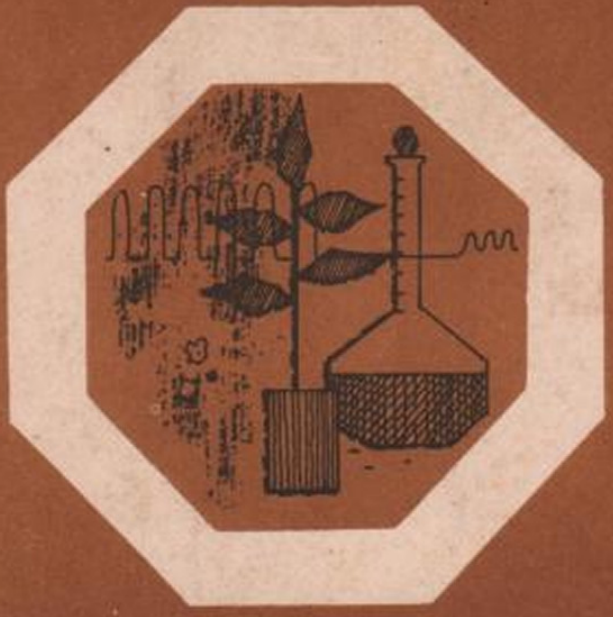
revista THEOBROMA



V. 8 – ABRIL – JUNHO 1978 Nº 2

Ilhéus – Brasil

REVISTA THEOBROMA

Publicação trimestral do Centro de Pesquisas do Cacau (CEPEC) da Comissão Executiva do Plano da Lavoura Cacaueira (CEPLAC), vinculada ao Ministério da Agricultura, Brasil.

Comissão Editorial: Paulo de Tarso ALVIM, Ph.D., Diretor Científico da CEPLAC; Fernando VELLO, M.S., Diretor Técnico da CEPLAC; Luiz Ferreira da SILVA, Eng. -Agrº, Diretor do CEPEC; José Correia de SALES, Eng. -Agrº, Editor.

Assessores cientfficos que participaram da revisão dos trabalhos contidos no presente número: Forbes Peter BENTON, Ph.D.; Rafael E. CHEPOTE, Eng. -Agrº; Joseph ITURBE G., Bioquímico; Mohamed Kamal El KADI, Ph.D.; José Oliveira LEITE, Eng.-Agrº; Max de MENEZES, Ph.D., Maria Neide SILVA,Bibliotecária; Pedrito SILVA, Eng.-Agrº.

Distribuição por permuta

Endereço para correspondência (address for correspondence): Divisão de Comunicação da CEPLAC (DICOM); Caixa Postal 7; 45.600 - Itabuna, Bahia. Brasil.

Tiragem: 4.000 exemplares

Revista Theobroma, v. 1. nº 1 1971
Ilhéus, Comissão Executiva do Plano
da Lavoura Cacaueira, 1971 –
v. 22,5 cm

1. Cacau – Periódicos. I. Comissão Executiva do Plano da Lavoura Cacaueira, ed.

CDD 630.7405

REVISTA THEOBROMA

V. 8 Abril – junho 1978 N. 2

CONTEÚDO

REVISTA THEOBROMA

V. 8 April – June 1978 No. 2

CONTENTS

LABORATORY REARING OF *Forcipomyia* spp. MIDGES (DIPTERA, CERATOPOGONIDAE): 1. ADULT FEEDING LARVAL FEEDING AND COPULATION TRIALS; A REVISION OF SAUNDERS METHOD OF REARING

Hugo Albert Besemer *
Saulo de J. Soria **

ABSTRACT

Laboratory mass rearing of *Forcipomyia* may be important to increase the pollination of *Theobroma cacao* L. The Saunders rearing method permits the rearing of only one generation.

Several compounds were tested as adult food, in order to increase the reproductive capacity. The value of several microorganisms as larval food was tested, as well as the effect of larval density on larval growth. The effect of temperature and humidity was tested on adult longevity, and the degree of fertilization in emergence boxes was compared to the degree in the field.

