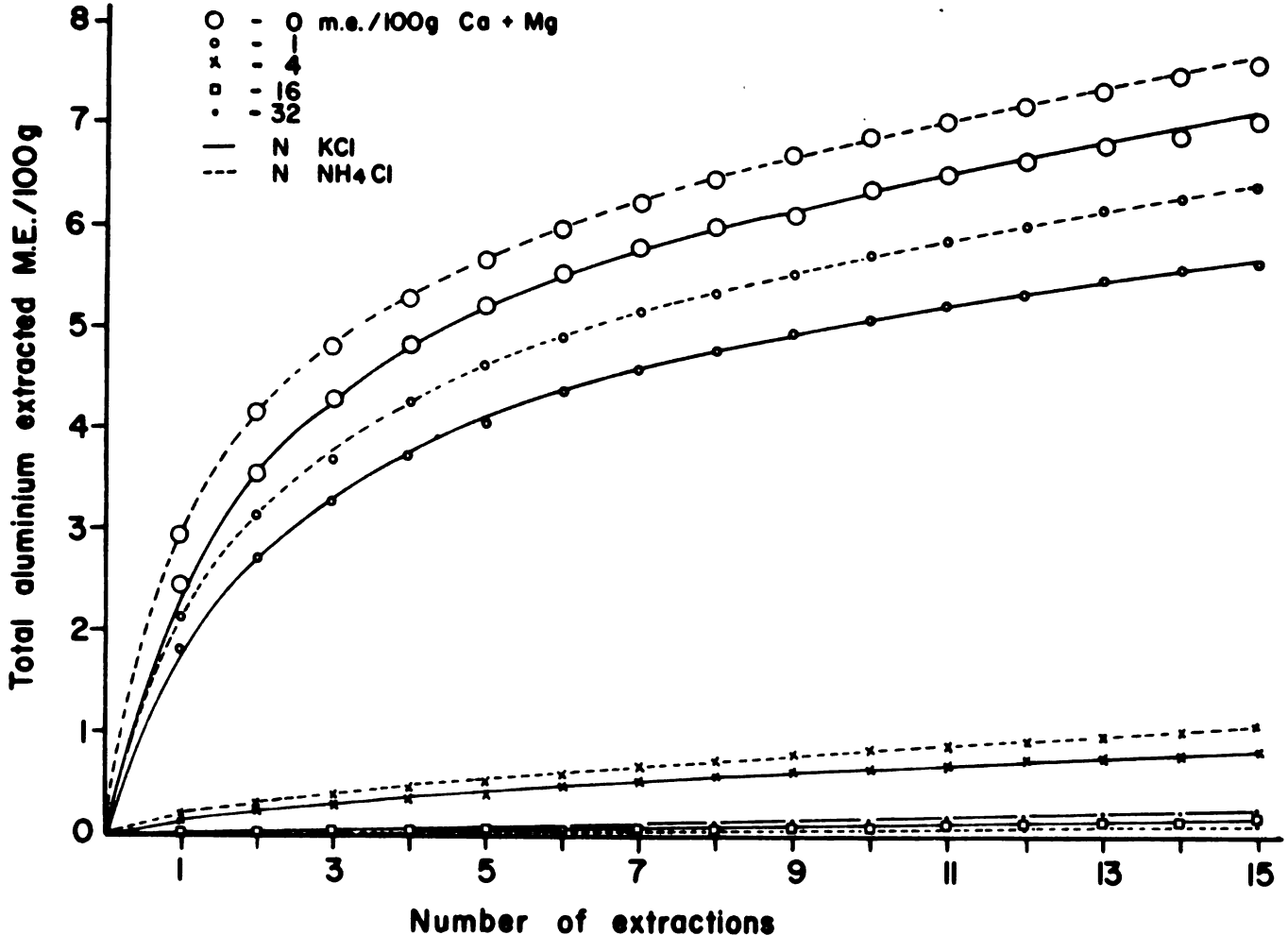


Fig. 43. Effect of liming on KCl extractable Al.

in extractable Al is observed (Table 59). In a typical Andosol (Birrisito) the high proportion of that non-exchangeable Al or low-pH-extractable Al seems to come from organic matter complexes. This Al cannot be extracted by N KCl, but seems to be extracted by cation exchange resin, as shown in Table 60.

Table 59. Effect of H_2O_2 treatment on Al extracted by N KCl and N NH_4OAc, pH 4.8

Soil	Extractable KCl*	Exchangeable	Extractable NH_4OAc**	Extractable Total
	Al meq/100 g			
CERVANTES				
Untreated	0.72	tr	1.44	2.16
H_2O_2 treated	0.62	tr	15.16	15.78
BIRRISITO				
Untreated	3.18	2.25	5.38	8.56
H_2O_2 treated	9.28	7.30	8.22	17.48
COLORADO				
Untreated	4.74	2.65	1.66	6.40
H_2O_2 treated	5.09	4.20	1.61	6.70
SAN ISIDRO				
Untreated	6.35	4.30	1.44	7.79
H_2O_2 treated	7.05	4.95	1.00	8.05

* Exchangeable according to the definition of successive extraction-extrapolation method.
** Extractable following fifteen successive N KCl extractions, as indicated in the 'residual' procedure.

As a conclusion we may indicate that well developed allophanic soil (Birrisito*) presents low exchangeable Al, high amounts of organically complexed Al. When weathering advances, an increase in exchangeable forms of Al is noticed. In the

* Besoain, E. Clay mineralogy of volcanic soils of Costa Rica. (unpublished)

Table 60. Effect of lime applications on CEC, and extractable Al from Birrisito soil

Limestone ton/ha	pH	CEC			Al	
		N KCl	N NH_4OAc	Resin	KCl	Resin
		meq/100 g				
0.00	4.24	7.4	58.3	23.7	4.19	16.1
3.78	5.26	11.9	63.4	20.8	0.80	11.1
7.56	5.72	15.7	61.7	23.5	0.05	5.7
15.12	5.86	20.1	63.9	22.3	0.05	3.9
30.24	6.06	22.9	57.2	23.4	0.00	2.7

initial stage of allophane development (allophane B of Fieldes (1)) organically complexed Al seems to be low as well as exchangeable Al.

References

1. Fieldes, M. 1955. Clay mineralogy of New Zealand soils. Part 2. Allophane and related mineral colloids. N. Zel. Jour. Sci. & Technology 37:336-350.

2. Lin, C. and Coleman, N. T. 1960. The measurement of exchangeable Al in soils and clays. SSSA P 29:444-446.

3. McLean, E. O., et al. 1959. Aluminum in soils. III. A comparison of extraction methods in soils and clays. SSSA P 23:289-293.

4. Pratt, P. F. and Bair, F. L. 1961. A comparison of three reagents for the extraction of Al from soils. Soil Sci. 91:357-359.

5. Skeen, J. B. and Sumner, M. E. 1967. Exchangeable Al. I. Efficiency of various electrolytes for extracting Al from acid soils. S. Afr. J. Agri. Sci. 10:3-10.

b. Cation exchange capacity (CEC) of Andosols (K. Igue, R. Fuentes and M. Morelli)

Cation exchange and retention properties are associated with negative charge developed by soil colloids. In

soils of tropical regions the negative charge developed by soil is quite variable and it is affected by soil pH (5). When conventional methods, such as buffered salts or unbuffered neutral salts are used to measure CEC, two problems arise: a) the retention of excess saturating salts which gives erroneous values of CEC, and b) the hydrolysis of adsorbed cation due to the washing of excess salts (4). This problem is aggravated when we deal with allophanic soils high in organic matter content. The reliable method for measuring CEC is therefore lacking.

To obtain this information a series of studies were conducted by comparing several conventional methods and compared with isotopic methods using ^{45}Ca (1).

pH-variable CEC: When buffered N NH_4OAc, N $Ca(OAc)_2$ was used at different pH's the CEC increases with pH, as shown in Fig. 44. Different values are obtained for different methods. Higher values are obtained for NH_4OAc due to an incomplete washing of excess salts (4), and lower values in the case of $Ca(OAc)_2$ due probably to hydrolysis (4). In both cases excess salts are washed with ethanol (3 times) and water (twice in the case of $Ca(OAc)_2$ and once for NH_4OAc). When isotopic method was used the washing steps were avoided. The soils were saturated previously with Ca and then equilibrated with ^{45}Ca. If we assume that isotopic exchange takes place following the equation:

$$^{45}Ca\text{-solution} + {}^{40}Ca\text{-soil} \rightleftharpoons {}^{45}Ca\text{-soil} + {}^{40}Ca\text{-solution},$$

so that,

$$\frac{(^{45}Ca\text{-soil})}{(^{45}Ca\text{-sol.})} = \frac{(^{40}Ca\text{-sol.})}{(^{40}Ca\text{-soil})}$$

the CEC can be calculated (1):

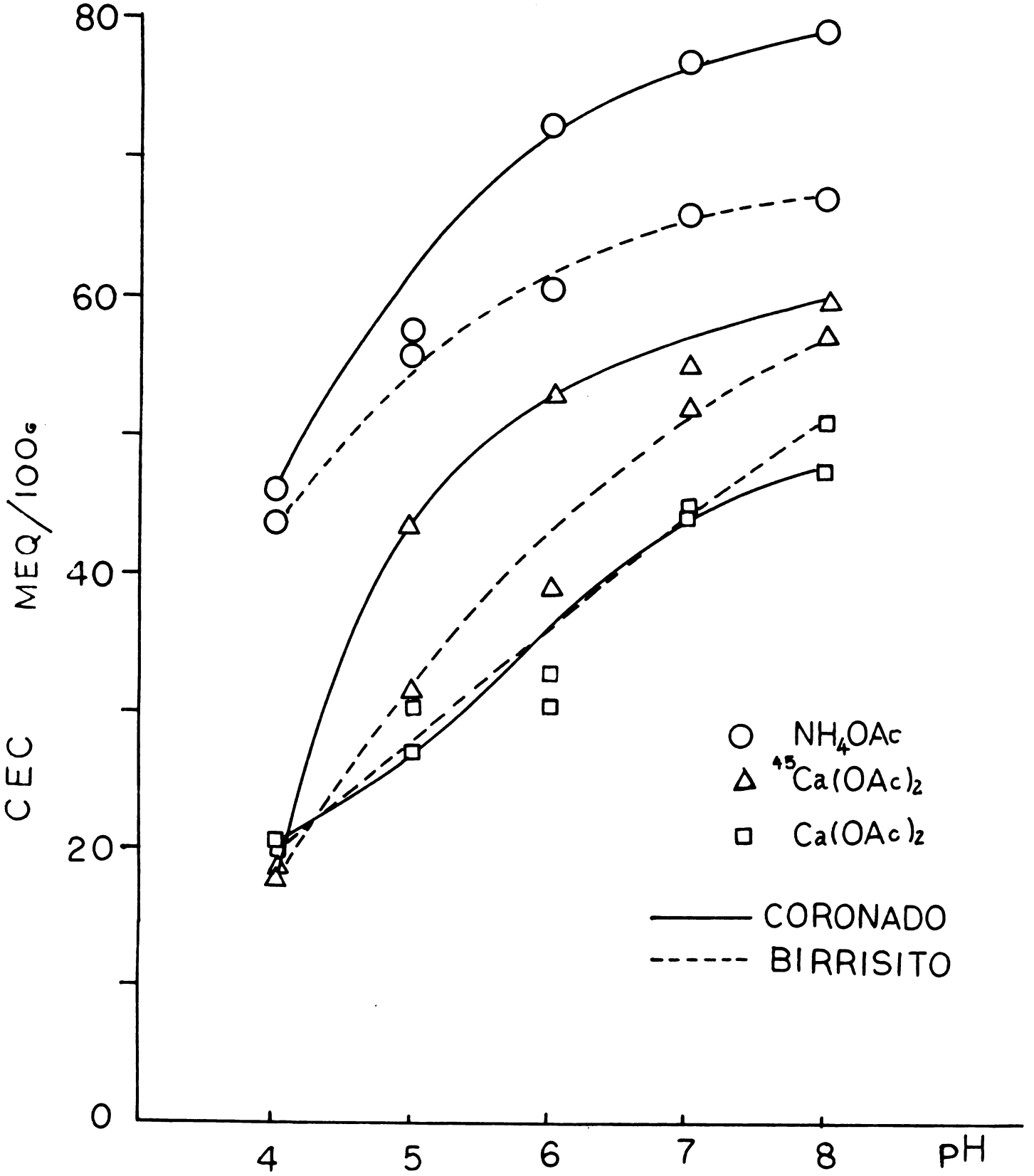


Fig. 44. Cation exchange capacity of two allophanic soils as a function of pH and different methods.

CEC meq/g = $\frac{A}{B}$ x C - C, where

A = initial activity CPM/ml

B = final activity CPM/ml

C = total Ca in equilibrium solution.

This method eliminates the error due to excess salt by subtracting the cation associated with solution retained by the soil (weighing process); and eliminates error due to hydrolysis by avoiding washing. Thus, Fig. 44 shows CEC-values by $^{45}Ca(OAc)_2$ method to be intermediate, and increases mostly in the range of pH 4.0 to 6.0.

According to Wada and Ataka (6), the exchange of ions in allophane is due to two mechanisms: a) electrostatic nature (coulombic) which is due to dissociation of H^+ or OH^- group from -SiOH and -Al.OH in the range of pH 4.0 to 7.0. Therefore, this process is highly pH dependent and not dependent on salt concentration; and b) physical retention of salt molecules with equivalent amounts of cations and anions, which is dependent on salt concentration and independent of pH.

CEC of mineral and organic fractions: Fig. 45 shows the effect of pH on mineral and organic fractions of these soils by the isotopic method.

A higher contribution of organic matter to total CEC is observed as compared to mineral fraction. Furthermore, the increase in CEC of organic matter is pronounced in the lower range of pH, 4.0 to 6, whereas the mineral fraction increases in the range of 6 to 8.0.

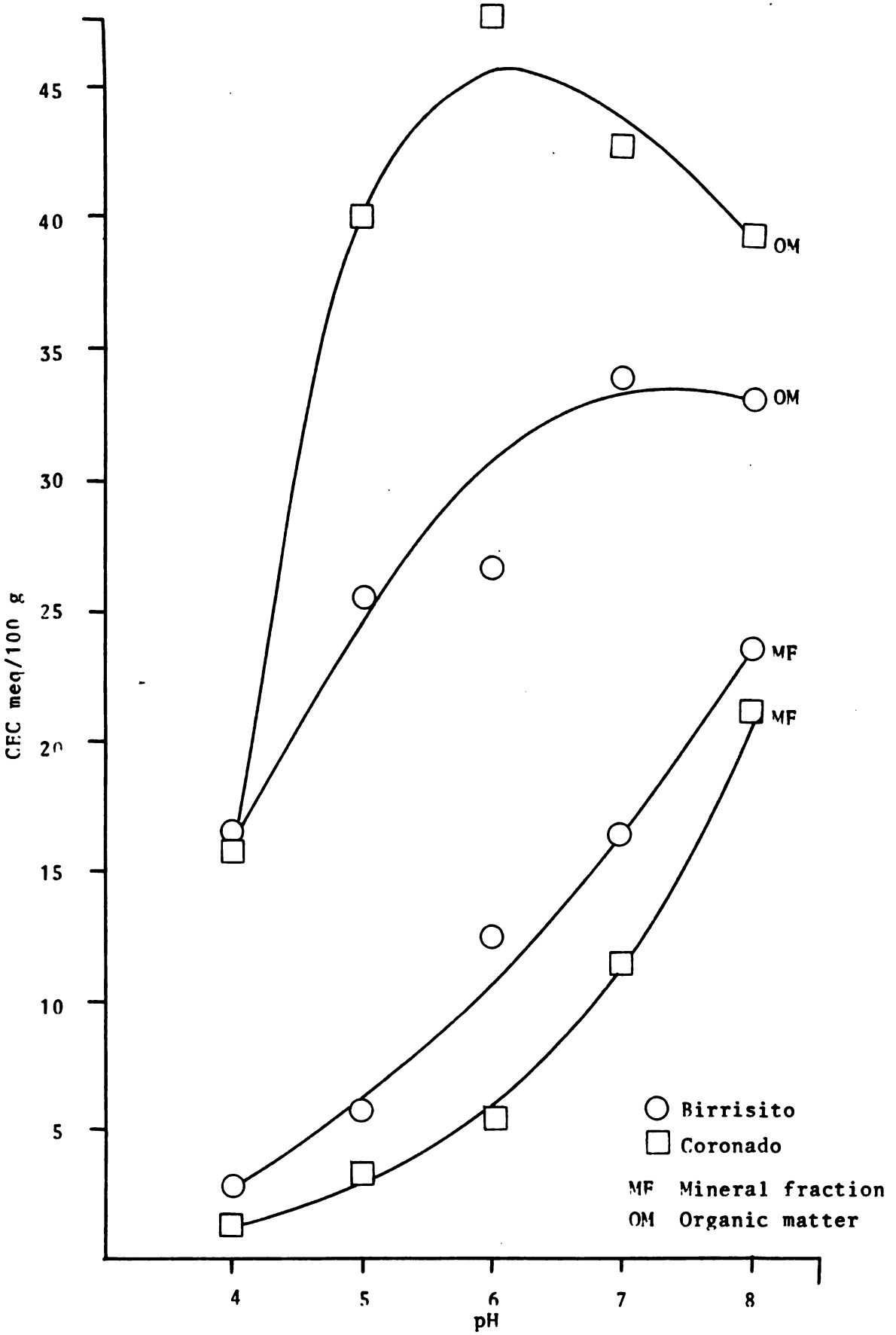


Fig. 45. Cation exchange capacity of mineral and organic fractions in Birrisito and Colorado soils.

Between pH 4.0 and 5.0 the CEC of inorganic fraction of these soils does not exceed 5 meq/100 g, which suggests the non-existence of permanent charge as in the crystalline clay minerals and most of the charges are variable in nature. At low pH, high positive charge may develop (5) with high positive adsorption. The isoelectric pH of these soils should be in the range of pH 5.0 to 6.0 (Basoain, E. 1971 personal communication), and at this point the retention of salts seems to be critical. As the pH increases from isoelectric pH, negative charges increase considerably for inorganic fractions due to deprotonation of the -OH group of SiOH - Al - OH (2, 3). Since the CEC of organic matter is obtained by the difference between total and mineral fraction, the result is the apparent decrease in the CEC of organic matter at higher pH's. When the CEC of the soil is measured part of the charges developed in organic matter-free soils is blocked.