Feeding on a combination of sugar and a cacao flower increased the percentage of females ovipositing from 33.3 to 71.4% and the size of the batches from 55.5 to 117.3 eggs, as compared to sugar only. This effect could not be imitated by adding casein, cholesterol or calcium oxalate to the adult diet.

Larvae grew quickest on malt agar dishes with *Pseudomonas* sp. and *Xanthomonas* sp. Saprophytic fungi appeared to inhibit growth. There is a density above which growth is inhibited. Within the ranges above and below this density there is no clear density effect.

Received for publication on October, 20, 1977, and in revised form on August 2, 1978.

* Agr.-Eng. Candidate, Agricultural University of Wageningen, Holland.

** Ph.D., Divisão de Zoologia Agrícola, Centro de Pesquisas do Cacau, C.P. 7, 45.600, Itabuna, Bahia, Brasil.

No correlation was found between average maximum and minimum daily temperature and humidity, and longevity. A high correlation was found between the longevity of the female and the number of days which preceded the maximum air temperature.

Sperm was found in the spermathecae of females emerging from emergence boxes. The percentage of females copulated was low, below 10%, which was also the case in the field.

It was concluded that emphasis should be put on the possible role that vitamins from cacao flowers have on the reproductive capacity. Larvae feed mainly on bacteria. Copulation can take place in dark, moist circunstances, shortly after emergence. Peak temperatures are an important factor determining adult mortality.

INTRODUCTION

Obtaining successive generations and large numbers of *Forcipomyia* midges in the laboratory is the goal to be achieved, because of the potential use of these insects to increase cacao pollination, either by liberation of these insects when populations are scarce or by introduction into areas where they are absent.

Rearing of *Forcipomyia* midges has been done in the past using a simple empirical method devised by L. G. Saunders (5) for cacao pollinating species. Adult females collected in the field are fed on 20% sucrose solution, in single rearing cages until oviposition (3, 6). The eggs are recovered from the cages and placed on malt agar medium inoculated with washings from decaying organic material from the leaf mat of the cacao plantation. Emerging larvae are reared to pupation, and adults emerge a few days after pupation from the same environment. The culture however stops at this stage:.females fed on sugar solution survive until oviposition but the eggs never hatch.

Whereas population growth and mass rearing is the general aim, the specific objectives of this work were to study several compounds as diets for adults and to investigate several culture media and microorganisms for larvae in order to optimize population growth and overcome the problem of non-viability of the eggs of females, obtained in the laboratory. Additional observations are included regarding effects of larval crowding on larval growth rates and on preliminary copulation trials.

MATERIAL AND METHODS

Experiments were carried out in the Cacao Research Center (CEPEC – CEPLAC) during 1977.

Feeding experiments with adults. Females collected from the field were put into single rearing cages as described by Soria and Wirth (6). They were fed

using little discs of purple blotting paper, thoroughly washed before in order to avoid contact with possible toxic compounds. These discs were dipped in either a 20% sucrose solution, a 10% casein hydrolysate solution, or water. One group of caged females was supplied with both a sucrose disc and a casein disc, all other females received only one type of disc.

To evaluate the treatments, the longevity of the female, the number of eggs oviposited and the number of hatched eggs were determined.

Other feeding experiments used females obtained from emergence boxes, as described by Winder and Silva (8). The boxes were filled with one year old, rotten cacao fruits.

The following series of treatments were compared in the order enumerated below.

1. Sucrose 20%, cholesterol 50 ppm solution in physiological serum (1/1000 NaCl solution), a combined solution of 20% sucrose and 50 ppm cholesterol in physiological serum, and the latter solution combined with a 10% casein disc.

2. The same treatments as in 1., using 100 ppm cholesterol instead of 50 ppm cholesterol.

3. A mixed solution of 20% sucrose and 100 ppm cholesterol in physiological serum, cholesterol 100 ppm in physiological serum, sucrose 20% solution in physiological serum, and physiological serum only.

4. Sucrose 20%, a combined solution of 20% sucrose and 0.1% calcium oxalate, the latter solution combined with a 10% casein disc, and a 0.1% calcium oxalate solution.

5. Sucrose 20%, sucrose 20% combined with a cacao flower, and a cacao flower only. The cacao flower was supplied stuck in a small dentist anaesthesia tube hanging on copperwire and filled with cotton dipped in Hoagland solution.

All solutions were supplied on little discs of blotting paper. These treatments were submitted to the same procedures as in the casein experiment above. The chemicals used were the commercial types for laboratory use manufactured by Merck, except for the calcium oxalate manufactured by Carlo Erba.

Feeding experiments with larvae. *Forcipomyia* larvae were removed from rotten cacao beans. The beans were wetted (avoiding excess water) and then put into petri dishes (9.6 cm diam.). These were autoclaved for 20 minutes at 120°C and 1 kg/cm^2 pressure. Ten larvae were later placed in each petri dish. The larvae were treated differently as follows. Five dishes were supplied with larvae washed in water and rinsed in 1/1000 $HgCl_2$ solution in order to prevent contamination of the beans by microorganisms. The larvae placed in five other dishes were washed with water only. As a test, ten larvae washed and rinsed in

$HgCl_2$ were placed in a malt agar dish inoculated with washings. Ten other larvae, washed in water only, were placed in another malt agar dish. In a similar pair of dishes, 10 larvae were placed in each one; all larvae were washed in water; the larvae of one of the dishes were also rinsed in $HgCl_2$ and both dishes were sprayed with an extract from autoclaved rotten beans.

In another experiment different microorganisms were isolated from petri dishes, inoculated with washings, in which larvae were living for some time, which were obtained by means of the Saunders rearing method, as described in the Introduction.

Isolations were made by dissolving inoculum from the dishes in distilled water, and inoculating autoclaved malt agar dishes with these solutions. The same procedure was repeated, taking inoculum from one colony, until dishes were obtained which, at least seemed to contain only colonies of one type of microorganism.

These different types of microorganism were screened for their value in the feeding of *Forcipoymia* larvae. This was done by placing larvae, obtained by means of Saunders rearing method (descendants of one pair of parents) on petri dishes (9.6 cm diam.) with a culture of a certain microorganism. The average larval growth rate was determined by measuring the lenght of the living larvae as they were stretched on dry paper. Measurements were carried out once every two days, and the everage lenght of the larvae of one dish was taken as a measure for the growth within this population. The whole experiment was executed in duplo. It should be noted that change of weight would have been a better measure for growth in the population. but any weighing procedure might be too prejudicious for the very vulnerable larvae.

Milk agar and peptone agar instead of malt agar were used when the latter experiment was repeated. In these cases only bacteria were isolated. This experiment was done only once. Milk agar and peptone agar were manufactured by Merck, malt agar by Difco.

The different microorganisms were given names on the basis of the shape and color of the colonies.

Density effects on larval growth. Larvae, obtained from one batch of eggs by means of the Saunders rearing method, were placed at different densities in malt agar dishes (5.4 cm diam.), one day after hatching. The dishes were prepared and inoculated with washings from rotten cacao leaves. Dishes were prepared and inoculated five days before the start of the experiment, and were chosen to have as near possible the same stage of microbial growth. The recently hatched larvae, descending from one pair of parents, were set at densities of 5, 10, 15, 20 and 25 larvae per dish.

Growth was measured by the same procedure as in the experiment of

screening of microorganisms, and special emphasis was given to the difference between the initial linear stage of growth and the later nearly asymptotic stage of growth.

Effects of temperature and humidity on adult longevity. Females emerging from emergence boxes (8) were placed in single rearing cages (6) and fed by means of little discs of blotting paper with 20% sucrose solution, and their longevity was determined. All material in the emergence boxes was collected, several times, from one place. From the rotten fruits, that were collected, mainly *F. (F.) genualis* and *F. (F.) harpegonata* emerged.

A thermohygrograph (R. Fuess, portable) – at 20 centimetres from the tested midges – recorded the daily maxima and minima of temperature and humidity. This gave a general idea of the room climate of the experiment.

Correlation coefficients and regression coefficients were calculated between longevity and the everage maxima and minima of temperature and humidity to which the midges were exposed during their life. It should be noted that the midges were not exposed to the humidities as measured, since the cages were placed on wet paper towels.