Effect of liming on CEC: Liming application in acid soils is a common practice, and should change the soil pH and Al content. Fig. 46 shows the values of CEC as measured with unbuffered N KCl, sum of bases and exchangeable acidity, and resin methods as well as the conventional N NH_4OAc method. Liming had no effect on CEC-N NH_4OAc nor on CEC-resin, but markedly affected the CEC-KCl and the sum of cations. Here again, CEC-NH_4OAc does not show any definite tendency. As was observed, in the range of pH 4.22 to 6.07 due to liming, most of the CEC variation must be due to organic fraction. High proportion of Al that occupied exchange sites of organic

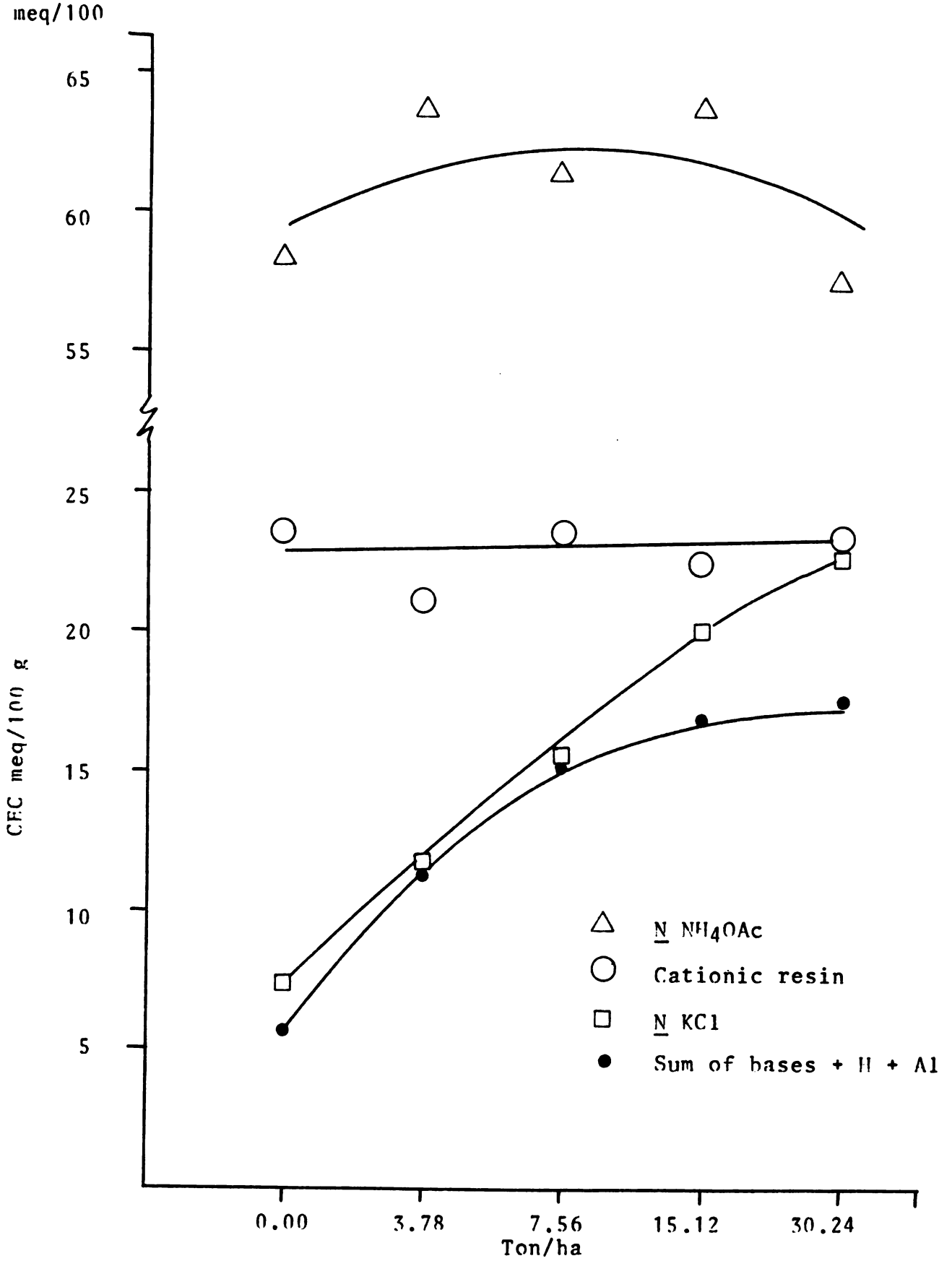


Fig. 46. CEC values obtained by different procedures in Birrisito soil with increasing lime applications.

matter was substituted by Ca. While exchange resin was able to extract that Al to give no change in CEC with pH (CEC-resin), unbuffered KCl detected the difference in CEC due to liming, according to the pH of the system. Unbuffered N KCl was not able to exchange with Al-complexed by organic matter, but upon liming K exchanged with Ca. Therefore, the difference in CEC-resin and CEC-KCl seemed to be accounted for by Al^{+++} extracted by resin.

Conclusion: Based on these studies following were the conclusions:

a) to determine the CEC in allophanic soils, the process of washing excess salt can be avoided to eliminate hydrolysis, and the excess salt can be corrected by substracting the volume of salt retained by the soil after saturating the soil with the cation and equilibrating with a solution of known concentration.

b) Buffered N NH_4OAc methods are not adequate methods under the prevailing conditions.

c) Unbuffered salt solutions ($CaCl_2$, KCl) are useful in determining the CEC at soil pH, but do not eliminate the Al^{+++} occupying the exchange sites of organic matter.

d) Higher percentage of CEC in these soils is due to organic matter.

References

1. Blume, J. M. and Smith, D., 1954. Determination of exchangeable Ca and CEC by equilibration with Ca^{45}. Soil Sci. 77:9-17.

2. DeVilliers, J. M. and Jackson, M. L., 1967. Aluminous chlorite origin of pH dependent CEC variations. SSSA Proc. 31:614-619.

3. Jackson, M. L., 1963. Aluminum bonding in soils: A unifying principle in soil science. SSSA Proc. 27:1-10.

4. Rich, C. C., 1962. Removal of excess salts in cation exchange capacity determinations. Soil Sci. 93:87-94.

5. Schofield, R. K., 1949. Effect of pH on electric charges carried by clay particles. J. Soil Sci. 1:1-18.

6. Wada, K. and Ataka, H., 1958. Ion uptake phenomena of allophane. Soil Sci. and Plant Food 4(1):12-18.

2. Phosphate Chemistry in Tropical Soils

a. Isotopically exchangeable P. I. Measurement of E value in soils of Central America (K. Igue and R. Fuentes)

The use of tracers to study the exchange reactions of phosphates in soils was first indicated by McAuliffe *et al.* in 1947 (4). Based on the isotopic exchange process, radioactive ^{32}P was used to measure the extent of the following reaction:

$$\text{Surface-}^{31}P + \text{solution } ^{32}P \leftrightarrow \text{Surface } ^{32}P + \text{solution } ^{31}P$$

at equilibrium,

$$\frac{\text{Surface-}^{32}P}{\text{solution}^{32}P} = \frac{\text{Surface-}^{31}P}{\text{solution}^{31}P} \qquad (1)$$

Based on exchange study, McAuliffe *et al.* indicated that two distinct reactions are involved in the kinetics of the changes of ^{32}P between solution and surface. They indicated also, that the amount of rapidly equilibrating surface P parallell levels of available P. Russell *et al.* (9) later on introduced the following expression to determine isotopically

exchangeable P

$$E_t = \frac{X_t}{Y_t} y-x \qquad (2)$$

where:

X_t and Y_t = amounts of ^{31}P and ^{32}P in the solution after time t.

x and y = quantity of soil P which has undergone change.

Several other expressions had been derived with the purpose of determining the labile phosphate in soils (1, 6, 5). Isotopically exchangeable P in soils had been indicated as varying with extrinsic factors and intrinsic soil properties as discussed by Mattingly and Talibudeen (6). Russell et al. (9) indicated that sorption of P during the experiment may cause error if sorbed P exchanged slowly. McAuliffe et al. (4) also indicated erratic values when handling dilute solutions such as the case of soils of low phosphate status. More recently, Amer (1) indicated the difficulty presented in measuring 'E' in high P fixing soils. He indicated that tracer losses are significant and the carrier-free method is considered unsatisfactory for a soil having more than 90% fixation percentage. When carrier was used, the author recommended 0.2 ppm solution which proved to be satisfactory for soils having a P fixation percentage of 20 or below. Tracer losses seem to be negligible in the case of low P fixing soils, and carrier-free methods can be used.

The soils of Central America, as most of the tropical soils, require heavy P fertilization in order to achieve

adequate yield. This study was undertaken with the purpose of gaining information of labile phosphate on these soils, and how this can be related to available P index.

Isotopic Exchange Measurement

Soil samples used in this study were collected from Guatemala, Salvador, Honduras, Nicaragua and Costa Rica by Müller et al. (7). Most of these soils are influenced by volcanic ash. Isotopically exchangeable P measurement was carried out by using carrier-free and carrier method. The concentration chosen for carrier method was 1.0 ppm.

Table 61 presents the 'E' values obtained by both the carrier and the carrier-free methods. It also shows surface-^{31}P according to McAuliffe et al. (4) and IDF (isotopic dilution factor) and Pm values, according to McConaghy et al. (5). Table 62 shows the correlation coefficients between different methods obtained for these soils. The 'E' values measured with the carrier and carrier-free methods are both closely associated (r^2=0.956). The 'E' values are highly associated with ^{31}P-surface (carrier) and ^{31}P-surface (carrier-free), Pm, IDF. Both 'E'values measured are associated with tests for available P in the following sequence: Olsen>Bray>Egner-Rheim>Mehlich. Among P fractions Ca-P>Al-P>Fe-P. There was relatively high association between resin extracted P and 'E' values (r^2>0.7).

When P-absorbed by the plant was related to those different methods the highest correlation was observed for resin-P. According to the r^2 values obtained, it can be grouped in the

Table 61. Origin and phosphate fractions as measured by different methods in soils of Central America, after Müller et al (1968)

Soil series	Classification	No.	Carrier-free			Carrier				Resin-P		Retention capacity	Plant test	
			E_1	$^{31}P_1$	C_p	E_2	$^{31}P_2$	P_m	IDF	$P_{res.}$	$P_{eq.}$	MgP/100g	dry wt. g/pot	P-absorb. mg/pot
			ppm											
GUATEMALA														
Mongoy	Brunizen	G-1	14.0	0.9	0.8	1.0	1.8	-	-	46.5	15.0	101	0.3	1.1
Toltecate	Brunizen	G-2	18.0	0.8	0.6	21.3	0.6	22.8	0.3	58.5	17.0	112	0.2	0.6
Cauque	Andosol	G-6	2.3	0.5	0.9	-	1.5	-	-	68.0	22.5	91	0.3	0.3
Valle	Andosol	G-7	30.0	3.5	4.0	27.5	7.3	27.3	4.6	164.0	52.0	49	20.0	41.4
Tequisate	Brunizen	G-11	38.1	8.0	11.3	26.0	1.3	26.8	10.8	145.0	51.0	88	14.0	47.3
Palin	Andosol	G-17	5.0	0.5	0.5	-	1.3	-	-	36.0	11.0	103	5.0	11.5
Camancha	Andosol	G-21	33.0	2.0	2.0	1.0	3.0	1.0	0.1	63.0	21.0	100	0.9	1.1
Mongoy	Litosol	G-31	13.8	1.3	1.3	5.8	6.0	5.8	1.6	78.5	22.5	72	2.6	8.9
Techan	Andosol	G-8	11.0	0.8	0.8	7.3	3.0	6.8	0.8	47.0	12.5	52	1.0	1.4
EL SALVADOR														
Pasaquina	Andosol	S-2	64.8	7.8	9.1	55.8	14.8	55.8	13.3	145.0	51.5	141	15.0	54.7
Ozatlan	Brunizen	S-7	9.8	2.0	2.9	26.8	5.8	27.0	3.5	68.7	18.5	156	19.0	19.4
Azuaqualpa	Brunizen	S-20	2.5	0.8	0.8	-	1.0	-	-	31.1	7.5	156	0.3	0.6
Aramanca	Andosol	S-25	0.5	0.5	0.4	0.3	4.5	-	-	35.0	11.0	78	0.8	1.3
HONDURAS														
Talanga	Fluvisol	H-8	4.3	0.8	0.9	-	0.5	-	-	46.8	12.5	152	4.7	9.4
Talanga	Regosol	H-12	2.0	0.5	1.0	-	4.8	-	-	28.5	8.5	132	1.4	2.5
A-S	Planosol	H-15	2.8	0.4	0.5	3.3	3.0	3.3	3.8	68.5	18.5	95	8.2	18.9
NICARAGUA														
Estelí	Fluvisol	N-5	3.0	0.5	0.8	-	4.5	-	-	39.0	11.0	166	8.5	17.8
Concordia	Fluvisol	N-6	2.0	0.5	0.5	3.5	4.5	2.8	0.5	41.0	14.0	124	4.6	10.1
Estelí	Fluvisol	N-7	34.5	7.0	9.5	25.3	12.3	25.5	10.3	170.0	51.0	41	15.0	51.0
Estelí	Fluvisol	N-8	37.0	6.0	7.6	40.8	11.3	40.3	9.0	215.0	81.0	115	20.6	76.2
Estelí	Fluvisol	N-9	81.3	15.0	19.5	53.3	17.3	53.5	17.8	306.0	91.0	156	23.5	96.3
Tipitapa	Andosol	N-17	3.8	0.9	0.8	-	0.5	-	-	27.5	7.5	168	0.8	1.6
La Virgen	Vertisol	N-23	168.3	31.5	42.0	158.3	33.8	158.0	38.3	295.5	84.0	80	18.7	127.1
COSTA RICA														
Siquirres	Fluvisol	CR-8	2.3	0.5	0.5	-	1.0	3.3	0.2	93.8	30.0	158	9.2	24.8
Nicoya	Planosol	CR-33	tr	tr	tr	-	0.5	-	-	89.5	27.5	300	4.0	10.4
Reventazón	Cambisol	CR-44	18.5	4.3	6.5	37.5	6.3	35.5	4.0	175.5	66.0	212	17.9	53.7
Palmar Sur	Fluvisol	CR-47	21.3	3.5	4.5	24.0	7.0	24.0	4.1	244.5	80.0	218	16.8	60.5
Purires	Cambisol	CR-55	2.8	0.5	0.8	-	3.5	-	-	61.0	17.0	109	1.6	3.7
Térraba	Fluvisol	CR-66	3.8	0.8	1.0	6.8	3.0	5.8	0.6	81.5	27.5	100	18.7	28.1
Encanto	Cambisol	CR-68	3.0	0.8	0.8	-	0.5	-	-	75.0	25.0	-	6.5	15.0

Table 62. Correlation matrix for different variables

	Isotopical exchange		Surface P		P_m	IDF	Resin-P		Retention % P	Available Test				Phosphate fractions			
	E_1*	E_2**	$^{31}P_1$*	$^{31}P_2$**			P_{res}	$P_{eq.}$		Bray	M.C.	Olsen	F.R.	P_{NH_4Cl}	P_{Al}	P_{Fe}	P_{Ca}
E_1*	1.000	0.956	0.979	0.928	0.957	0.970	0.775	0.714	-0.130	0.859	0.693	0.896	0.845	0.51	0.524	0.461	0.656
E_2**		1.000	0.958	0.932	0.999	0.961	0.781	0.733	-0.057	0.909	0.686	0.952	0.849	0.494	0.641	0.576	0.791
$^{31}P_1$*			1.000	0.928	0.959	0.987	0.789	0.724	-0.104	0.893	0.736	0.899	0.857	0.538	0.541	0.504	0.672
$^{31}P_2$**				1.000	0.930	0.938	0.778	0.719	-0.106	0.780	0.722	0.872	0.865	0.627	0.509	0.439	0.700
P_m					1.000	0.962	0.781	0.733	-0.057	0.908	0.699	0.951	0.850	0.493	0.636	0.575	0.789
IDF						1.000	0.800	0.740	-0.115	0.894	0.801	0.916	0.892	0.594	0.520	0.487	0.696
P_{res}							1.000	0.986	0.079	0.724	0.721	0.702	0.890	0.663	0.616	0.435	0.675
$P_{eq.}$								1.000	0.102	0.702	0.678	0.673	0.860	0.670	0.615	0.407	0.675
% P									1.000	-0.086	-0.169	0.047	0.049	-0.012	0.256	0.342	0.254
P-absorbed										0.890	0.793	0.830	0.930	0.725	0.599	0.463	0.739
Dry matter										0.632	0.652	0.613	0.740	0.652	0.496	0.380	0.642

* Carrier-free
** Carrier

following sequence: P-resin, Egner-Rheim (r^2>0.9)>IDF, ^{31}P, E_2, Pm, $^{31}P_2$, E_1, Bray, Olsen (r^2>0.8)>Mehlich, NH_4-P, Ca-P (r^2>0.7)>Al-P, Fe-P (r^2>0.6).

In general carrier method associated better with different parameters. This supports previous work by Amer (1), indicating that carrier method is more reliable in measuring E value in high-P fixing soils. In fact, 'E' values are negatively correlated with percentage of P retention (%P). The range of P retention capacity varies from 10 to 60%. The highest retention capacity among these soils was found to be those from Costa Rica, which also showed the lowest P concentration in the solution.

Fig. 47 shows the adsorption isotherm for three soils of Costa Rica. The characteristics of these soils are indicated in Table 63. Birrisito, which is a typical allophanic soil, showed highest adsorption capacity as compared to the other two soils.

To further verify how these soils release P, samples of Birrisito and Colorado soils were first saturated with carrier-free ^{32}P solutions. Following ^{32}P fixation by the soil, a second equilibration was carried out with solutions of increasing P concentration. From Table 64 it is possible to observe that almost 100% of ^{32}P was retained by the soil. The calculated 'E' value shows a marked variability indicating the unreliability of the method. When a second equilibration was carried out only a little ^{32}P was released from the soil. The soil continued to gain phosphorus from the solution. This is

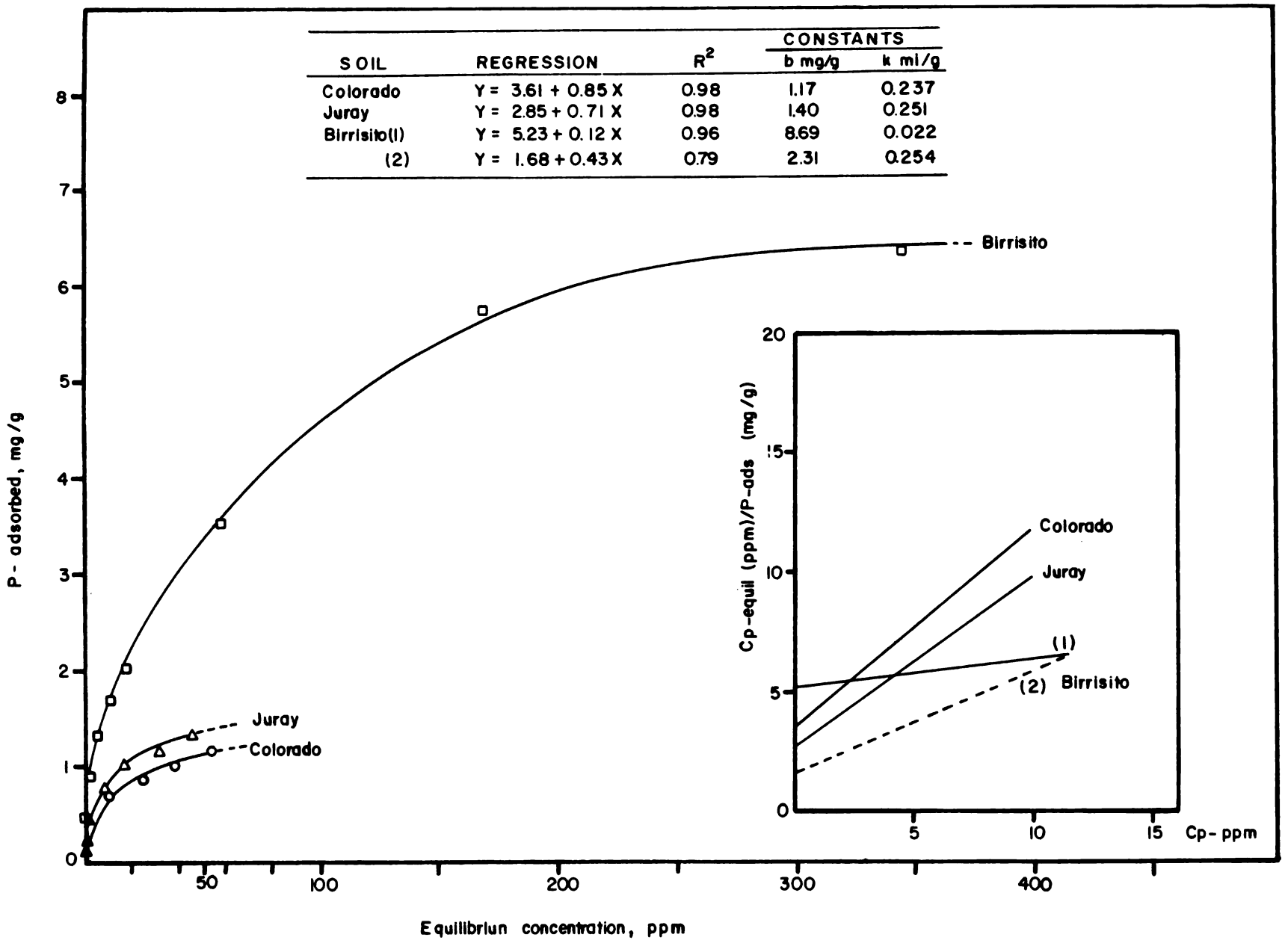


Fig. 47. Adsorption isotherm for three highly P fixing soils of Costa Rica.

Table 63. Chemical, physical and mineralogical characteristics of the soil used in phosphate studies

	Juray (Distropepts*)	Birrisito (Dystrandepts*)	Colorado (Distropepts*)
pH	6.2	5.7	5.2
Organic matter %	12	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
Mineralogy[3]	Allophane (d) Gibbsite	Allophane (d) Gibbsite (c) Ferric gels (p)	Allophane (p) Metahalloysite (d) Gibbsite (p) Gethite/hematite (p) Ferric gels (p)

* Classified by Knox and Maldonado, and Aguirre.
** Hashimoto and Jackson's method. Bornemisza and Igue.
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 Perrot.
d = dominant; p = present; c = common.

Table 64. Variability of E value by carrier-free method in two highly P retentive soils of Costa Rica

First Equilibration			Second equilibration			
Equilibrium ppm	Concentration* CPM/ml	E value	Initial concentration ppm	Final concent. ppm	CPM/ml recovered	P ppm retained or released
			C O L O R A D O S O I L			
0.015	66	25	0.0	0.005	23	-0.005
0.015	55	30	0.0	0.015	92	-0.015
0.025	48	58	0.5	0.015	21	+0.485
0.015	62	27	0.5	0.015	21	+0.485
0.015	67	25	1.0	0.015	21	+0.985
0.015	71	24	1.0	0.005	18	+0.995
0.025	62	44	2.0	0.015	34	±1.985
0.010	70	16	2.0	0.015	26	±1.985
0.005	68	8	4.0	0.025	34	+3.975
0.005	75	8	4.0	0.035	36	+3.965
			B I R R I S I T O S O I L			
0.015	42	35	0.0	0.015	50	-0.015
0.025	41	62	0.0	0.005	56	-0.005
0.035	35	100	0.5	0.005	20	+0.495
0.020	36	55	0.5	0.015	27	+0.485
0.015	52	29	1.0	0.025	23	+0.975
0.015	39	38	1.0	0.015	21	+0.985
0.005	49	10	2.0	0.020	23	+1.980
0.015	44	33	2.0	0.015	20	+1.985
0.025	38	66	4.0	0.035	22	+3.965
0.035	43	83	4.0	0.005	23	+3.995

* Initial activity added: 110×10^3 CPM/ml. Twenty five ml of solutions was equilibrated with 1 g of soil. First equilibration - 3 hours, and the second, 30 minutes.

in agreement with previous observations of McAuliffe *et al.* (4), Russell *et al.* (9) and Amer (1) that indicated erratic values for high retentive soils.

Quantity/intensity relationship

Since the P retentive properties of soil were known,

it was necessary to know next how the P fixed by the soil can be exchanged, or how it affects the immediately labile pool of P. Beckett and White (2) introduced the quantity-intensity concept and found that quantity parameter (Q_o) is smaller than the 'E' value measured.

The second part of our study was primarly concerned with the pool of 'immediately labile' phosphate at different regions of Langmuir's isotherm, and how it is related with E, Q_o and plant available P.

In order to obtain Q/I relationship the soils were initially treated with P, by applying doses that corresponded to an arbitrary chosen region of isotherm. Thus Birrisito soil adsorbed 0.50, 1.50, and 2.48 mg P/g soil; Colorado adsorbed 0.25, 0.78 and 1.320 mg P/g of soil, respectively, in regions I, II and III. Following the first treatment, they were equilibrated with ^{32}P labelled solutions of increasing concentrations: 0, 0.1, 0.2, 0.5, 1.0, 2.0 and 4.0 ppm. Second equilibration time was 30 minutes to minimize diffusion and microbial activity. Following equilibration, the amount of P adsorbed or released by the soil was determined and the plot of Q/I relationship obtained as shown in Fig. 48.

Table 65 indicates the values of Q/I parameters (Q_o, AR_o, PBC), E values, extractable P, and P-absorbed by corn and bean plants.

The data shows that E values, Pm, and ^{31}P-surface increases from region I to III for both soils. Quantity (Q_o) and intensity (I_o) also increase, whereas the PBC ($\Delta Q/\Delta I$) decreases with P saturation. Under this highly fixing soils E values by carrier-

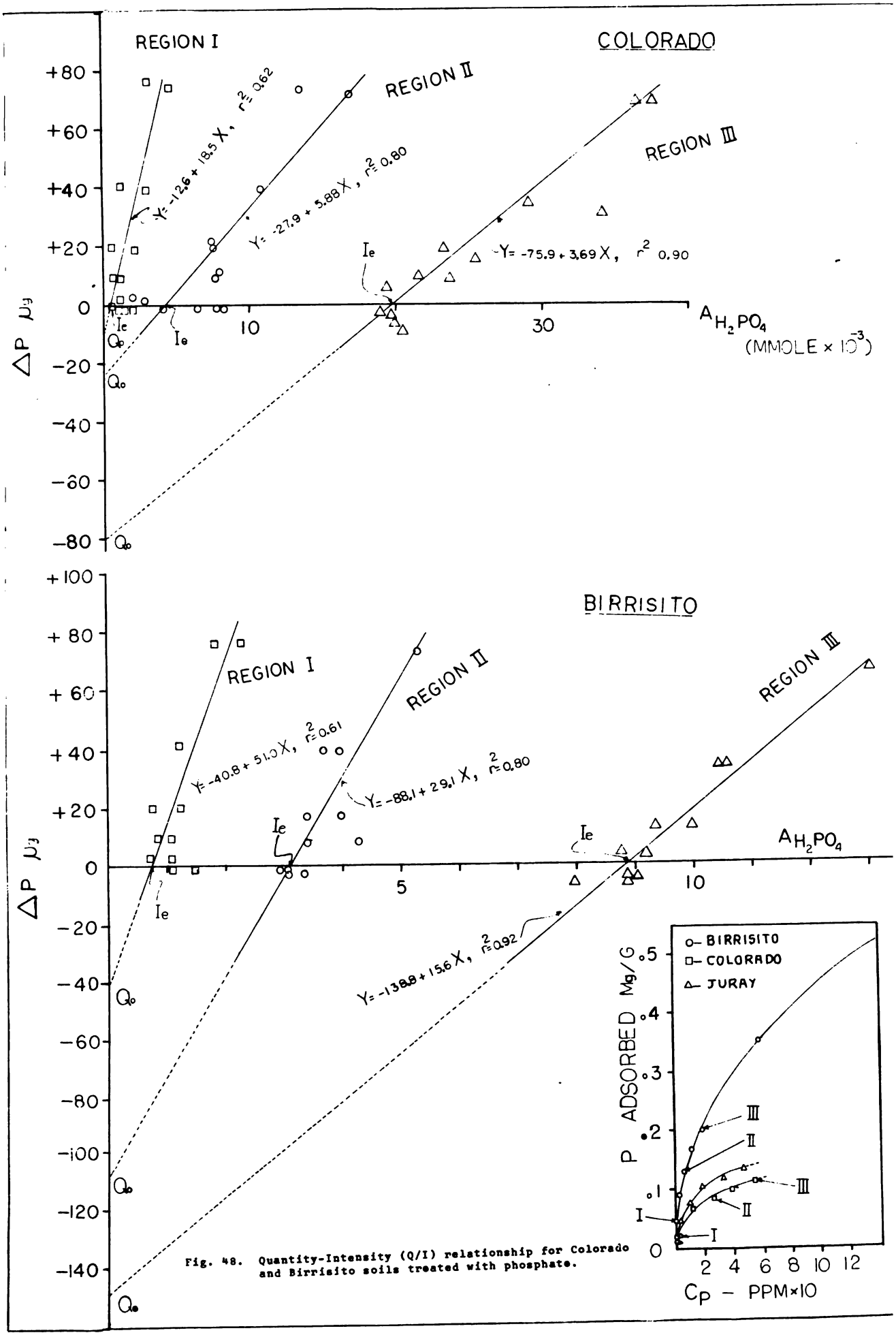


Fig. 48. Quantity-Intensity (Q/I) relationship for Colorado and Birrisito soils treated with phosphate.

Table 65. Available P index as measured by plant uptake, Q/I parameter, isotopic method and extraction method

Isotherm regions	Mg/P g soil	P-uptake corn mg/pot Total	P-uptake corn mg/pot $P_{fert.}$	P-uptake beans mg/pot Total	P-uptake beans mg/pot $P_{fert.}$	Dry matter g/pot Bean	Dry matter g/pot Corn	Q/I Parameter ug/g $-\Delta Q_o$	Q/I Parameter mmole $AR_o \times 10^{-3}$	Q/I Parameter PBC^P	Extractable P Resin ppm	Extractable P KCl ppm	Extractable P K_2SO_4 ppm	E value ppm	^{31}P surface ppm
I_B	0.50 (500)**	6.7	2.63	6.7	0.95	4.1	5.1	41	0.80	51	42	8	9	1.3* 145	32* 170
II_B	1.50 (1500)	20.8	18.7	16.8	10.3	7.1	11.0	88	3.03	29	34	25	38	3.4* 239	85* 262
III_B	2.48 (2480)	36.5	41.9	21.5	19.0	8.2	14.1	139	8.90	16	134	74	91	6.9* 408	169* 449
I_C	0.25 (250)	7.7	2.64	7.1	1.52	3.1	4.9	13	0.68	19	20	9	11	7.8* 91	193* 91
II_C	0.87 (870)	34.7	24.7	23.1	13.2	6.1	11.4	28	4.75	6	66	39	73	225	271
III_C	1.32 (1320)	33.9	30.8	24.5	18.9	5.0	9.1	76	20.6	4	116	87	142	437	439

* Carrier-free method
** Number in parenthesis in mg/pot
B = Birrisito soil; C = Colorado soil.

free method are much lower than carrier method. In accordance with Beckett and White (2) Q_o values are smaller than E values (carrier), which indicates over-estimation of available P by the tracer technique. As indicated by Amer (1) tracer losses may occur.

Table 66 shows the correlation matrix for different values obtained. The Q_o value shows relatively good association with E value (carrier) followed by P-resin>P-KCl>P-K_2SO_4> P-adsorbed corn>P-adsorbed bean. When plant adsorbed P was related with other parameter the following order was observed: P-K_2SO_4>P-KCl>E>P-resin>$AR_o(I_o)$>Q_o for both corn and beans. The fact that E value under present conditions are highly associated with P-KCl, P-resin, and P-K_2SO_4 indicates that adsorption of P are mostly on the surface of the colloids which can partially exchange with ^{32}P, Cl^-, $SO_4^=$ or can be extracted by resin.

The P-absorbed by crops are somewhat low, and this can be attributed to a short period of growth (4 weeks). It is possible that a successive cropping could give values rather better correlated.

White (10) indicated that during early growth period (28 days) depletion fell mainly on the net-exchange phosphate (Q_o) and once net-exchange sites were depleted, there was some alteration of crystalline phosphate lattices so that some of isotopic-exchange sites were eliminated. This indicates the existence of relationship between Q_o and E. The PBC in this case decreased as the P saturation of the soil increased. White (10) observed

Table 66. Correlation matrix

	P-absorbed		Dry matter		Q/I parameter		Extractable P			Isotopic P
	corn	bean	bean	corn	ΔQ_o	AR_o	P_{resin}	P_{KCl}	$P_{K_2SO_4}$	(E)
Absorbed P										
corn	1.000	0.978	0.725	0.870	0.573	0.685	0.822	0.867	0.872	0.841
bean		1.000	0.666	0.816	0.512	0.740	0.754	0.857	0.899	0.837
Dry matter										
bean			1.000	0.961	0.834	0.211	0.577	0.520	0.422	0.629
corn				1.000	0.756	0.350	0.663	0.647	0.582	0.702
Q/I parameter										
ΔQ_o					1.000	0.429	0.725	0.658	0.499	0.782
AR_o						1.000	0.787	0.919	0.952	0.878
Extractable-P										
P_{resin}							1.000	0.944	0.864	0.933
P_{KCl}								1.000	0.971	0.974
$P_{K_2SO_4}$									1.000	0.926
Isotopic P										
E										1.000

also that upon depletion of net-exchange sites the PBC increased due to the mobilization of non-labile P.

References

1. Amer, F., 1962. Determination of ^{32}P exchangeable phosphorus in soils. In Radioisotopes in soil-plant nutrition studies. IAEA, Proc. Symposium, Bombay, 1962. pp. 43-58.

2. Beckett, P.H.T. and White, R. E., 1964. Studies on the phosphate potential of soils. III. Measurement of phosphate potential. Plant and Soil 20:253-282.

3. Fassbender, H. W., Müller, L. and Balerdi, F., 1968. Estudio de P en suelos de América Central. II. Formas y su relación con las plantas. Turrialba 18:333-347.

4. McAuliffe, C. D., Hall, N. S., Dean, L. A. and Hendricks, S. B., 1947. Exchange reactions between phosphates and soils: Hydroxylic surfaces of soil minerals. SSSA Proc. 21:119-123.

5. McConaghy, S. J., Stewart, W. B. and Malek, M., 1966. Soil phosphate status as measured by isotopic exchange and other techniques. In Soil chemistry and fertility meeting, Inter. Soc. Soil Sci. Aberdeen, 1966. pp. 151-160.

6. Mattingly, G.E.G. and Talibudeen, O. Isotopic exchange of phosphate in soil. Rep. Rothamst. Exp. Sta., 1960. pp. 246-265.

7. Müller, L., Balerdi, F., Díaz-Romeu, R. and Fassbender, H. W. 1968. Estudio de P en suelos de América Central. I. Ubicación, características físicas y químicas de los suelos estudiados. Turrialba 18:319-332.

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

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

10. White, R. E. Buffering capacity of soil on uptake of P by plants. In Int. Congr. Soil Sci., 9th, Trans. Adelaide, Australia, 1968. v.2:787-794.

b. Reactions and efficiency of phosphate fertilizers in volcanic ash soils
(K. Igue, D. Suarez, J. Urrutia and R. Fuentes)

As a consequence of the high P fixing capacity of allophanic soils, an improvement on fertilizer management seemed to be of primary importance under tropical conditions.

The effectiveness of phosphate fertilizers depends upon several factors such as degree of solubilization, size of granules, rate of application, and placement. Several of these factors are interrelated. For instance, the dissolution of monocalcium phosphate decreased 15 times as the granule size increased from 16-20 mesh to 4-5 mesh (8), whereas the effectiveness of insoluble phosphates increased as the granule size decreased (4). The rate of dissolution of fertilizers and subsequent movement affects their efficiency. Under conditions of high P fixing soils the proportion of P reacted with the soil should increase with the solubilization of phosphate (1, 3, 10).

This study was undertaken with the purpose of verifying:

a) the reaction of monocalcium phosphate monohydrate (MCP-C), simple superphosphate (MCP-S), and dicalcium phosphate anhydrous (DCPA) near the site of application, and

b) the efficiency of different granule size on availability of MCP-C in these soils.

The phosphates used were labelled with ^{32}P (0.436 mCi/g P_2O_5) and prepared by TVA. The characteristics of the soil used in this study were presented in Table 63. Phosphate adsorption capacity was already presented in Fig. 47.

Reactions of fertilizers

To achieve the first objective, the original technique of Lindsay and Stephenson (6) which allowed to reproduce the zone near the site of P application was adapted. Fig. 49 shows the schematic diagram of the column used in the experiment. Soils were packed in the column (57 cm^2 cross section) by separating each layer (0.5 cm) with nylon screen. Once the column was set up it was allowed to react for 12 days (moisture 1/3 bar suction). Following that period the columns were leached with 120 mm of water (50 in). The annual rainfall within this area is equivalent to 100-150 in. per year.

When the columns were analyzed for P there was 3 to 7.5% of the original applied P left at the site of application in the case of MCP-C and MCP-S, but as much as 97% remained in the case of DCPA. The distribution of P in different layers of the column is shown in Fig. 50. Most of P remained in the layers close to the site of application and decreased with depth. Only a small fraction of P from DCPA reacted with the soils even under leaching conditions. The fraction of P leached throughout the soil column was small, less than $1^{o}/_{oo}$ for Juray and Birrisito, and 2.5% in the case of Colorado, the less fixing soil.