Copulation trials. Emergence boxes, as described by Winder and Silva (8) appeared to yield females which were able to lay fertile, hatchable eggs. These boxes are made of wood, each consisting of five compartments, with a layer of 3 cm of wet sand at the bottom of each compartment in order to maintain humidity and prevent drying out of the contents. The emerging insects are collected in glass tubes with a baffle, screwed into the lid of each compartment. In the present study the boxes were filled with rotten cacao fruits, which proved to be an excellent breeding medium for *Forcipomyia* subgenus *F.* sensu stricto.

In order to investigate, whether copulation had taken place in these boxes, samples of about 20 females per day per box were examined for the presence of sperm cells in the spermatheca.

This was done by drowning the midges in physiological serum (1/1000 NaCl solution), putting them on slides in a drop of serum, separating out the spermathecae, and squashing these under a cover slide. The spermathecae were then examined under a compound microscope (800x). The sperm cells are unmistakable when present.

The percentage of copulation was compared with a parallel sample, made at the same time, of 20 females collected in the field from flowers. These were examined in the same way.

RESULTS AND DISCUSSION

Adult feeding. No evidence could be found that adding a protein source

(casein) to the adult diet improved the reproduction of *Forcipomyia* (Table1) It should be noted that females, handpicked in the field, may already have fed before they arrived in the laboratory. This must be taken in account when comparing Table 1 with Tables 2 and 3. The age of the midges when they arrived in the laboratory was unknown. This resulted in a lesser longevity, when they were fed on sucrose only, as compared to midges obtained from emergence boxes.

The suggestion, in the literature, that the females need a protein source in their diet (6) could not be confirmed.

Omitting a sugar source in the diet appears to be prejudicial to the fitness of the females and their reproductive capacity (Table 2). The effect of cholesterol cannot be ascribed to the serum in which it was diluted, since feeding on serum only appeared to be less favourable to reproduction than feeding on cholesterol diluted in serum.

Feeding on the flower only had a remarkably favourable effect on reproduction. Also the longevity in this

Table 1 – Effect of feeding females, hand picked in the field, with sucrose and casein hydrolysate solutions, on oviposition capacity and hatchability of eggs.

Parameter measured	Sucrose (20%)	Casein (10%)	Combined	Water
Percentage ♀♀ ovipositing	12.9	8.8	16.3	12.3
Average number of eggs per batch	72.1	48.4	73.0	67.1
Average longevity of ♀♀ (in days)	2.48	1.15	1.98	1.67
Percentage of eggs hatching	31.4	13.0	25.0	1.45
Size of sample (number of ♀♀)	77	79	73	73

Table 2 – Effect of feeding on oviposition capacity, egg hatchability and female longevity, of adult females, obtained from emergence boxes, fed with cholesterol 50 ppm and 100 ppm solutions in physiological serum, physiological serum only, and flower, as compared to controls fed on 20% sucrose solutions only from the same populations (values for sucrose between brackets).

Parameter measured	Cholesterol 50 ppm	Cholesterol 100 ppm	Serum	Flower
Percentage ♀♀ ovipositing	6.3(31.5)	6.1(31.1)	0(53.3)	31.2(39.1)
Average number of eggs per batch	56.0(37.0)	69.7(48.2)	-*(75.8)	92.0(57.8)
Percentage of eggs hatching	0.00	0.00	0.00	1.55(11.6)
Size of sample (♀♀)	32	40	15	30

* Not recorded.

treatment was relatively high: 5.2 days whereas females of the same population on which this specific experiment was carried out lived 6.7 days when fed on 20% sucrose only.

If a sugar source is included, by far the best combined diet appeared to be flower + sugar: 71.4% of the females oviposited with an average of 117.3 eggs per batch (Table 3). The percentage of hatching in this treatment however was lower than in the treatment fed on sucrose only. This may be due to a lower average of fertilisation in the specific population on which the treatment was applied, or a lack of spermcells compared to the large number of eggs produced. In this respect it is interesting to note that with a diet of 50 ppm cholesterol, 20% sucrose and 10% casein, a very high percentage of the eggs hatched. More experiments should be done before stating anything definitely about this.

The treatments with calcium oxalate could not be included in the Tables. since the eggs in this treatment were of an immature appearance. Whereas eggs are normally chitinized, octaedric, uniform in size, and gen-

Table 3 – Effect of feeding adult females, obtained from emergence boxes, with a combination of sucrose and several other food substances. Effects on oviposition capacity, egg hatchability and female longevity.

Parameter measured	Sucrose alone	Cholesterol (50 ppm)	Cholesterol (100 ppm)	Cholesterol (50 ppm) + 10 % casein	Cholesterol (100 ppm) + 10 % casein	Serum (1/1000 Na Cl)	Flower
Percentage ♀♀ ovipositing	33.3	25.0	30.0	21.8	20.0	20.0	71.4
Average number of eggs per batch	55.5	49.8	52.6	39.2	56.4	57.8	117.3
Percentage of eggs hatching	15.5	1.54	5.12	59.0	-*	0.0	5.80
Average longevity of ♀♀ (in days)	5.9	4.9	4.8	3.1	3.94	4.53	6.2
Size of sample (♀♀)	54	52	37	32	35	15	30

* Not recorded.

erally laid in strings, these eggs were whitish, shining, horse shoe shaped, and laid in a droplet of fluid. Since they were very variable in size, it was impossible to decide, what could be considered as an egg, and what not. The percentage of females ovipositing was rather high, 60%. Perhaps calcium oxalate plays a role in stimulating oviposition, but in that case the concentration used in the experiment was too high.

In general it can be concluded, that a sugar source, other than cacao flowers only, is necessary for the survival of the adults. Some substances in the cacao flower are beneficial to the reproductive capacity of the midges. This effect could not be imitated by adding a protein source, (casein) or cholesterol to the diet.. It is known that for some insects casein has too low a content of certain amino acids, such as cystine and glycine (7). On the other hand no beneficial effect could be found at all, as casein contains all amino acids essential for insects.

Cooke and Sang (1) found that phytosterols were superior to cholesterol, for *Drosophila,* but the present trials did not show any beneficial effect of adding cholesterol to the diet. So it seems most worthwhile to put emphasis on watersoluble vitamins to explain the beneficial effect of feeding on cacao flowers on the reproduction. The highest longevity occurs if females are fed on 20% sucrose only.

Larval feeding. Only one larva pupated when fed on the medium of autoclaved rotten beans. Presumably this larva was already fed sufficiently before being placed on this medium. In factum pupation may also be reached when larvae are put between wet filter papers, in order to recover faeces. Mortality did not occur in any of the malt agar dishes. So the mortality of the larvae fed on autoclaved beans cannot be ascribed to the treatments of washing and rinsing, and also not to a watersoluble substance from the autoclaved rotten beans. Therefore the most probable conclusion is that the larvae live on the biomass of the microorganisms involved in the decomposition of the material, and not on the biomass of the rotten beans.

If reared on malt agar dishes with different cultures of microorganisms, the highest growth rates can be found feeding with cultures of *Pseudomonas* sp. and *Xanthomonas* sp. (Table 4), 1.1 mm/day. A lower growth rate was observed in cultures of *Mucor* sp. (0.60 mm/day). This was the only fungus which could sustain growth of the larvae. No growth at all could be observed in cultures of saprophytic fungi such as *Penicilium* sp., *Aspergillus niger,* and *Bothryodiplodia theobromae.* In these cases mortality occurred within 2 days. If larvae were reared on a mixture of *Pseudomonas* sp. and *Penicilium* sp. a lower growth rate was found than in the pure culture of *Pseudomonas* sp. (0.45

Table 4 – Growth rates in the initial growth stage and length of larval stage, of *Forcipomyia* larvae reared on malt agar dishes with colonies of different microorganisms.

Microorganism	Initial growth rate (mm/day)	Length of larval stage (days)*
Pseudomonas sp.	1.10	7
Xanthomonas sp.	1.10	6
Penicillium/Pseudomonas combination	0.45	8
Mucor sp.	0.60	8
Penicillium sp.	0.00	-**
Aspergillus niger	0.00	-**
Bothryodiplodia theobromae	0.00	-**

* Difference between first day on which hatching and the first day on which pupation occurred.