A higher percentage of P reacted with Al (72-82%) and less with Fe. Only small fractions of Ca-P (4%), and occluded forms of P were found as reaction product. Fig. 51 shows the ratio of Al-P/Fe-P as the distance from the site of application increases. Since Al is the dominant constituent of these soils it reacts quickly with saturated metastable triple point solution

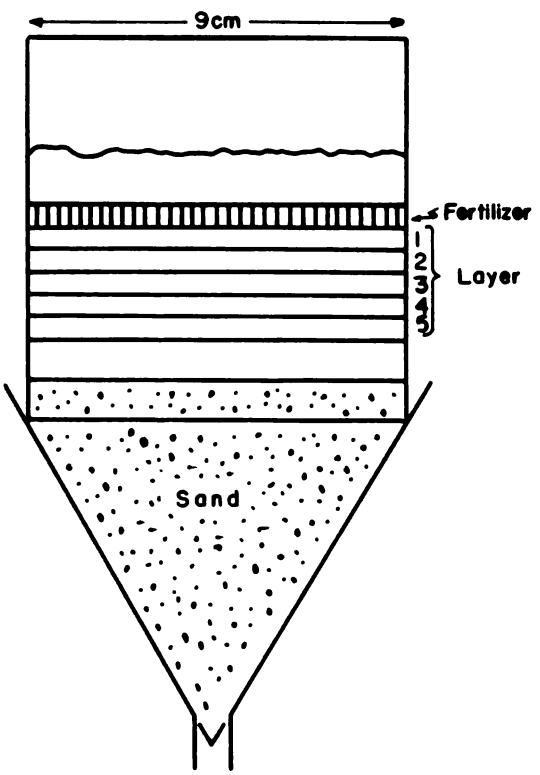


Fig. 4 Layered soil column to study reaction and movement of fertilizer phosphate

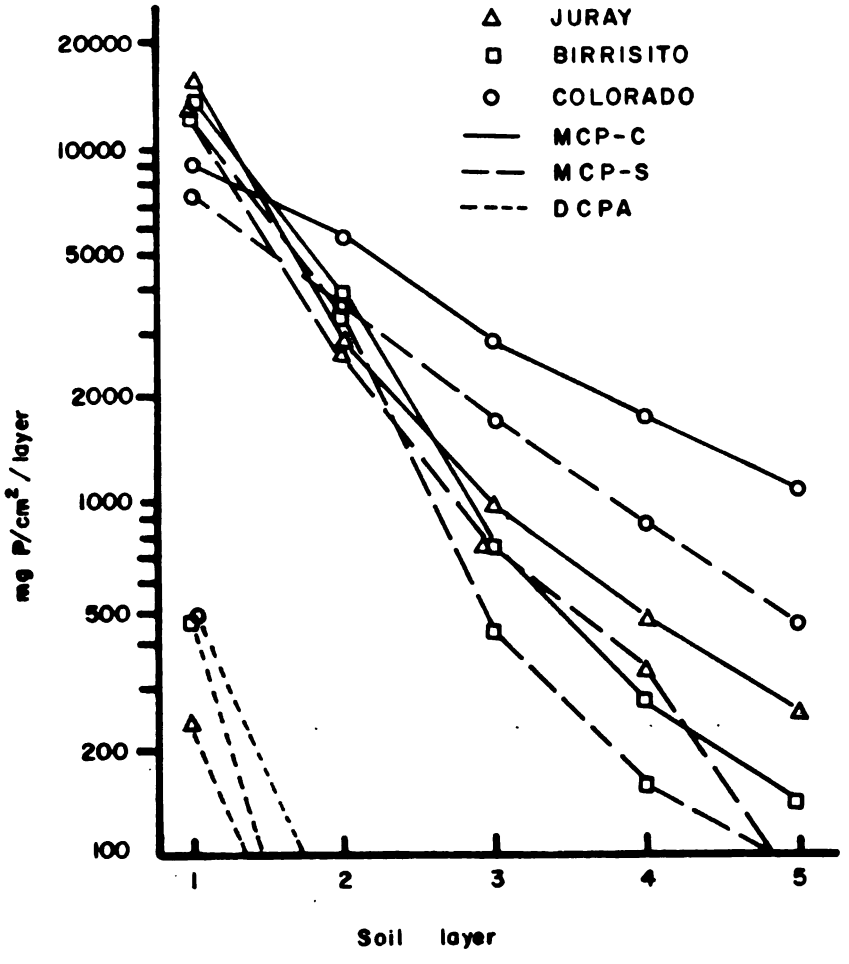


Fig. 50 Distribution of P in successive soil layers following reaction and leaching period

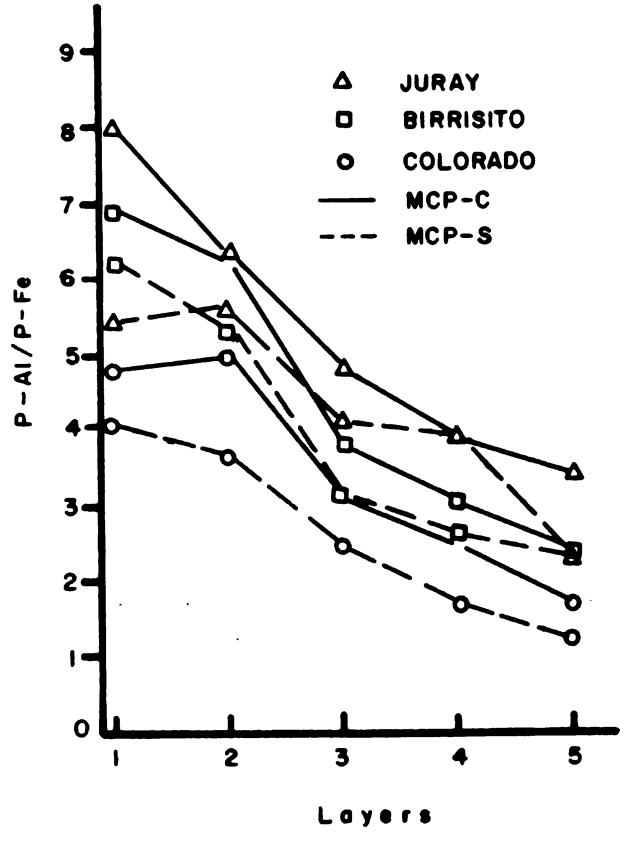


Fig. 51 Ratio of P-Al / P-Fe in successive soil layers of the column.

(MTPS). Chang and Chu (2) found that Fe-P increases with time following P application. They indicated that P-fixation is a surface phenomena, where ionic activity of Al, Fe or Ca had no effect, but only the specific surface. Since Al is the dominant cation on allophanic soils, it is expected that P reacts first with Al. On aging, Al-P tends toward a more stable Fe-P. The relative decrease in Al-P with distance can be explained also as a function of pH, which is lower at the site of application, and solubilizing higher proportion of Al as compared to Fe.

Figs. 52 and 53 show the effect of fertilizers on extractable Al and Fe. Less Al and Fe are extracted from layers adjacent to fertilized zone. Most of this Al and Fe must have reacted with P, relatively higher proportion as Al-P. The DCPA affected Al considerably only for Birrisito soil. As shown by Lindsay and Stephenson (7) MTPS solution due to its high acidity (pH 1.48) dissolves high amounts of Al, Fe, Ca, Mn and K, and the pH is decreased considerably. As shown in Figs. 52 and 53, DCPA did not show significant effect on soil components and increased slightly the pH near the zone of application.

Fertilizer effectiveness

The second part of this study was carried out at the greenhouse. A factorial experiment was used to determine the efficiency of MCP-C in five different granule sizes (-12 mesh, 9-12, 6-9, 3-6, and 1/2 inch size). The fertilizers were mixed with the soil, except for 1/2inch size pellets that were placed at 3 cm depth. Corn was grown for 30 day period and

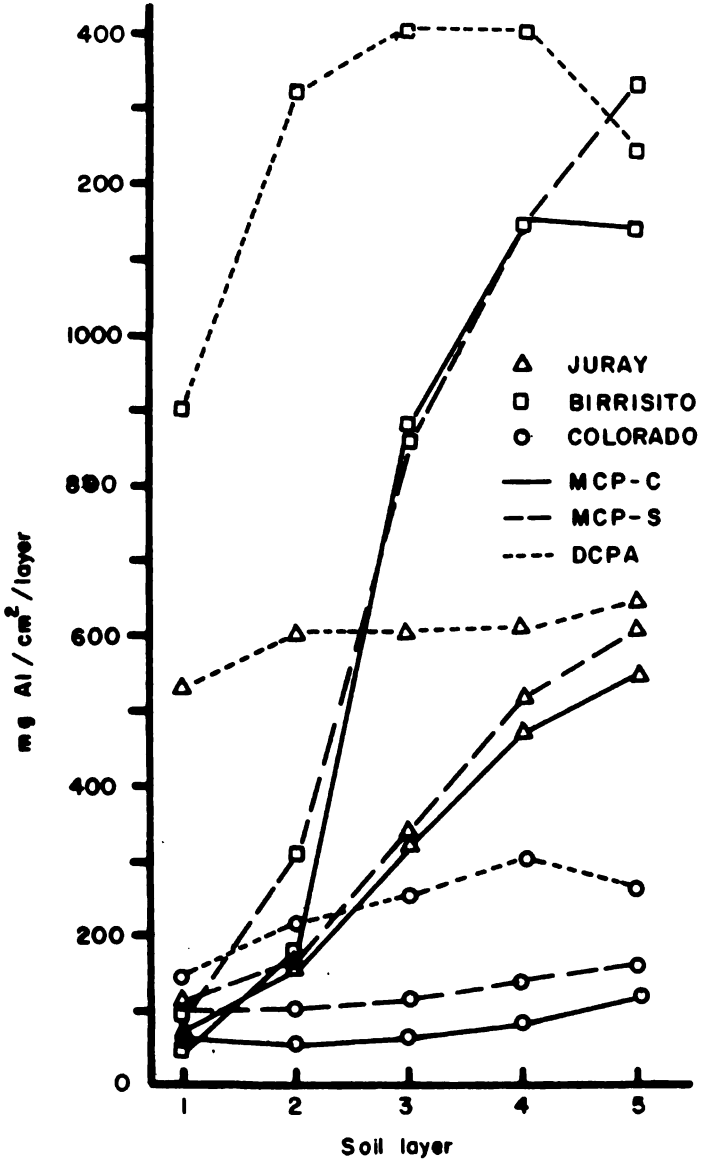


Fig. 52 Effect of different phosphate fertilizers Al by N NH_4 OAc, pH 4.8

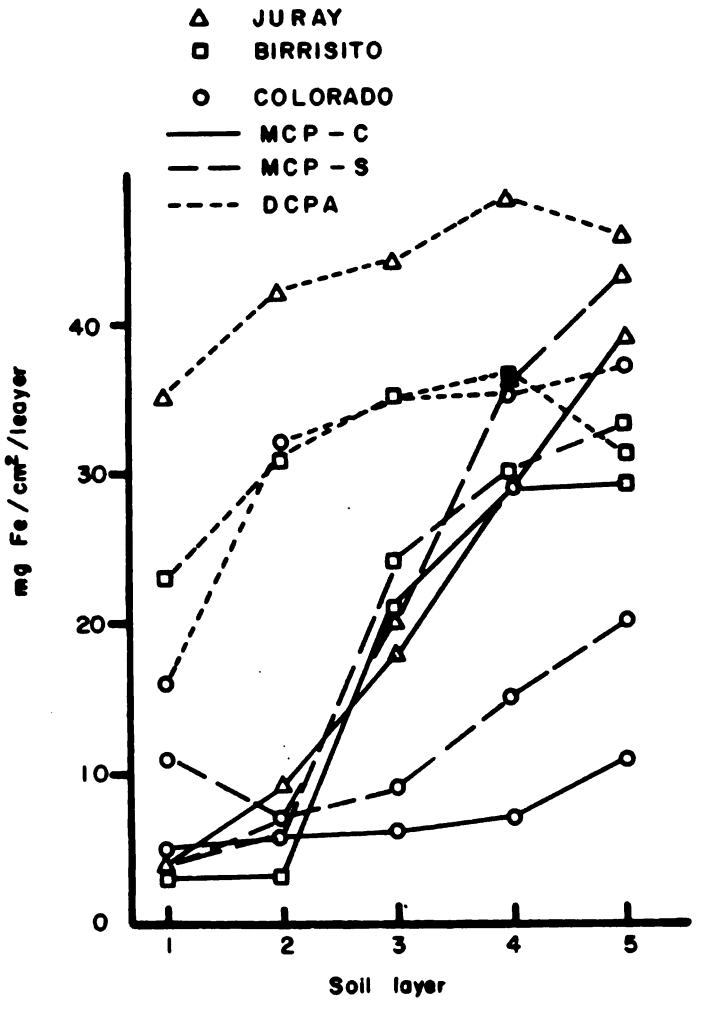


Fig. 53 Effect of different phosphate fertilizers on extractable Fe by N NH_4 OAc, pH 4.8

reseeded for residual phosphate analysis. The effectiveness of fertilizers was calculated as follows:

$$\% \text{ efficiency} = \frac{^{32}\text{P-absorbed}}{^{32}\text{P-applied}} \times 100.$$

The 'A' value according to Terman and Khasawneh (9):

$$A = R\left(\frac{1-y}{y}\right), \text{ where}$$

$$R = \text{fertilizer applied (mg } P_2O_5\text{/pot)}$$

$$y = \frac{\text{P-plant from soil}}{\text{P-plant from fertilizer}}$$

The effect of P doses was highly significant on dry matter, total P uptake, absorption efficiency, and affected the A value. The effect of granule size was also highly significant for all soils. As shown in Figs. 54 and 55, increasing granule size the effectiveness of MCP-C increased for both Birrisito and Colorado soil. In both soils an increase in doses decreased the efficiency, whereas in the case of Juray soil a minimum efficiency was obtained at intermediate dose.

Based on multiple regression analysis the relative effect of granule size on efficiency was calculated and indicated on Table 67. The granule size that maximizes efficiency tends to increase with successive croppings, which indicated that, for long term effect the bigger the size of the granule the higher will be the efficiency of MCP-C in these soils.

Granule size showed also significant effect on calculated 'A' value. It decreased abruptly with an increase in granule size from <12 mesh to 6-9 mesh, and tended to be somewhat

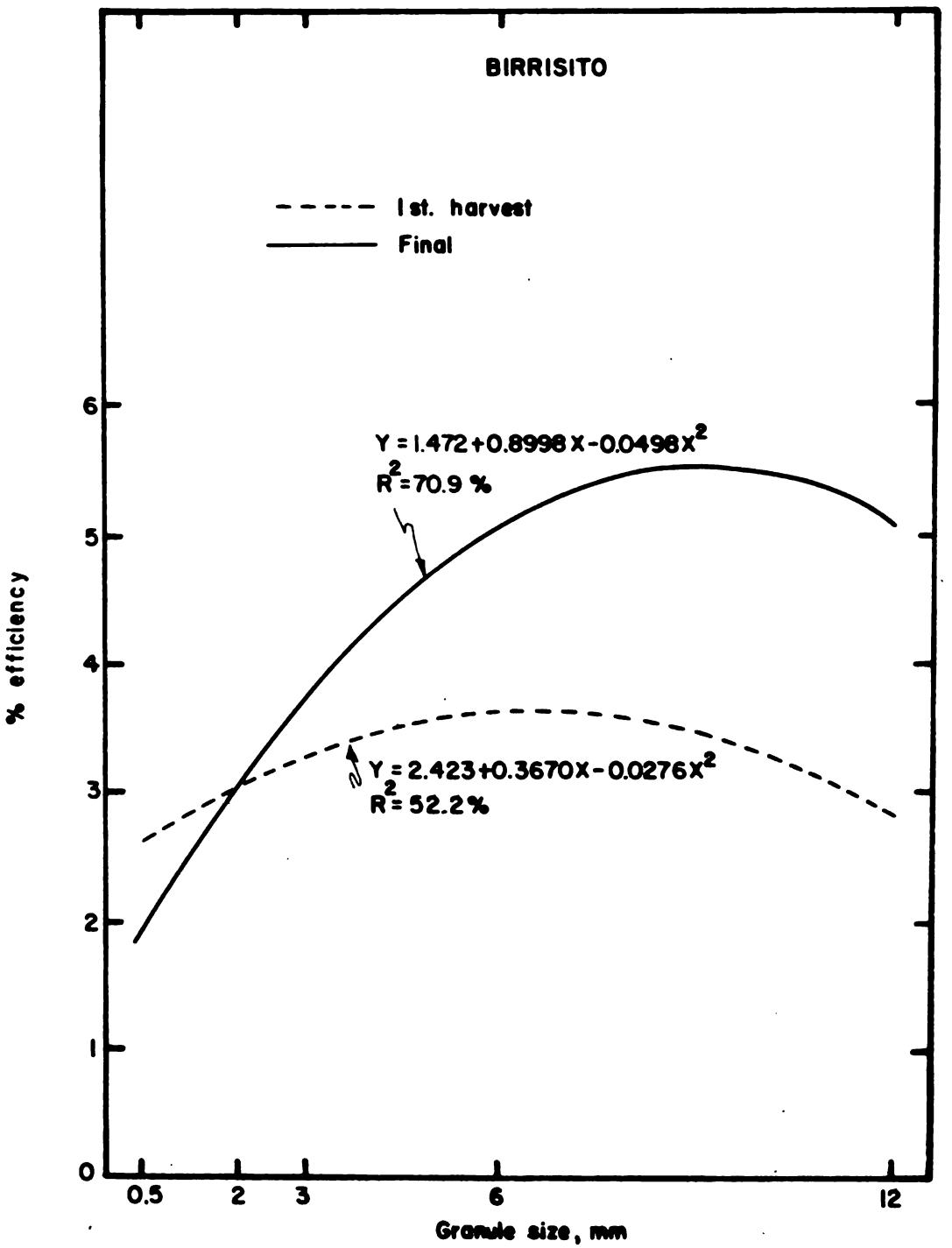


Fig. 54. Response curve for efficiency as a function of granule size

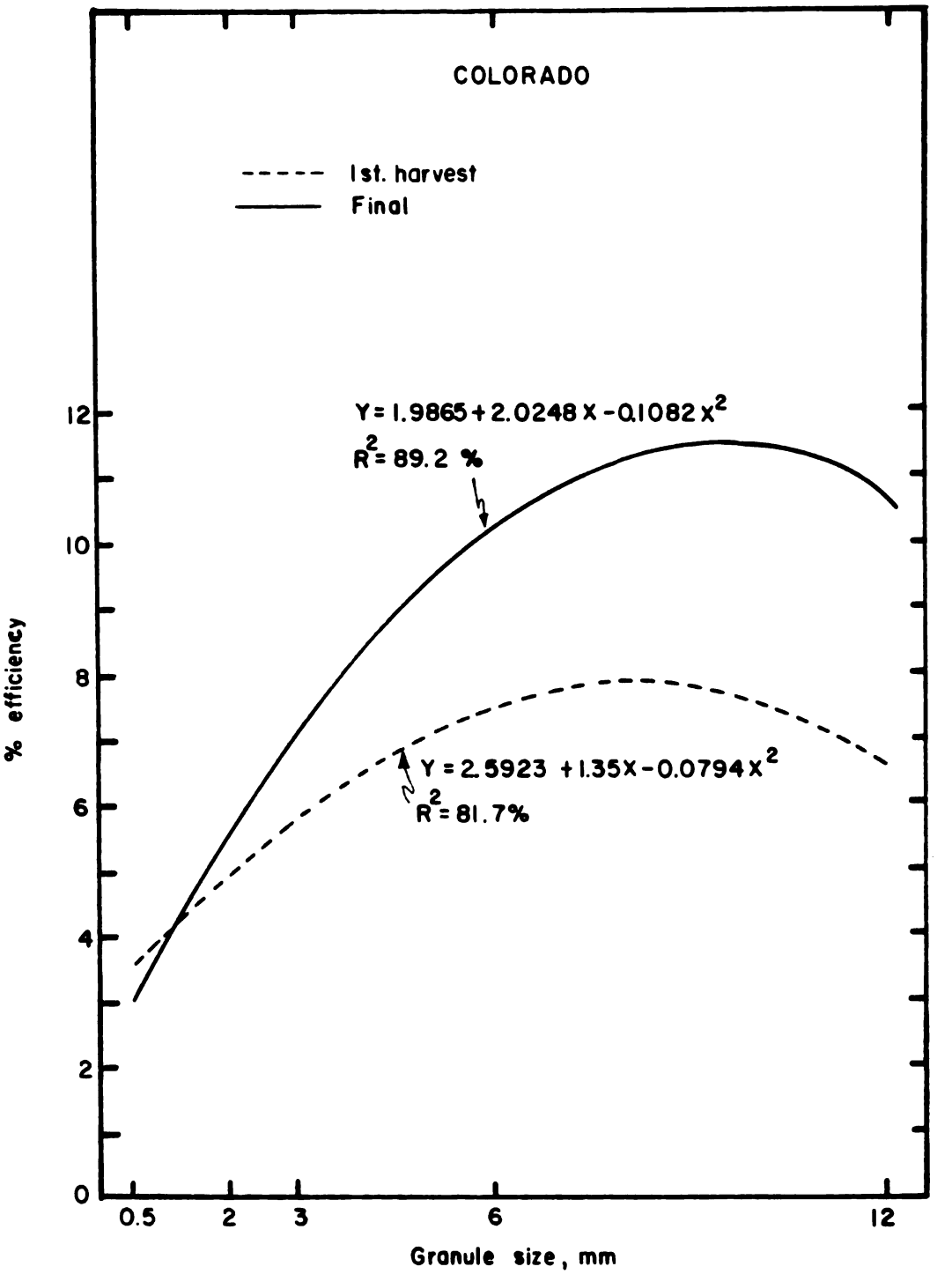


Fig. 55. Response curve for efficiency as a function of granule size

Table 67. Granule size and doses that maximize the efficiency of MCP

Soil	1st. Harvest			1st + 2nd Harvest		
	% effic.	mg P_2O_5 /liter	Size mm	% effic.	mg P_2O_5 /liter	Size mm
Birrisito	3.64	456	6.65	5.82	142	8.54
Colorado	7.89	412	9.06	11.45	344	9.37
Juray	Maximization not possible					

Regression (1st. cropping)

$$Y_1 = 1.09 + 6.12 \cdot 10^{-3}X_1 + 1.35X_2 - 6 \cdot 10^{-6}X_1^2 - 7.94 \cdot 10^{-2}X_2^2 - 1.47 \cdot 10^{-4}X_1X_2$$

$$Y_2 = 1.69 + 2.53 \cdot 10^{-3}X_1 + 4.15 \cdot 10^{-1}X_2 - 2 \cdot 10^{-6}X_1^2 - 2.76 \cdot 10^{-2}X_2^2 - 1.06 \cdot 10^{-4}X_1X_2$$

$$Y_3 = 4.21 - 2.43 \cdot 10^{-3}X_1 + 4.94 \cdot 10^{-1}X_2 + 4 \cdot 10^{-6}X_1^2 - 3.05 \cdot 10^{-2}X_2^2 - 4.17 \cdot 10^{-4}X_1X_2$$

Regression (Sum of 1st and 2nd cropping)

$$Y_1 = 1.04 + 1.22 \cdot 10^{-2}X_1 + 2.22X_2 - 1 \cdot 10^{-5}X_1^2 - 1.08 \cdot 10^{-1}X_2^2 - 5.73 \cdot 10^{-4}X_1X_2$$

$$Y_2 = 1.52 + 3.06 \cdot 10^{-3}X_1 + 9.21 \cdot 10^{-1}X_2 - 3 \cdot 10^{-6}X_1^2 - 4.98 \cdot 10^{-2}X_2^2 - 2.49 \cdot 10^{-4}X_1X_2$$

$$Y_3 = 6.07 - 3.26 \cdot 10^{-3}X_1 + 5.43 \cdot 10^{-1}X_2 + 4 \cdot 10^{-6}X_1^2 - 2.68 \cdot 10^{-2}X_2^2 - 5.13 \cdot 10^{-4}X_1X_2$$

Y_1 = Efficiency Colorado — X_1 = doses

Y_2 = Efficiency Birrisito — X_2 = granule size

Y_3 = Efficiency Juray

constant as the size increased further (Fig. 56). The calculated A values are very high as compared to other previous works, indicating high relative unavailability of P in these soils.

When P sources were compared in separate experiment (<12 mesh), the DCPA tended to maintain a higher efficiency than other two sources (Table 68). Again, the A value decreased with an increase in fertilizer efficiency. Therefore, DCPA the most efficient source, presented the lowest A value. This result is in close agreement with a previous study where it was shown that DCPA is the less reacting phosphate source.

The following conclusions can be drawn from this study:

1. Under prevailing conditions - phosphate fixation increases as phosphate solubility increases. As the allophane content of the soil increases a higher proportion of P reacts with the soil.
2. The relative efficiency of fertilizer increased for less soluble and less reactive source (DCPA)
3. An increase in granule size of highly soluble P (MCP-C) the efficiency is increased. Under conditions of high P fixing soils the placement of fertilizer can overcome the problem.

References

1. Bornemisza, E. and Fassbender, H. W., 1970. Uptake of fertilizer phosphate from nine soils from humid tropics. Agrochimica 14:259-268.
2. Chang, S. C. and Chu, W. K., 1961. The fate of soluble phosphate applied to soils. J. Soil Sci. 12:286-293.

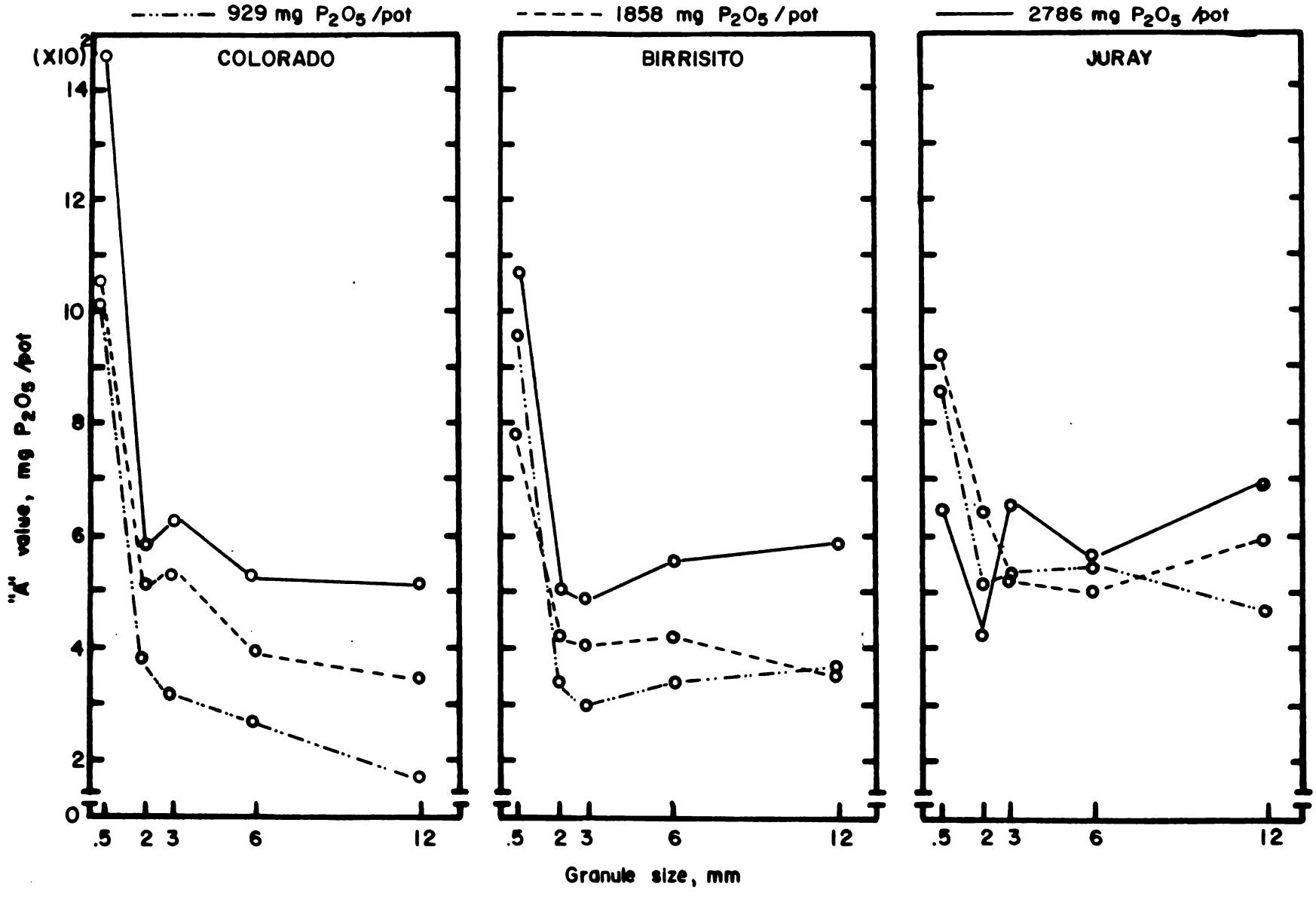


Fig. 56. Effect of granule size on 'A' value.

Table 68. 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_6 /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

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

* Colorado soil - 2 kg/3 liter pot

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

3. Hsu, P. H., 1965. Fixation of phosphate by Al and Fe in acid soils. Soil Sci. 99:398-402.

4. Lawton, K. and Cook, R. L., 1955. Interaction between particle size and water solubility of phosphorus in mixed fertilizers as factor affecting plant availability. Farm Chemicals 118(4):44-46.

5. Lawton, K., et al., 1956. Influence of particle size, water solubility, and placement of fertilizers in the nutrient value of phosphorus in mixed fertilizers. Soil Science 82(6):465-476.

6. Lindsay, W. L. and Stephenson, H. F., 1959. 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.

7. Lindsay, W. L. and Stephenson, H. F., 1959. Nature of the reactions of monocalcium phosphate monohydrate in soils. II. Dissolution and precipitation reactions involving Fe, Al, Mn, Ca. Soil Sci. Soc. Amer. Proc. 23:18-22.

8. Starostka, R. W., Caro, J. H. and Hill, W. L., 1954. Availability of phosphorus in granulated fertilizers. SSSA Proc. 28(1):49-52.

9. Terman, G. L. and Khasawneh, F. E., 1968. Crop uptake of fertilizer and soil P in relation to calculated A values. Soil Sci. 105:346-354.

10. Wada, K., 1959. Reactions of phosphate with allophane and halloysite. Soil Sci. 87:325-330.

3. Trace Elements in Tropical Soils

a. Factors affecting zinc absorption by corn from volcanic ash soils.
(K. Igue and M. L. Marinho)

The specific objective of this study was to investigate the effect of free Al_2O_3 and Fe_2O_3 content and P applications on availability of native and applied zinc in soils previously indicated as deficient (6).

The soils were selected from areas of known Zn deficiency of the Meseta Central, Costa Rica. Characteristics of these soils are indicated in Table 69. A factorial design was used to study the effect of P and Zn applications. Radioactive ^{65}Zn was used to trace Zn absorption by corn plant. Available Zn was determined by two methods: 0.1 N HCl and 0.01 M Na_2-EDTA.

The results indicated that as the free R_2O_3 content of the soil increased the recoveries of applied Zn decreased for 0.01 M Na_2-EDTA method. This was not the case for the 0.1

Table 69. Chemical and physical characteristics of soils used in the experiment

Soil Properties	Soil		
	Barrial	Capri	Juan Viñas
pH H_2O (1:2.5)	5.4	5.2	5.0
Organic matter %	8.3	12.6	13.0
* CEC meq/100 g	42.3	47.2	48.0
** Free Al_2O_3%	5.42	7.78	16.64
*** Free Fe_2O_3%	4.85	4.43	5.65
Total Zn ppm	44.0	42.0	37.0
Zn 0.1 N HCl ppm	8.1	4.3	2.2
Zn 0.01 M EDTA ppm	1.8	1.3	0.7
P-Mehlich ppm	1.5	0.8	tr
* Ca ppm	315	160	90
* K ppm	180	197	50
* Mg ppm	507	192	95
Sand %	47.3	51.0	51.5
Silt %	27.0	21.0	18.5
Clay %	26.0	24.0	29.5

* NH_4OAc N pH 7.0
** Hashimoto and Jackson, 1960 (18)
*** Kilmer, 1960 (18)

N HCl method. Incubation time decreased Zn recovery in the case of Na_2-EDTA. This seems to be a good evidence of interaction between free R_2O_3 and Zn.

P applications increased Zn-extracted by Na_2-EDTA for all soils, but there was no effect with 0.1 N HCl (Fig. 57).

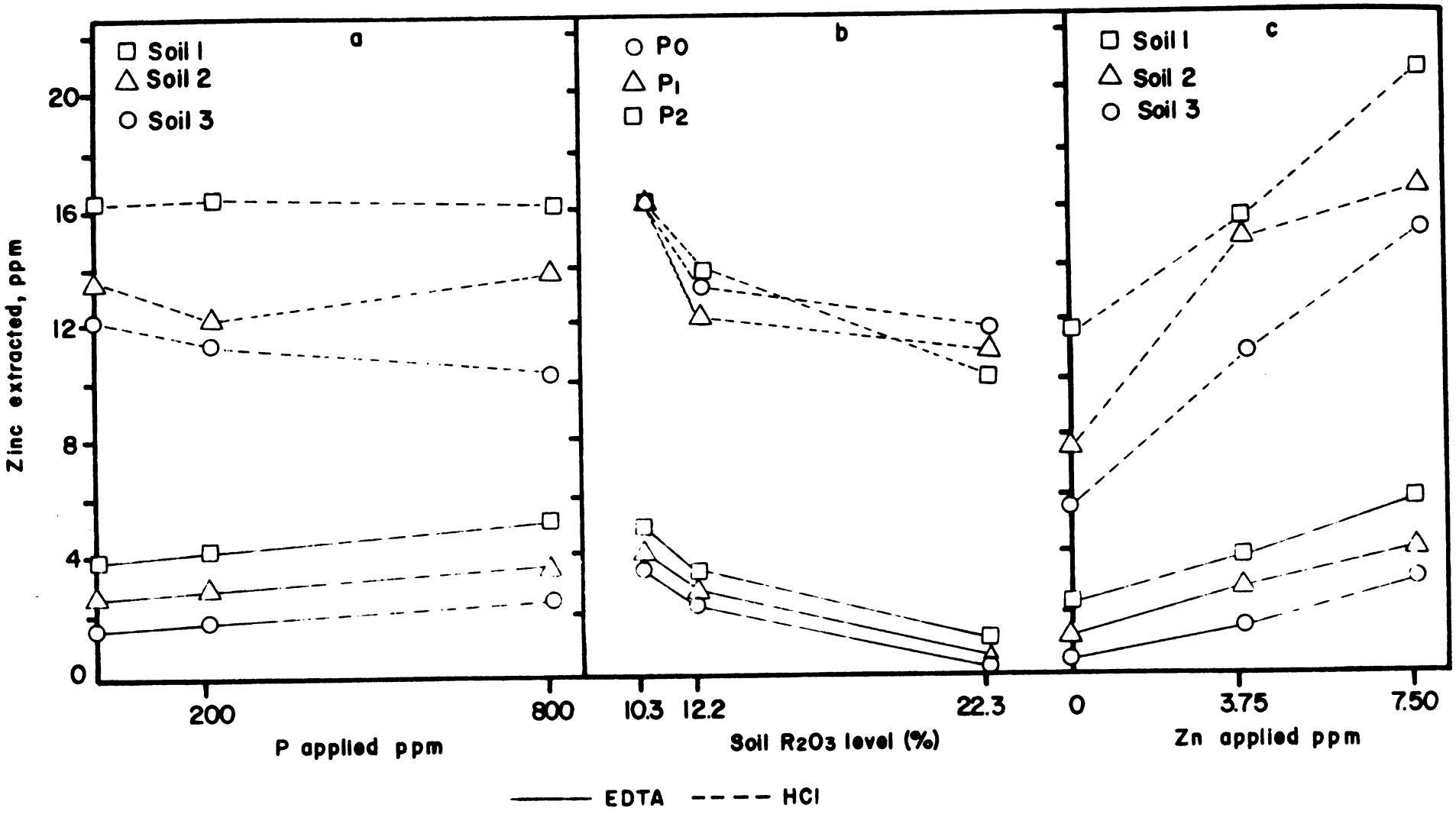


Fig. 57. Zinc extracted with 0,01 M Na_2 EDTA and 0,1 N HCl as function of a) P applied, b) level of free R_2O_3, and c) zinc applied

There was a highly significant effect of applied Zn and soil differences upon corn growth and the absorption (0.01 level) as shown on Table 70. Total uptake was related to available Zn in the soil. Zn absorbed from fertilizer is lower for soil high in free R_2O_3 (soil 3) than for soils low in R_2O_3 (soils 1 and 2). Total Zn uptake decreased with P application in the soil 1, but had no effect on soils 2 and 3. The concentration of Zn in the tissue, decreased with P applications in all cases, probably due to an increase in growth caused by P applications. When no P was added the concentration of Zn was higher for soil 3(low in available Zn) which was explained by poor growth due to acute P deficiency. Table 71 shows the calculated 'L' value for the three soils as a function of P and Zn applied.

The 'L' value decreased as the soil available Zn decreased and followed the same tendency as the extractable Zn by 0.1 N HCl and 0.017 M Na_2-EDTA.

The 'L' values were also affected by P applications. The variations of 'L' value in the soil as function on nutrient applied is clearly explained by Terman et al. (8), in which they showed three different situations for the calculated 'A' value for P. mathematically 'L' value is similar to 'A' value proposed by Fried and Dean (2). The difference between 'A' and 'L' values rests on the equilibrium between added nutrient and that of the soil (1).

Better correlation was obtained with EDTA method as compared to the HCl method for total Zn absorbed by corn. The EDTA method also showed a higher correlation coefficient with the 'L' value than did the HCl method. The correlation was higher

Table 70. Effect of P and Zn application upon plant growth, absorption and Zn concentration in three soils

Treatments* (R_2O_3:Zn:P)	Dry matter g/pot	Zn absorbed mg/pot			Zn concentration ppm tissue
		Total	From soil	From fertilizer	
101	11.88**	0.202	0.202	-----	17
102	13.77	0.165	0.165	-----	12
110	4.93	0.168	0.144	0.024	34
111	12.18	0.256	0.225	0.031	21
112	14.59	0.204	0.170	0.034	14
121	12.76	0.345	0.280	0.065	27
122	16.03	0.337	0.274	0.063	21
201	8.60	0.129	0.129	-----	15
202	14.42	0.114	0.114	-----	10
210	3.08	0.102	0.078	0.024	33
211	8.94	0.179	0.142	0.036	20
212	12.64	0.190	0.158	0.032	15
221	8.80	0.378	0.298	0.080	43
222	13.28	0.359	0.296	0.063	27
301	5.09	0.076	0.076	-----	15
302	9.98	0.070	0.070	-----	7
310	2.40	0.091	0.071	0.020	38
311	5.98	0.114	0.085	0.029	19
312	9.28	0.102	0.076	0.026	11
321	6.35	0.165	0.104	0.061	26
322	9.86	0.197	0.140	0.057	20

* First number refers to soil, the second to Zn and the third to P.
** Average of 3 replications.

Table 71. Calculated 'L' value for the three soils as a function of P and Zn applied

	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
Average	23.0	32.5	15.3	31.5	11.7	15.6

with P application as compared with treatment without P application in accordance with Trierweiler and Lindsay (9).

To further study the adsorption characteristics of these soils, the samples with different rates of P were equilibrated with solutions of increasing Zn concentration, from zero up to 400 ppm. Equilibration time was 6 hours. Fig. 58 shows the plot of Langmuir's isotherm for the three soils and different P concentrations.

At higher zinc concentration, the Langmuir adsorption equation described the adsorption by all soils.

The adsorption maxima (b) and the intercation energies or affinities (k) of the soils for Zn^{+2} were calculated from the data in Fig. 58 and are shown in Table 72.