** Complete mortality after 2 days.

mm/day). Also the lenght of larval stage was longer in the dishes where growth occurred in the presence of fungi, indicating that fungi delay larval development.

If reared on milk agar or peptone agar dishes with cultures of bacteria, the larvae did not survive. Probably this was due to the fact that these media soon became very soft, and the larvae drowned in these media. In earlier experiments it was observed that larvae of the subgenus *Euprojoannisia* are more tolerant to a medium with a consistency of ooze than larvae of the subgenus *Forcipomyia* sensu stricto.

Contradictory conclusions can be found in literature about larval feeding. Saunders (5) concluded from the occurrence of microoorganisms in the stomach that the larvae feed on these "molds, yeasts and bacteria". Dessart (2) concluded from the fact that also decayed plant tissues could be found in the stomach, that the larvae feed on these tissues. The present study gives an indication that the larvae feed mainly on the plant pathogenous bac-

teria in their habitat. The intestinal tract after dissection appeared to contain the bulk material of the rotten cacao beans, on which the dissected larvae lived. The faeces, which can be recovered easily by keeping the larvae a few days between wet filter papers appears to consist of the same material.

Density effects. Growth rates at densities 5 and 10 are much higher than the growth rates occurring at densities 15, 20 and 25 (Table 5). Within the two categories, however, growth rates were similar to each other. A possible explanation is that at a certain density food (microorganisms) become a limiting factor, and that above this density there is an equilibrium between the amount of microorganisms consumed and the growth of the microorganisms.

Below the interference density there is still considerable growth after 5th day after hatching, whereas above this density hardly any growth occurs after the 5th day. Consequently the lenght of larval stage was longer at higher densities. Figure 1 shows the relation between density and the time needed to reach 50% pupation. This value was obtained by interpolation.

In general in any *Forcipomyia* breeding system the optimal density should be chosen in relation to that system.

Effects of temperature and humidity. The experiments described in this paper were executed in physical conditions which are generally considered as unfavourable for keeping adult diptera alive. In the course of 20

Table 5 – Growth rates of *Forcipomyia* larvae on inoculated petri dishes with malt agar (5.4 cm diam.) at deliberatively chosen densities, in the linear and asymptotic growth stage.

Density (larvae per dish)	Growth rate before fifth day (mm/day)	Growth rate after fifth day (mm/day)
5	0.93	C.21
10	0.94	0.21
15	0.68	0.08
20	0.68	0.00
25	0.69	0.02