Table 72. Regression equation for Langmuir's isotherm, and adsorption constant for three soils at different doses of P

Soil	Regression	r^2	Constants		
			b(adsorption maxima) mg/g	meq/100 g	k(energies) ml/g
100	Y= 4.59+0.27X	0.989	3.70	11.6	0.59
101	Y= 4.83+0.26X	0.991	3.85	12.0	0.53
102	Y= 3.95+0.26X	0.998	3.85	12.0	0.66
200	Y= 9.21+0.47X	0.896	2.13	6.6	0.51
201	Y= 8.63+0.47X	0.888	2.13	6.6	0.55
202	Y= 7.67+0.31X	0.964	3.22	10.1	0.41
300	Y=11.57+0.62X	0.966	1.61	5.0	0.54
301	Y=11.26+0.52X	0.907	1.92	6.0	0.46
302	Y= 6.27+0.57X	0.879	1.75	5.5	0.91

Adsorption maxima (b) is higher for soil 1, and decreases as the R_2O_3 content of the soil increases. Phosphate application seemed to have no effect on adsorption maxima, except in

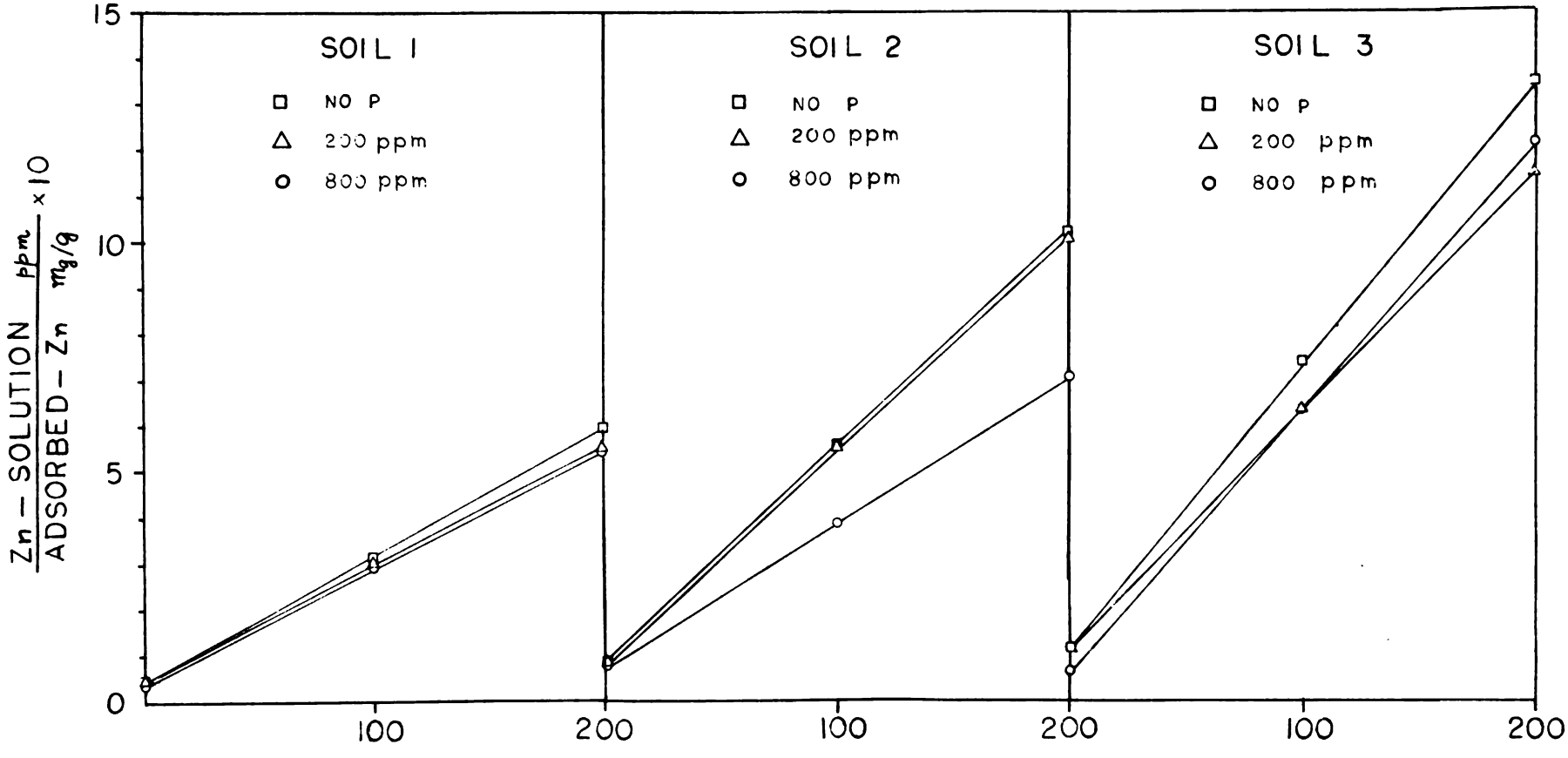


Fig. 58. Plot of Langmuir's isotherm for zinc adsorption in three volcanic ash soils deficient in zinc.

soil 2 with 800 ppm of P. Soil 1 that presents higher values for available Zn showed higher adsorption capacity. The data suggested that adsorption can be due to an exchange mechanism, or to a precipitation reaction. The soil 3, high in free Al_2O_3, must have the exchange positions occupied by Al to allow less amount of Zn to be adsorbed, whereas soil 1 had more exchange sites to be occupied by Zn. Soil 3 is considered a typical andosol (allophane is the dominant clay) and higher percentage of exchange sites due to organic matter is occupied by Al (2). This is also related with low available Zn in this soil. Sharpless, Wallihan and Peterson (7) indicated that 75% of Zn retained by southern California soils was present in exchangeable forms, 15-20% in acid extractable form, and the remainder are not extractable by either method. On the other hand, Udo et al. (10) showed that carbonates clay and organic matter are responsible for Zn retention in calcareous soils. They indicated also that in carbonate-free soils the amorphous silica seemed to be the main factor.

Under the present conditions, retention of Zn as silicates seems to be probable, since amorphous silica is present in relatively high concentrations in soils 1 and 2 due to recent volcanic ash (3). This is supported by high value of 0.1 N HCl extractable Zn as compared to Na_2-EDTA. Lindsay and Norrell (5) presented evidence that Zn reacts with amorphous SiO_2 in soils to form $ZnSiO_3$.

In conclusion it can be said that:

a) Free R_2O_3 content is not related either to Zn availability

or to Zn adsorption capacity.

b) Zn availability increased with Zn retention capacity of the soil, which suggested formation of compounds like $ZnSiO_3$

c) Phosphate application had no direct effect on P-adsorption, but it affects Zn adsorption by increasing plant growth.

References

1. Fried, M., 1964. "E", "L", and "A" values. In Trans. 8th Inter. Congr. Soil Sci. Bucharest, 1964. pp. 28-29.

2. Fried, M. and Dean, L. A., 1952. A concept concerning the measurement of available soil nutrients. Soil Sci. 73: 263-271.

3. Gonzalez, S. Caracterización de minerales de arcilla en suelos volcánicos de Costa Rica. M.S. thesis. Turrialba, Costa Rica, IICA, 1972. 68 p.

4. Igue, K. and Fuentes, R., 1972. Characterization of Al in volcanic ash soils. SSSA Proc. 36(2): (in press).

5. Lindsay, W. L. and Norrell, W. A. Equilibrium relationships of Zn^{+2}, Fe, Ca, H with EDTA and DTPA in soils. SSSA Proc. 33:62-68. 1969.

6. Rubinstein, J. K., 1968. Determinación de Cu y Zn en suelos y plantas de café por espectrofotometría de absorción atómica. Thesis Ing. Agr. San Jose, Costa Rica, University of Costa Rica, 1968. 68 p.

7. Sharpless, R. G., Wallihan, E. F. and Peterson, F. F., 1969. Retention of zinc by some acid zone soil materials treated with zinc sulphate. SSSA Proc. 33:901-904.

8. Terman, G. L. and Khasawneh, 1968. Crop uptake of fertilizer and soil phosphorus in relation to calculated "A" values. Soil Sci. 105(5):346-354.

9. Trierweiler, J. T. and Lindsay, W. L., 1969. EDTA-ammonium-carbonate soil test for zinc. SSSA Proc. 33:49-54.

10. Udo, E. J., Bohn, H. L. and Tucker, T. C., 1970. Zinc adsorption by calcareous soils. SSSA Proc. 34:405-407.

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

The purposes of this study were to a) evaluate the total and available forms of Zn, Fe, Mn, Ca, and Mo in eight soil profiles from the cocoa region of Eastern Brazil, and b) observe their distribution in those different soil types.

Soil samples were collected at different depths representing different horizons (Table 73). For the total trace-element content, digestion with $HClO_4/H_2SO_4$ (1) and a terciary acid mixture were used as described by Ulrich (2). For the available fractions the following extractants were used: 0.1 N HCl, 1% Na_2-EDTA, N NH_4OAc at pH 7 and 4.8.

Table 73 indicates the results of trace element contents in these soils at different depths. There was no significant difference between the method described by Jackson and Ulrich for Fe, and Zn, whereas for Mn higher values were observed in the Jackson method and lower values in the case of Cu as compared with Ulrich.

Table 74 shows the available fractions as determined by different extractants. Higher amounts were extracted by 0.1 N HCl and 1% Na_2-EDTA as compared with N NH_4OAc, even at pH 4.8.

The distribution of these elements in the profile were associated with clay content. In most soils analyzed the concentration of available forms was higher at surface horizon in association with organic matter.

Table 73. Concentration of total Zn, Cu, Mn, Fe and Mo determined by two methods

Soils	Horizon	Zn (ppm)		Cu (ppm)		Mn (ppm)		Fe (ppm)		Mo (ppm)	
		Jackson	Ulrich	Jackson	Ulrich	Jackson	Ulrich	Jackson	Ulrich	Jackson	Ulrich
CEPEC	A_1	82.6	86.4	19.3	tr	4082.4	2160.0	7776.0	23598.0	2.82	tr
(Alfisol)	A_3	69.9	99.9	16.0	tr	3030.3	2081.0	9590.4	28416.0	2.50	tr
	B_1	58.3	48.6	15.6	tr	2021.8	1420.0	7646.4	23760.0	2.56	tr
	B_2	51.5	16.2	12.8	27.0	1283.0	594.0	6998.4	21330.0	2.68	tr
	B_3	47.1	37.8	14.6	43.2	745.2	432.0	6156.0	27108.0	2.43	tr
	C_1	61.2	43.2	13.6	tr	596.2	432.0	7322.0	24300.0	2.27	tr
	C_2	62.2	27.0	16.8	tr	855.4	475.2	8942.0	25812.0	1.94	tr
ITABUNA	A_1	24.8	10.3	13.0	tr	877.6	406.9	8961.0	7622.0	1.73	tr
(Alfisol)	A_2	20.4	25.8	12.2	tr	815.8	283.3	7416.0	7725.0	1.85	tr
	A_3	19.5	tr	13.0	tr	494.4	139.1	11124.0	8240.0	2.16	tr
	B_1	25.2	tr	13.9	tr	339.9	92.7	15141.0	9785.0	3.71	tr
	B_2	22.2	tr	14.2	tr	63.0	tr	12285.0	9450.0	2.13	tr
	C_1	20.6	tr	10.6	tr	49.9	tr	10171.2	8112.0	2.18	tr
	C_2	14.3	tr	12.4	tr	49.9	tr	12552.0	5980.0	2.11	tr
NAZARE	A_1	28.6	87.6	16.9	77.3	512.9	309.0	19158.0	11485.0	2.09	tr
(Ultisol)	A_3	36.2	77.3	19.0	51.5	139.9	309.0	27686.0	14008.0	2.83	tr
	B_1	32.3	77.3	18.4	51.5	216.3	195.7	22742.0	17252.0	3.95	tr
	B_2	32.6	93.6	20.2	52.0	174.7	119.6	20592.0	18616.0	4.21	tr
	B_3	26.7	105.0	23.2	52.5	113.4	89.3	14238.0	19950.0	2.84	tr
	C_1	24.6	94.5	24.9	94.5	63.0	52.5	16632.0	19005.0	2.84	tr
	C_2	34.3	73.5	22.2	52.5	63.0	42.0	20979.0	20475.0	4.49	tr
HIDROMORFICO	A_{11}	37.5	tr	12.1	tr	247.2	901.2	4944.0	13132.0	1.58	tr
	A_{12}	45.8	tr	10.7	tr	239.4	908.2	5922.0	14332.0	1.09	tr
	A_{1g}	16.4	10.5	2.7	tr	409.5	971.3	5544.0	14332.0	2.13	tr
	B_{2g}	26.0	tr	1.9	tr	756.0	551.3	3465.0	11035.0	2.60	tr
	B_{3g}	24.9	tr	1.9	tr	579.6	498.7	2583.0	12600.0	1.89	tr
	C_{1g}	28.1	41.6	2.6	tr	517.9	494.0	4618.0	13832.0	1.59	tr
	C_2	28.6	42.4	6.9	tr	203.9	695.3	5747.0	17922.0	1.58	tr
RIO BRANCO	A_1	65.5	42.8	19.7	tr	590.6	363.8	12198.0	22898.0	2.25	tr
(Inceptisol)	A_3	39.6	68.9	12.6	tr	222.6	137.8	20988.0	22790.0	1.91	tr
	B_1	26.5	68.9	12.6	tr	209.8	137.8	14755.0	24645.0	1.37	tr
	B_2	37.5	37.1	13.4	15.9	178.1	127.2	15900.0	25705.0	1.62	tr
	B_3-C	55.9	tr	12.7	42.8	179.8	96.3	13033.0	26108.0	1.93	tr
	C-D	55.9	tr	15.4	26.8	147.7	69.6	12712.0	24610.0	2.25	tr
ITAMIRIM	A_{11}	53.8	87.6	16.9	25.8	815.8	957.9	18540.0	20497.0	1.85	tr
(Vertisol)	A_{12}	49.1	41.2	20.8	25.8	815.8	798.3	11124.0	20600.0	1.85	tr
	A_3	51.5	15.6	24.6	26.0	811.2	520.0	15101.0	22984.0	1.87	tr
	B_1	41.4	64.2	40.9	26.8	385.2	310.3	12326.0	26910.0	2.41	tr
	B_2	43.7	43.6	51.0	98.1	215.8	316.1	10791.0	28340.0	2.94	tr
	B_3	60.8	70.8	61.8	98.1	327.0	327.0	9810.0	29975.0	3.43	tr
VALENCA	A_1	36.4	41.2	16.0	25.8	339.9	247.2	11804.0	20343.0	1.58	tr
(Oxisol)	A_3	39.3	41.6	18.5	15.6	218.4	275.6	15600.0	24024.0	1.87	tr
	B_1	38.3	52.5	19.4	15.8	252.0	278.3	20349.0	24570.0	5.39	tr
	B_{21}	39.3	52.0	22.9	26.0	218.4	208.0	12168.0	26780.0	4.45	tr
	B_{22}	37.4	78.0	18.5	26.0	205.9	182.0	24710.0	26936.0	5.34	tr
	B_3	37.1	52.5	30.2	26.3	176.4	94.5	23625.0	27720.0	5.67	tr
COLONIA	A_{11}	35.8	tr	16.8	tr	110.2	76.5	3488.0	8058.0	1.56	tr
(Oxisol)	A_{12}	13.7	10.2	3.5	tr	244.8	127.5	5386.0	13158.0	2.14	tr
	B_1	14.1	tr	3.5	tr	171.4	117.3	5263.0	9945.0	1.96	tr
	B_{21}	14.1	tr	2.6	tr	201.9	86.7	12118.0	9435.0	2.07	tr
	B_{22}	21.6	tr	1.8	tr	201.9	40.8	9180.0	8160.0	2.07	tr
	B_3	11.9	tr	4.4	tr	201.9	20.4	7466.0	7650.0	1.84	tr
	C	7.7	tr	5.1	tr	142.1	tr	5129.0	5665.0	2.09	tr
General Average		36.4	37.1	15.9	19.3	515.6	388.1	11896.1	18070.0	2.50	tr
Average	A	40.4	39.3	15.0	13.0	800.9	630.2	11702.6	16749.0	1.98	tr
Average	B	34.4	37.4	18.0	25.1	392.4	264.0	12639.2	19739.7	2.98	tr
Average	C	33.8	32.2	13.1	17.4	268.9	226.1	10480.4	16571.3	2.33	tr

tr = traces

Table 74. Concentration of available Zn, Cu, Mn, Fe and Mo as determined by various methods.

Soils	Horizons	Zn (ppm)				Cu (ppm)				Mn (ppm)				Fe (ppm)			
		HCl 0.1 N	Na_2EDTA 1%	NH_4OAc pH 4.8	NH_4OAc pH 7.0	HCl 0.1 N	Na_2EDTA 1%	NH_4OAc pH 4.8	NH_4OAc pH 7.0	HCl 0.1 N	Na_2EDTA 1%	NH_4OAc pH 4.8	NH_4OAc pH 7.0	HCl 0.1 N	Na_2EDTA 1%	NH_4OAc pH 4.8	NH_4OAc pH 7.0
CEPEC	A_1	44.8	32.4	17.8	0.3	5.2	6.6	0.9	1.6	2646.0	2246.0	383.4	68.0	250.6	907.2	30.8	tr
(Alfisol)	A_3	16.1	13.3	6.0	0.3	2.2	2.8	tr	0.4	129.9	2053.0	59.9	1.1	269.7	888.0	14.7	tr
	B_1	9.2	5.4	3.2	tr	1.6	1.6	tr	0.4	91.8	1263.6	60.5	2.5	237.6	646.0	13.0	tr
	B_2	4.3	2.2	1.6	tr	1.1	1.1	tr	0.4	108.0	475.2	76.8	26.5	210.6	318.6	18.9	tr
	B_3	3.9	1.7	1.6	tr	1.6	0.8	tr	tr	36.9	62.6	39.9	15.3	196.6	169.0	29.2	tr
	C_1	5.4	1.9	2.4	tr	1.4	0.8	tr	tr	22.7	22.9	21.6	10.3	312.1	230.0	50.8	tr
	C_2	8.1	1.9	2.5	tr	1.9	0.8	tr	tr	34.6	24.4	20.5	10.3	766.8	308.9	56.2	tr
ITABUNA	A_1	7.4	4.6	2.4	0.8	1.6	2.6	0.8	1.9	812.9	825.3	133.8	65.9	396.6	483.5	5.5	7.2
(Alfisol)	A_2	3.3	2.1	1.0	0.3	1.3	2.3	1.3	1.9	232.8	412.0	206.0	135.9	69.0	343.0	30.9	8.8
	A_3	3.3	1.4	0.9	0.3	1.4	2.3	2.1	1.2	167.9	379.9	159.6	103.0	46.4	240.0	21.6	11.1
	B_1	3.1	1.6	0.6	0.2	1.3	2.3	1.9	1.2	89.6	173.0	80.3	57.7	40.7	150.4	13.7	11.9
	B_2	3.2	1.5	0.6	tr	0.8	0.7	1.7	tr	2.3	5.3	4.2	1.7	23.6	76.8	7.4	tr
	C_1	3.1	1.4	tr	tr	0.8	0.7	0.7	tr	1.6	2.8	1.8	0.8	18.5	60.3	2.6	tr
	C_2	2.9	1.4	tr	tr	1.0	0.7	0.8	tr	0.7	2.6	1.8	0.8	13.5	52.0	1.6	tr
NAZARE	A_1	3.1	2.4	0.8	0.3	3.1	3.4	tr	0.4	401.7	350.2	126.7	54.6	148.4	272.9	7.7	tr
(Ultisol)	A_3	1.3	1.2	tr	tr	2.1	2.3	0.3	0.4	191.6	245.1	120.5	75.2	162.7	257.5	18.9	tr
	B_1	1.6	0.8	tr	tr	2.9	2.3	1.7	0.4	64.7	66.8	46.7	21.6	126.7	123.6	11.9	tr
	B_2	1.6	1.3	0.5	tr	2.4	2.3	0.5	0.6	35.2	33.3	26.5	11.4	101.9	68.4	5.7	tr
	B_3	1.4	0.9	0.2	tr	2.0	2.3	1.9	1.0	9.9	6.8	9.6	3.5	97.6	65.1	5.8	5.4
	C_1	1.4	0.4	tr	tr	2.5	2.3	1.7	1.3	6.3	4.7	6.6	2.4	101.9	65.1	2.6	6.3
	C_2	0.8	1.1	tr	tr	2.1	1.6	tr	tr	6.5	4.7	6.8	0.8	120.8	50.9	10.3	8.9
HIDROMORFICO	A_{11}	2.6	3.1	1.4	0.6	2.1	3.6	1.9	1.3	53.6	41.7	37.1	16.5	136.9	329.6	4.4	1.9
	A_{12}	2.9	3.1	1.5	0.5	1.8	2.8	tr	1.4	46.7	40.4	33.6	13.4	197.4	357.0	11.6	1.1
	A_{1g}	2.4	2.5	1.1	0.6	1.4	0.7	tr	0.4	51.5	75.6	17.0	3.7	66.1	81.9	3.2	1.1
	B_{2g}	2.3	2.4	0.8	0.2	1.4	0.7	tr	1.3	42.0	136.9	12.4	1.3	71.4	86.1	2.1	tr
	B_{3g}	2.3	2.1	0.5	0.3	1.1	0.7	0.3	tr	22.0	38.3	6.3	1.3	81.9	74.5	3.7	0.5
	C_{1g}	3.7	2.5	0.9	0.3	1.0	1.6	tr	0.4	16.6	17.2	5.7	1.2	110.2	73.8	4.4	0.5
	C_2	6.6	0.8	0.9	tr	1.9	1.5	tr	0.6	17.0	2.8	4.1	2.4	311.1	144.2	9.1	0.5
RIO BRANCO	A_1	8.6	3.5	0.9	0.5	4.8	2.3	0.6	1.9	369.2	401.3	96.3	19.8	105.4	428.0	16.1	tr
(Inceptisol)	A_3	4.2	1.6	0.4	0.3	1.4	1.1	0.3	0.6	19.7	17.5	7.8	3.2	132.5	259.7	16.9	tr
	B_1	3.5	1.0	tr	tr	1.4	1.1	1.1	0.6	7.2	5.5	3.5	1.3	80.6	220.5	28.1	tr
	B_2	4.2	0.7	tr	tr	1.6	1.1	1.1	0.6	5.3	5.5	3.0	0.5	56.2	125.1	10.6	tr
	B_3-C	5.4	0.8	0.3	tr	1.4	1.1	1.3	1.3	4.5	5.6	3.0	1.1	48.2	108.1	5.6	tr
	C-D	6.3	1.4	0.5	tr	1.9	1.6	1.9	1.1	6.6	5.4	3.0	0.9	43.3	88.8	4.6	tr
ITAMIRIM	A_{11}	3.4	4.4	1.8	0.3	1.9	2.6	0.3	1.2	319.3	504.7	180.3	11.3	129.8	200.8	9.3	tr
(Vertisol)	A_{12}	156	1.8	1.0	0.3	2.9	2.6	0.3	0.6	94.8	339.9	54.6	5.2	111.2	164.8	8.8	tr
	A_3	1.4	1.4	0.4	tr	2.9	2.5	0.3	0.6	49.9	211.1	34.3	3.6	122.7	114.4	7.6	tr
	B_1	1.8	0.9	0.5	tr	4.8	4.9	0.3	0.6	19.3	25.2	15.0	5.4	141.2	56.9	9.6	tr
	B_2	0.3	0.4	0.2	tr	6.5	7.4	0.3	0.7	2.0	1.1	3.6	1.7	100.3	61.8	12.0	tr
	B_3	1.3	0.4	1.1	tr	7.1	6.2	1.1	tr	2.0	1.1	3.3	1.1	86.1	82.8	11.5	tr
VALENCA	A_1	1.0	1.6	0.9	tr	2.4	2.6	tr	0.4	88.0	37.6	40.2	16.5	173.0	236.9	23.5	tr
(Oxisol)	A_3	0.5	0.5	tr	tr	3.1	3.1	tr	0.6	20.8	7.6	9.4	2.6	121.7	114.4	18.9	tr
	B_1	0.4	0.6	tr	tr	2.1	1.9	tr	0.4	4.6	2.8	5.3	1.1	118.6	64.1	6.3	0.5
	B_{21}	0.6	0.8	tr	tr	3.4	2.8	0.8	tr	10.0	5.7	5.7	1.7	96.8	42.6	1.0	6.2
	B_{22}	0.5	0.7	tr	tr	1.9	1.6	tr	tr	4.0	2.4	3.1	0.8	111.3	46.8	tr	18.0
	B_3	0.5	0.7	tr	tr	3.2	1.6	tr	tr	8.2	2.4	3.2	0.8	123.9	44.6	tr	16.3
COLONIA	A_{11}	1.2	0.8	tr	tr	1.3	2.2	tr	1.2	2.1	2.6	2.2	1.2	255.0	622.2	112.0	7.1
(Oxisol)	A_{12}	1.4	1.0	tr	tr	1.3	2.2	tr	1.2	1.5	2.8	1.5	1.2	327.4	678.3	108.9	4.4
	B_1	1.4	3.8	tr	tr	1.0	2.2	tr	tr	1.8	14.3	1.5	1.0	216.2	127.5	183.6	5.6
	B_{21}	1.4	0.8	tr	tr	0.9	1.8	tr	tr	0.7	2.0	1.5	0.8	83.6	57.1	91.6	5.6
	B_{22}	1.4	1.0	0.5	tr	0.8	1.5	0.3	0.6	1.0	2.0	1.5	tr	31.4	23.5	19.4	4.4
	B_3	tr	0.4	0.2	0.2	0.2	0.7	0.3	0.4	0.7	tr	1.5	tr	21.4	11.2	8.7	4.4
	C	tr	0.3	tr	tr	0.8	0.7	0.4	0.4	0.7	tr	2.0	0.8	18.2	10.7	6.7	5.2
General Average		3.9	2.2	0.1	0.1	2.1	2.2	0.6	0.7	116.4	184.8	41.5	14.8	142.7	206.8	22.6	2.7
Average	A	5.6	4.4	2.0	0.3	2.3	2.7	0.5	1.0	288.4	413.4	89.7	31.7	170.7	366.3	28.8	2.2
Average	B	2.3	1.3	0.5	0.1	2.2	2.2	0.6	0.5	223.8	88.4	17.5	6.7	104.4	121.5	20.8	3.3
Average	C	3.8	1.3	0.7	0.1	1.5	1.2	0.6	0.4	11.8	8.8	7.4	2.9	181.3	108.5	14.9	2.1

tr = traces

Among the trace elements analyzed for total content, Fe presented the highest concentration and Mo the least. The following order can be indicated: Fe>Mn>Zn>Cu>Mo. The total content of Mo was relatively low and was detected only by $HClO_4$ 60% method.

In the case of available fraction, Mo and Fe showed the highest concentrations, followed by Zn and Cu, whereas Mo was not detected in a significant amount. Among the extractants for available fractions, the following general order can be indicated: 0.1 N HCl≥1% Na_2-EDTA≥N NH_4OAc pH 9.8≥N NH_4OAc pH 7.0.

References

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

2. Ulrich, B., Hempler, K. and Benzler, J. H. Zur analitischen Bestimmung von Gesamt Phosphorsaeure und Laktatloeslicher Phosphorsaeure im Bodenproben. Die Phosphorsaeure 20: 344-347. 1960.

E. COLLECTION OF RAINFALL FOR FALLOUT ANALYSIS

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

II. PLANS FOR THE CONTINUATION OF OBJECTIVES AND POSSIBLE NEW OBJECTIVES IN CONSIDERATION OF PAST RESULTS

The general objectives of the Nuclear Energy Project in the Training and Research Center of IICA at Turrialba, are twofold: To carry out basic or applied agricultural research in the American tropics using nuclear energy as a tool; and to train students from Latin America in the use and application of nuclear energy techniques to agriculture.

The research activities of the project consist of the following three fields: 1) radiation botany and plant genetics - use of induced mutations in plant breeding, 2) entomology - application of male sterilization method for insect control, and 3) radiobiology in insect pathology - use of radiation to induce mutations of insect pathogens. For the coming years, more specific plans for the continuation of the current research and for the new lines of research are presented below.

A. Radiation Botany and Plant Breeding

1. Mutation breeding in Manihot

Because Manihot is a basic food crop in the tropics and has a phenomenal yield which could meet the need of the growing population in the tropical areas, we have explored the methods of mutation induction in this crop as an improvement measure. As one can find in this research progress report, pollen irradiation appears to be a promising method which 1) eliminates the problem of chimera; 2) induces a reasonably high frequency of mutations in the R_1 generation with a relatively low dose of

radiation; 3) allows us to self the R_1 plants and to bring out the recessive mutations; and 4) provides an opportunity for quickly evaluating the field performance of the desirable mutants.

Since last year, we have obtained several hundreds of the R_1 plants from pollen irradiation. All of them are being vegetatively propagated for field evaluation. Some of the R_1 appeared to have vigorous growth and are being selected for yield trials in the coming year.

One of the weaknesses of Manihot is that it has a long life cycle which makes it difficult to compete with other agricultural crops. Especially in the dry areas such as the Pacific Coast of Central America where there is a dry period of 6 months a year, the Manihot usually takes 2 years for growth. It would be extremely desirable to have an early mutant which produces edible roots in 6 to 7 months. We are now selecting the fast-growing and most vigorous R_1 plants for the breeding project. If the vigorous plants produce sizable edible roots in this short period, we can consider them as early mutants.

Another weakness of Manihot is that the plant contains cyanogenetic glucoside. The hydrocyanic acid (HCN) free from the glucoside is extremely poisonous. A quantity of 50-60 mg of HCN is considered as a lethal dose to man. The roots of all Manihot cultivars, so far as we know, contain a certain amount of the cyanogenetic glucoside. The HCN content of some cultivars is as high as 300 mg or more per kilogram of fresh roots. Although the roots of low HCN content cultivars are safe to eat

after cooking, there is concern about the chronic effect of HCN poisoning. The real solution of this problem is to develop an HCN-free Manihot cultivar.

While the HCN-content of a Manihot plant is influenced by a number of environmental conditions, there is little doubt that the content is basically controlled by genetic factors. Thus, it is probable that a cultivar with extremely low HCN content or free of HCN can be found in the natural population or be induced by mutation method.

A fast and simple method for detecting the presence of HCN will greatly facilitate the selection of the desirable type from our mutant and cultivar collection. The Guignard test (the reddish color reaction in sodium picrate paper in the presence of HCN) may be used qualitatively to detect the HCN. This appears to fulfill our experimental requirement.

For the coming years, plans for the project of Manihot improvement will consiste of 1) looking for better methods for inducing mutations in the cultivars, 2) evaluating the yield performance of the vigorous R_1 mutants from pollen irradiation, and 3) screening the irradiated materials with the objectives of isolating earliness or HCN-free mutants.

2. Field trial of the coffee compact mutant

One of the plant characters that has merit in the green revolution is the dwarf or compact type of growth in the grain crops. Evidently, this type of plant, besides its good yield potential, can give rise to a high production in responding to heavy applications of fertilizer without causing lodging problems.

Since last year, we have isolated two homozygous lines of the compact mutant from the coffee irradiation experiment. While these mutants appeared to produce fruits heavily last year, the yielding records have not been obtained. Whether these mutants can stand heavy fertilizer applications for inducing a heavy crop has not been studied. Experiments designed to study the yield performance of the compact mutants, as compared with the local high yielding coffee varieties (Mundo Novo and Caturra), will be carried out in the coming year.

3. Nutrition study of the white bean mutant

A previous experiment carried out by INCAP showed that when the laboratory rats were fed with the white mutant beans for 21 days, the rats had a 70% increase of body weight over those fed with the beans of the original black parent. Although chemical analysis showed that the protein content of the white mutant was slightly but consistently higher than the black parent (1 to 3%), this slight increase in protein quantity cannot account for the large gain of body weight. In an agreement with INCAP, we decided to reconfirm the results of the feeding experiment and to look into the protein quality of the white mutant, especially several essential amino acids. It is known that bean proteins are deficient in methionine; an addition of a very small amount of this (0.15%) would increase the protein quality significantly.

B. Control of Insects by Male Sterilization Method

1. Sterilization of the Mediterranean fruit fly and its application to fly eradication

As reported earlier, this is a cooperative proyect with OIRSA and IICA (IICA is a contractor of the USAEC) to evaluate the eradication of the medfly in Central America using gamma-sterile-male technique. In this cooperative project, we are responsible for carrying out all basic laboratory studies necessary for the field trials for which OIRSA is responsible. With the basic information supplied by us and the technical and financial support of the IAEA, in 1968-1969, OIRSA carried out a field test in Nicaragua (48 km^2) to demonstrate the effectiveness of the sterile male technique for suppressing the medfly. The conclusion of the test was that the medfly can be eradicated in Central America through the use of sterile insect releases.

Throughout the past we have been actively engaged in carrying out the supporting research for the OIRSA-IAEA project. The current IAEA support to OIRSA will end this June. Recently, an USAID sponsored team concluded its visit in Central America with the following objectives: 1) to estimate the damage caused by the medfly in Central America and 2) to estimate the cost of its eradication from this area using the sterile male technique in combination with other control methods. Official report of this team is not yet out but it appears that the group is going to recommend its eradication.

If the OIRSA project for eradication of the medfly from Central America is extended then we will continue our cooperation with OIRSA in supplying the basic supporting research needed

for the actual eradication campaign. If not, we plan to phase out gradually the medfly research in the future. But meantime, we will do some basic research still lacking related to medfly control by sterile insects.

We have found that males of the medfly can be sterilized successfully with 10 kr by irradiating either mature pupae (1-3 days before adult emergence), or newly emerged adults (1-2 days after emergence). However, sterilization slightly reduces the mating competitiveness of treated males. Results of our experiments carried out in the laboratory indicate that the critical stage of this insect for sterilization is the pupal stage (24 hr before adult emergence). Males irradiated at an earlier stage, i.e. 48 hr before emergence were ca. 50% less effective in mating compared to those irradiated as pupae (24 hr before eclosion) or as 1-2 day old adults. We plan to carry out experiments in the coming years in small cages in the field to confirm the laboratory results under conditions more nearly approaching natural conditions.

Efforts will be continued to breed a good visible medfly mutant using gamma irradiation. Availability of a genetic marker can be very useful in ecological studies especially in a campaign using sterile male releases.

2. Biology and sterilization of the coffee leaf miner, Leucoptera coffeella (Guerin-Meneville)

Studies will be continued under this project to evaluate the effectiveness of the gamma-sterile-male technique for controlling the coffee leaf miner. Dose-response curves for induced

sterility have been worked out for males and females of this moth.

As reported earlier in this report, sterilization of the males with 90 kr, drastically reduced the insemination capacity as well as the mating competitiveness of treated moths. In the coming years experiments will be carried out to evaluate the mating effectiveness of sub-sterile males (with 5-20% residual fertility).

Preliminary results presented earlier indicated that F_1 inherited sterility can be induced in the coffee leaf miner with very low irradiation doses. Progeny of normal females crossed with males irradiated with 20 kr (having ca. 80% residual fertility) was more than 99% sterile. More experiments will be carried out to determine the lowest sterilization dose applied to males necessary for obtaining F_1 progeny with less than 1% residual fertility.

Phenomenon of the presence of F_1 sterility with low sterilization doses could perhaps add more value to sterile male control in the coffee leaf miner. By reducing sterilization dose of 90 kr chances of improving the mating competitiveness of the irradiated males are better. Thus in the coming years experiments will first be carried out in the laboratory to thoroughly evaluate the mating competitiveness of the F_1 males obtained from parent males irradiated with low sterilization doses. If laboratory results would be promising, then population suppression of this moth will be studied by releases of sub-sterile insects in the field in caged coffee plants.

Experiments will also be carried out to determine minimum sterile to normal male ratio required for suppressing the egg-hatch of normal females to an effective level necessary for its control by sterile-male releases.

3. Biology and sterilization of the shootborer, Hypsipyla grandella Zeller (Lepidoptera:Phycitidae)

Tree species of the Meliaceae family all over the tropics are well-known for their high timber quality. In Latin America, mahogany (Swietenia sp.) and Spanish cedar (Cedrela sp.) have, for centuries, been first class export timbers and are widely used within the countries. However, all attempts to establish large scale plantations have failed, due to the frequent attacks of the shootborer, Hypsipyla sp.

Over 100 years have elapsed since the description of H. grandella (1848), but so far no effective control measure is known. Control of this insect by means of insecticides is not considered feasible since the attack continues over a long period of time; the pest is inaccessible during most of its life cycle, and insecticides are costly and often have undesirable side effects. Basic information on the biology and behavior of H. grandella are still lacking; this fact has undoubtedly been one of the most important reasons why control attempts have been unsuccessful.

The fact is that the natural stock of the valueable Meliaceae is dwindling very rapidly throughout Latin America due to irrational cutting without reforesting of large size plantations. With a practical solution of the Hypsipyla problem, the

forthcoming benefits have been recognized all over Latin America.

Towards the end of 1970, the Department of Forestry of the Turrialba Center, under the directorship of Mr. Pieter Grijpma (under technical assistance of the Bureau of the Netherlands) initiated an Interamerican Working Group on Hypsipyla Problems. Recently, Dr. Edgar Clark of the U. S. Forestry Service has arrived (under FAO technical asiistance program) in Guatemala to work in forest insects especially Hypsipyla.

Recently, due to growing public concern about the safe use of chemicals for insect control, research has been diverted greatly towards finding non-chemical control methods. Because of its great economic importance throughout Latin America and the fact that biology and behavior of it offer promising aspects for control using sterile-male technique, we initiated work on H. grandella last year, in cooperation with the Forestry Department of this center. The main objective of the project is to evaluate the feasibility of its control by gamma sterilization method, which will involve work on the basic biology of this insect such as its mating and oviposition behavior.

As mentioned earlier in this report, some progress was made last year in rearing this insect on an artificial diet. This will enable a regular supply of a large number of insects through out the year for experiments. After resolving some basic biological techniques, eg. mating and oviposition problems, the studies will be carried out on the effects of gamma sterilization on the fertility, fecundity, longevity and mating competitiveness of treated males.

C. Radiobiology in Insect Pathology

This section has moved along the proposed lines, which are to study the radiation biology and the virulence of some insect pathogens like Metarrhizium anisopliae and Bacillus thuringiensis.

As a test insect we are using the Meliaceae shootborer, Hypsipyla grandella Zeller, considered the major detriment to the establishment of plantations of cedar and mahogany in tropical Latin America.

The main purpose of this section is to use radiation to induce mutants which are more radiation resistant and at the same time more pathogenic.

1. Pathological control of insect pests

a. Susceptibility of H. grandella to several gamma-radiation-induced mutants of M. anisopliae

If was shown in a previous experiment that color mutants FB-7-3 and FB-9-2 preserved their pathogenicity against H. grandella. Now we wish to include the gray-spore color mutant FB-13, and to characterize further these mutants to determine their LD_{50} pathogenic index, and nutritional requirements.

b. Susceptibility of H. grandella to several varieties of B. thuringiensis

It was also shown in a previous experiment that H. grandella is susceptible to B. thuringiensis var. thuringiensis and var. entomocidus. We plan next to standardize our bioassays following Dulmage et al.'s recommendations (J. Inv. Path. 18: 240;245. 1971), but utilizing H. grandella as a test insect. We will determine the LD_{50} of the following varieties:

thuringiensis, entomocidus, subtoxicus, sotto, alesti, HD-1, tolworth, ashman, dendrolimus, finitimus, morrison, limmasol, IICA-13-1-1-4 and IICA-13-1-1-5.

Our purpose is to find the most pathogenic strain against H. grandella.

2. Radiation biology and mutagenesis of insect pathogens

a. Survival of some gamma-radiation induced mutants of M. anisopliae after ultraviolet or gamma rays irradiation

This work is continuation on the radiation biology of M. anisopliae, We wish to compare the radiation resistance of the color mutants FB-7-3, FB-9-2 and FB-13 against the wild type. We'll also compare the radiation resistance of spores kept at room temperature and at 4°C.

b. Radiation biology and B. thuringiensis

So far we have determined the UV-254 survival of a few varieties of thuringiensis. Now we want to include strains like subtoxicus, morrison, ashman, tolworth, dendrolimus and limmasol, and to determine their resistance to 360 nm ultraviolet radiation and to Co-60 gamma rays. Our purpose is to determine which one is the most radiation resistant variety so we can select it and try to increase its pathogenicity by means of radiation induced mutations.

D. Training

In the training aspects of the Nuclear Energy Program, the staff members will give seminars, or lectures as to the use of

nuclear energy in agriculture. In addition, the staff members will direct the research of students for their thesis work at the M.S. level. It has been considered that the training center at Turrialba is a good stepping-stone to further higher learning in the Universities of the U. S. for the Latin American students. More than 30 percent of the graduate students and research assistants in this program have gone to U. S. Universities for further graduate studies at the Ph.D. level.

III. GRADUATE STUDENTS TRAINED, AND DEGREE GRANTED

Name	Nationality	Degree Granted	Period Trained
ARMENTA, Jorge[5]	Mexico		Sept.1971-
BERRIOS, Francisco[1,2]	Nicaragua	M. S.	Sept.1970-Mar. 1972
DELGADO DE LA FLOR, Luis[1]	Peru	M. S.	Sept.1968-Apr. 1970
DEL POZO, Jorge[5]	Peru		Sept.1971-
FUENTES, Raul[8]	Mexico	M. S.	Apr. 1968-Oct. 1971
GONZALEZ, Sergio[1,2]	Chile	M. S.	Sept.1970-Feb. 1972
GUERRA, Julio[1,2]	Peru	M. S.	Sept.1970-Feb. 1972
MARINHO, Murillo Lins[2]	Brazil	M. S.	Sept.1968-Aug. 1970
MIRANDA, Emo Ruy[3]	Brazil	M. S.	Sept.1970-Jan. 1971
MIRANDA, Paulo[1]	Brazil		Jan. 1970-Mar. 1970
ICAZA, Javier[6]	Nicaragua		Sept.1970-
MORELLI, Maurelio[2]	Brazil	M. S.	Sept.1969-Mar. 1971
PINEDA, Ricardo[1]	Peru	M. S.	Sept.1967-Jul. 1969
RAMIREZ, Eddie[8]	Venezuela	M. S.	Sept.1968-Dec. 1970
REYES, Jesus Antonio[1]	Colombia	M. S.	Sept.1968-Sept.1970
RIOS, Victoriano[1]	Panama	M. S.	Sept.1967-Dec. 1968
ROCABADO, José[7]	Bolivia		Sept.1970-
SANTANA, Charles[3]	Brazil	M. S.	Sept.1969-Apr. 1971
SUAREZ, Domingo[4]	Chile	M. S.	Sept.1969-May 1971
URRUTIA, Jorge[2]	Chile	M. S.	Sept.1969-Jan. 1971

1. On NEP Graduate Assistantship.
2. On IICA Fellowship.
3. On CEPLAC Fellowship (from the Brazilian Government)
4. On OAS Multinational Project Fellowship
5. Dutch Government Fellowship
6. On IICA-Northern Zone Fellowship
7. Special Student
8. Jr. soil chemist in NEP
9. Jr. entomologist in NEP

IV. BIBLIOGRAPHY

Berrios, F. and Hidalgo-Salvatierra, O. Susceptibility of Hypsipyla grandella Zeller to the fungus Metarrhizium anisopliae (in Spanish). Turrialba 21(2):214-219. 1971

_____ and Hidalgo-Salvatierra, O. Susceptibility of Hypsipyla grandella Zeller to the fungi Beauveria bassiana and Beauveria tenella (in Spanish). Turrialba 21(4): 451-454. 1971.

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

_____. Book review of: Water repellent soils. L. F. de Bano and J. Letey, eds. California University Press, Riverside, 1969. 354 p. Turrialba 19:438. 1969.

_____. Book review of: Soil biochemistry. A. D. McLaren and G. H. Petterson, eds. Marcel Dekker, N. Y., 1967. 509 p. Turrialba 19:438-439. 1969.

_____, and Fassbender, H. W. Uptake of fertilizer phosphate from nine soils from the humid tropics. Agrochimica XIV (2-3):259-268. 1970.

_____ and Pineda, R. The amorphous minerals and the mineralization of nitrogen in volcanic ash soils. In Panel on Volcanic Ash Soils in Latin America, held in the Training and Research Center, Turrialba, Costa Rica. July 6-13, 1969.

_____ and V. Rios. The movement of Ca, Sr, Mn and W in four Costa Rican Oxisols. Agronomy Abstracts, 1969:136.

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

Gonzalez, S., and Igue, K. Medición de la densidad de carga superficial en arcillas alogánicas por adsorción de Diquat^{+2} y Paraquat^{+2}. Turrialba 22(1):102-105. 1972.

Hidalgo-Salvatierra, O. Sex determination of Hypsipyla grandella pupae. Turrialba 21(2):221. 1971.

_____. Book review of: The genera of fungi sporulating in pure culture. Arx, J. A. von. Lehre, Germany, I. Cramer, 1970. (in Spanish). Turrialba 21(3):361-362. 1971.

Hidalgo-Salvatierra, O. Book review of: Biochemical interactions among plants. By National Academy of Science, Washington, 1971 (in Spanish). Turrialba 21(3):361-362. 1971.

_____. Susceptibility of Hypsipyla grandella Zeller to Bacillus thuringiensis thuringiensis and Bacillus thuringiensis entomocidus. Submitted to Turrialba for publication.

_____ and Madrigal, L., 1970. Trichogramma sp. an egg parasite of Hypsipyla grandella Zeller. Turrialba 20(4):513.

Hooper, G.H.S. and Katiyar, K. P. Competitiveness of gamma sterilized males of the Mediterranean fruit fly. Jour. Econ. Entomol. 64(5):1068-1071. 1971.

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

_____ 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).

_____ and Fuentes, R. Exchangeable Al in volcanic ash soils. SSSA Proc. (in press)

_____ and Fuentes, R. Retención y solubilización de ^{32}P en suelos ácidos de regiones tropicales. Turrialba 21(4): 429-434. 1971.

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

Katiyar, K. P. Aplicación de la energía nuclear en el control de insectos. Presentado en Segundas Jornadas Costarricenses de Microbiología, San José, Costa Rica, 3-6 Dic., 1969. Sin reimpresos.

_____. Comparación de dietas de zanahoria y de bagazo para la cría de larvas de mosca del Mediterráneo. Turrialba 20(2):217-222. 1970.

_____. Resultados más importantes de la investigación en entomología agrícola durante la década del 60. In Seminario Internacional sobre la Enseñanza de la Parasitología, IICA-ZN, Guatemala 20-24 marzo, 1972. (in press).

_____ and Ramírez, E. Some effects of gamma radiation on the sexual vigor of the Mediterranean fruit-fly, Ceratitis capitata (Wied.). In Sterile Male Technique for Control of Fruit Flies. Panel, Vienna, Austria, 1-5 Sept., 1969. Vienna IAEA, 1970. pp. 83-84 (Panel Proc. Series).

Katiyar, K. P. and Ramírez, E. Mating frequency and fertility of Mediterranean fruit fly females alternately mated with normal and irradiated males. Jour. Econ. Entomol. 63(4): 1247-1250. 1970.

_____ and Ramírez, E. Suppression of the reproductive potential of a wild strain Mediterranean fruit fly. Turrialba 22(2):156-159. 1972.

Marinho, M. and Igue, K. Effect of free sesquioxides and P upon Zn absorption by corn from volcanic soils. Agronomy Jour. 64:3-8. 1972.

Moh, C. C. Mutagenic effect of cycasin in beans (Phaseolus vulgaris L.). Mutation Research 10:251-253. 1970.

_____. Mutation in seed-coat colors of beans (Phaseolus vulgaris L.). Euphytica 20:119-125. 1971.

_____. Induced seed-coat color mutations in beans and their significance for bean improvement. In Induced Mutations and Plant Improvement, IAEA, Vienna, 1972. pp. 67-72.

_____ and Alan, J. J. Bean mutant induced by ionizing radiation. V. Curly leaf. Turrialba 20:120-121. 1970.

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

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

_____ and Alan, J. J. Bean mutant induced by ionizing radiation. VII. Compact mutant. Turrialba 21:478-480. 1971.

_____ and Alan, J. J. Pollen irradiation as a method for inducing mutations in cassava. Submitted to Euphytica for publication.

Morelli, M., Igue, K., and Fuentes, R. Efecto del encalado en el complejo de cambio de movimiento de Ca y Mg. Turrialba 21(3):317-322. 1971.

Santana, C. L. and Igue, K. Determinación de micro-elementos por absorción atómica. Turrialba 21(3):358-360. 1971.

_____ and Igue, K. Formas de micronutrientes em solos da região cacaueira da Bahia. Turrialba 22(1):73-80. 1972.

Urrutia, J. and Igue, K. Reacciones de los fosfatos monocalcio monohidratado y dicalcio anhidro en suelos volcánicos. Turrialba 22(2):144-149. 1972.

V. OPINION AS TO THE PRESENT STATE OF KNOWLEDGE IN THIS AREA OF RESEARCH, ITS SIGNIFICANCE IN THE FIELD OF BIOLOGY AND MEDICINE, AND NEEDED FUTURE INVESTIGATIONS.

According to the latest statistical projection of human population (cf. Liener: Toxic Constituents of Plant Foodstuffs, Academic Press, N. Y., 1969), there will be six billion people living on earth by the end of this century, and it will need many times more protein supply in order to keep the same nutritional level as we have at present. This implies that not only the protein supply but the foods serving as an energy source (carbohydrates) will also need to increase as well. Vital statistics in past years showed that the tropical regions have the highest birth rate. If this trend is continued, the food problem will be more acute in the tropics, since even now, many people are undernourished in many parts of the tropics.

Many countries in the tropical zone are agriculturally dependent. Because of the continuous growing season and the high humidity in many areas of the tropics, diseases and insect pests are the major problems in agriculture. These problems, in turn, affect the production of the crops. The danger of using pesticides as a control measure has further hampered the crop production.

The lines of research covered by the Nuclear Energy Project are of an agricultural nature. They include the improvement of tropical crops and the control of the insect pests.

Manihot is selected as our working crop because it is a basic food crop in the tropics and has large yield with the

great potential of meeting the needs of the growing population in the tropics. As has been pointed out in this report, there are some undesirable characters in this plant. One is its relatively long life cycle and the other is the presence of the cyanogenetic glucoside.

So far as we know, there are no Manihot cultivars producing mature roots in less than 8 months. By using pollen irradiation method, we were able to isolate a number of fast growing, vigorous R_1 mutants. These mutants are being tested for their production and earliness. By shortening the life cycle of Manihot, it is possible to make it more competitive with other crops.

Quite a number of food plants contain natural chemical constituents which are toxic for human consumption. Hemagglutinins in the legumes and cycasin in the cycads are the examples. The toxic chemical compound in Manihot plants is the cyanogenetic glucoside. The hydrocyanic acid (HCN) released from the glucoside is poisonous. A quantity of 50-60 mg of HCN is considered a lethal dose to man. The average HCN content of Manihot cultivars contains 50-100 mg per kilogram of fresh roots, but some cultivars contain more than 300 mg. While the roots of low HCN cultivars are considered to be safe to eat after cooking, there is great concern as to the pathological effect of accumulating low HCN concentrations. In tropical Africa, an individual consumes as much as one kilogram of Manihot roots per day, and it has been speculated that amblyopia in West Africa may be a manifestation of chronic Manihot poisoning. Recent studies in the

Manihot-eating area of Nigeria showed that the patients with the tropical atoxic syndrome had a high thiocyanic level in the plasma. This is the first evidence linking the consumption of trace amounts of HCN and the development of chronic diseases.

The fundamental solution of this problem is to cultivate the HCN-free Manihot. So far, no HCN-free cultivars are known. Whether an HCN-free clone can be induced by irradiation method is worth exploring.

The research program in entomology includes several tropical destructive insects.

The Mediterranean fruit fly is a very destructive pest of citrus throughout the tropical and subtropical regions of the world. Besides citrus, it attacks more than 200 other species of fruit and vegetable crops.

Presence of medfly in Central America was reported for the first time in 1955 from Costa Rica. Since then, the fly has moved to both neighboring countries of Nicaragua and Panama.

Data for calculating actual losses caused by this fly in Central America are not available. According to one estimate, actual and potential losses due to this fly amount to $298,699,400 in Central America including Mexico and Panama and in the U.S.A. it ascends to $1,137,312,000 (reported by Jarvis E. Miller, Consultant Economist of IAEA, "Studies on the actual and potential losses caused by Medfly in OIRSA region - Central America, Panama and Mexico - April, 1970).

Basic research carried out by us under medfly project enabled OIRSA to carry out a pilot test in Nicaragua in cooperation

with IAEA to demonstrate the effectiveness of sterile male technique to control medfly. The results showed that medfly can be eradicated from Central America by sterile male releases. However, present IAEA support to OIRSA will cease at the end of this June. But efforts are being made to continue the project and achieve medfly eradication from Central America. Very recently an USAID sponsored team has concluded its visit in Central America to assess the medfly situation. The official report of this team is not yet out, but it is expected that the team is in favor of recommending medfly eradication from Central America.

As can be seen, a good deal of progress has been made in medfly control but still a lot of basic information is pending if eradication is going to be affected. Studies will be needed to improve the mating vigor of the sterile male. Similarly, effect of sterilization on the dispersal habits of the treated insects under natural conditions will have to be studied in detail.

Hypsipyla grandella Zeller is a serious pest of the family Meliaceae which includes such classical timbers as mahogany (Swietenia sp.) and Spanish cedar (Cedrela sp.) both native species throughout most of Latin America. All attempts to re-establish large size plantations of these valuable Meliaceae have failed due to frequent attacks by the Hypsipyla species.

It is surprising to note that more than a century has passed since the description of H. grandella in 1848, yet very little progress has been made towards its control. So far, no effective

and economic means of control are known. Basic information on biology and behavior of H. grandella are still lacking. This fact undoubtedly has been one of the most important reasons why any control attempts have been unsuccessful.

Benefits forthcoming with a practical solution of the Hypsipyla problem have recently been recognized. Interest has been constantly growing in Hypsipyla research in all Latin American countries since last couple of years, when towards the end of 1970 an Interamerican Group on Hypsipyla Problems was organized at Turrialba Center. At the moment this group enlists 65 members from 25 different countries who are actively working or have shown some interest in Hypsipyla research.

At Turrialba Center about 12 members (scientists and students) are working on different aspects of Hypsipyla problem. We have made some progress in rearing this insect on artificial diet in the laboratory. But more work on the basic biology and ecology of the insect is needed in order to device some suitable control measures.

The coffee leaf miner, Leucoptera coffeella (Guerin-Meneville) is another important insect pest attacking coffee through out Latin America. The only satisfactory control of this insect is achieved through 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 non-chemical control method for this insect.

The dose and sterilization procedures, etc. have been worked out (as presented earlier in this report) for this insect. We have also found that the F_1 inherited sterility can be induced in the coffee leaf miner moths. In the future, therefore, work is needed to evaluate the practical use of sterile male technique (including use of F_1 sterility) in controlling the coffee leaf miner.

As far as the application of radiation biology in insect pathology is concerned, there is another laboratory, the All Union Research Institute of Plant Protection in Leningrad, where they study the lethal and mutagenic effects of UV and X-rays on some insect fungi. In the United States and Canada there is some work done along this line but it is scattered and not directly concerned with the applications of radiobiology in insect pathology.

This field has been touched only superficially. Its significance to biology and medicine is great if we think on the possibilities of reducing the use of highly toxic insecticides that contaminate air, soils and water and are the cause of so many cases of poisoned people and animals.

As an application of radiation and radioisotopes to agriculture, there is no doubt that this is a field where nuclear energy can find many applications directly or indirectly related to a particular problem.

As a problem of pure radiation biology, M. anisopliae, B. thuringiensis and the insect viruses can be used for dark repair

studies, photorecovery processes, chemical protection, mutagenesis, phage work, etc.

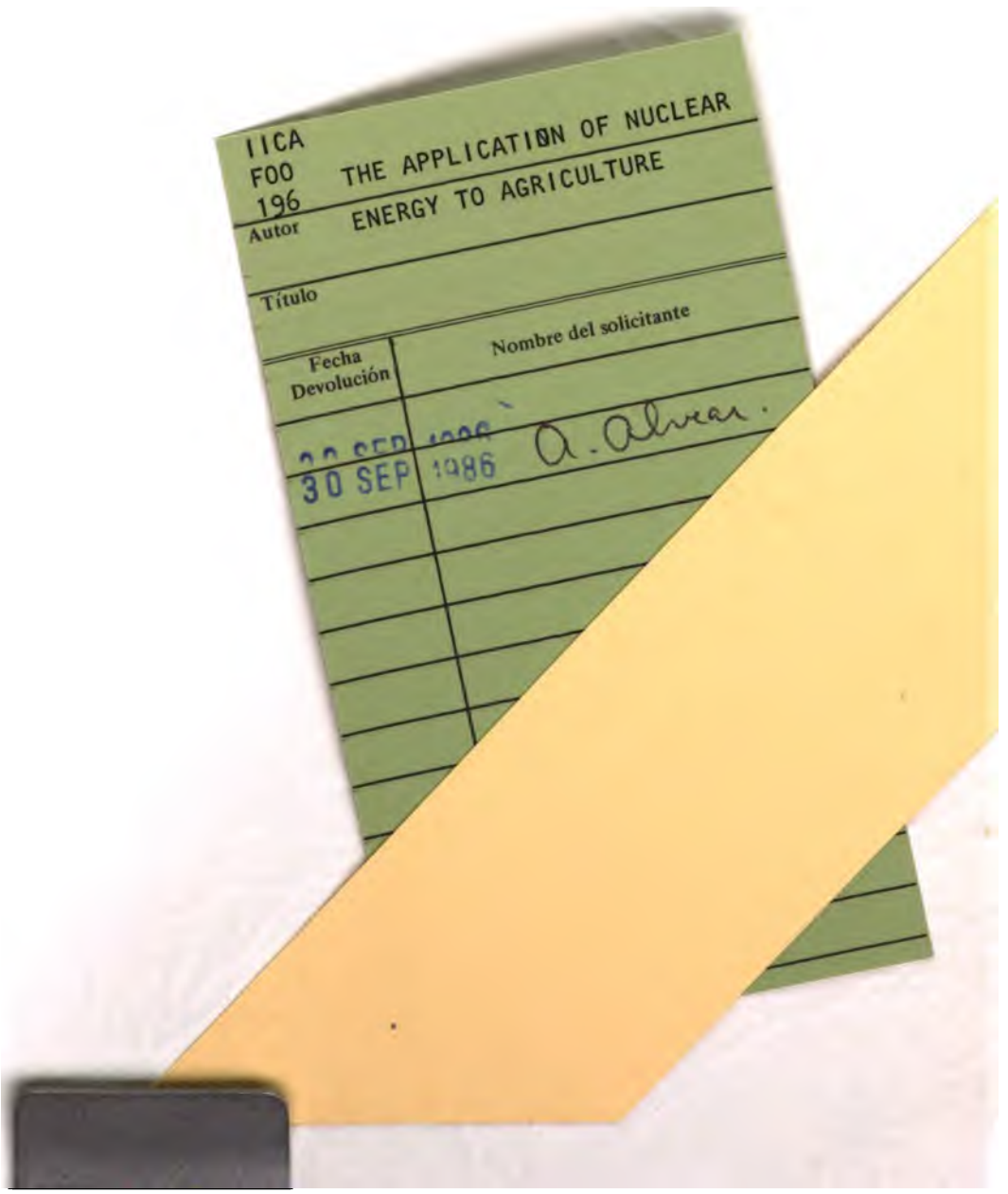
VI. THE PRESENT DIVISION OF FEDERAL SUPPORT FOR THE PROGRAM

The research and training activities in the Nuclear Energy Program at the Tropical Training and Research Center, Inter-American Institute of Agricultural Sciences of the O.A.S. are 100 percent support by the U. S. Atomic Energy Commission under Contract AT(11-1)-3217.

ESTA OBRA SE TERMINO DE IMPRIMIR EL VEINTE DE JUNIO DE MIL NOVECIENTOS SETENTA Y DOS EN LA IMPRENTA DEL IICA-CIDIA.

SE HIZO UN TIRAJE DE
200 EJEMPLARES

FECHA DE DEVOLUCION			
[illegible]			
30 SEP [illegible]			


IICA
F00
196
Autor
THE APPLICATION OF NUCLEAR
ENERGY TO AGRICULTURE
Título
Fecha Devolución
Nombre del solicitante
30 SEP 1986
A. Alvear.