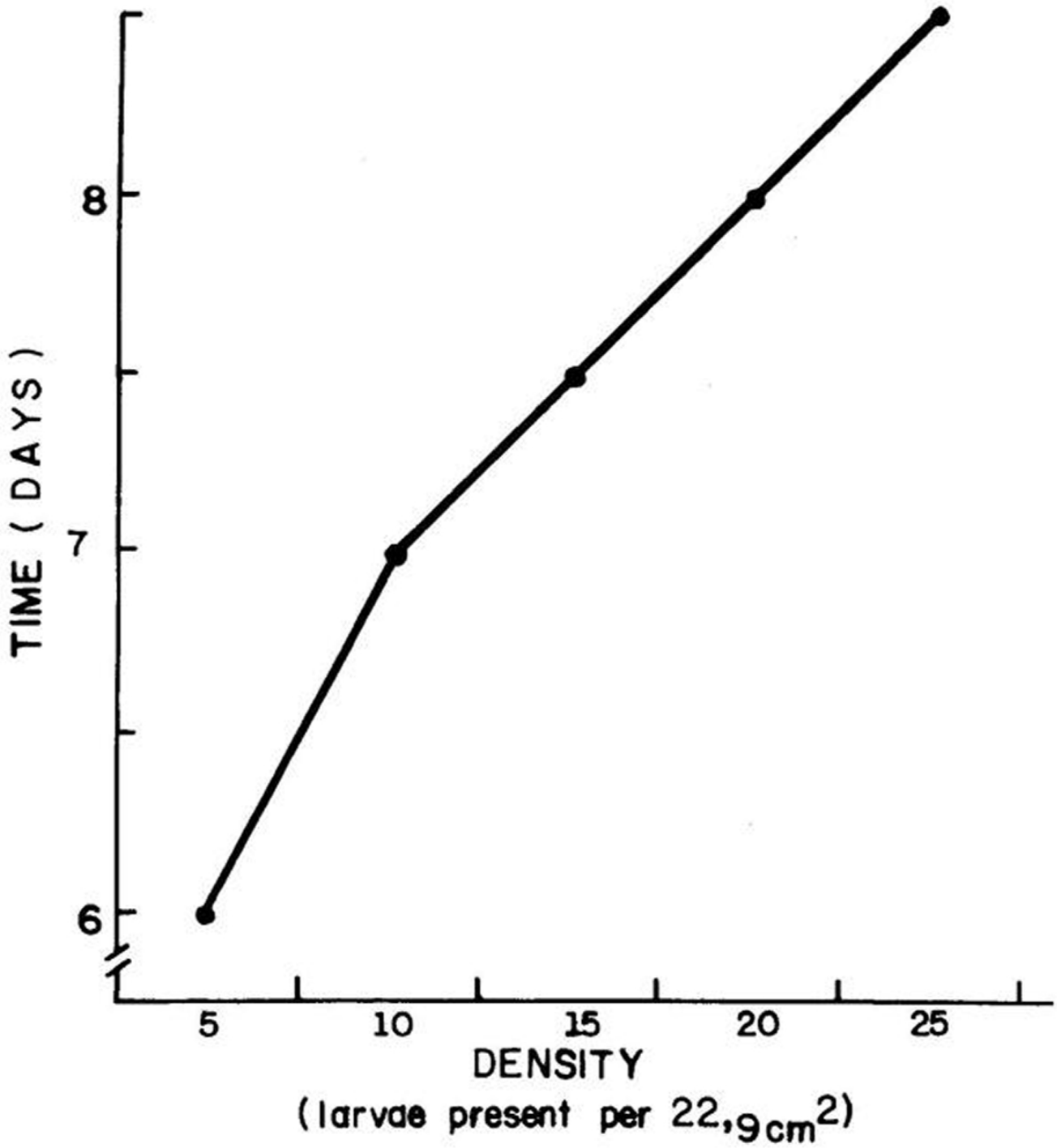


Figure 1 – Time needed to reach 50% pupation of larvae, related to the density of *Forcipomyia* larvae.

weeks the air conditioning was functioning 65% of the days.

During air conditioned days the maximum temperature occurs at about midnight, and maxima are between 26.5 and 30.7°C. At 8 a.m. there is a sudden fall in temperature, with stable minima between 24.0 and 24.5°C. The mean temperature is variable (a range of 2 – 3°C). After 4 p.m. the temperature rises steadily towards the

maximum at midnight. The relative humidity is clearly related to the temperature, maxima occur also at midnight; these may vary from 68 to 88%. At 8 a.m. a sudden fall in humidity occurs, to a minimum that varies between 45 and 57% After this minimum a steady rise of humidity occurs.

On abnormal days changes are smaller. Maxima in temperature and humidity are reached between 6 and 8 p.m., and range from 26.5 to 34°C. Minima are reached between 8 a.m. and noon, and vary from 24 to 31°C. Humidity does not follow a clear daily pattern on these days, and shows daily maxima between 78 and 99% and daily minima between 58 and 78%.

There appeared to be a very low correlation between average maximum temperature to which the midges were exposed, and their longevity ($r = 0.0041$). Also the correlation coefficient between average minimum temperature and longevity was very low ($r = 0.0466$). Correlations between longevity and average daily maximum and minimum humidity were not significant ($P = 0.01$).

On the other hand, there appeared to be a high correlation between number of days before the highest temperature during the life of the midge, and the longevity of the females. The correlation coefficient was 0.890, significant with a reliability of 0.01.

The regression coefficient was $b = 0.41$. This means that if the maximum temperature occurs one day later, longevity is to be expected 0.41 days longer.

The proportion of the sum of the squares of longevity, attributable to variation in time before the maximum temperature occurred is 78%.

It can be concluded, that peaks in temperature are highly correlated with mortality of adult midges in single rearing cages.

Copulation trials. By examining the contents of the spermathecae a positive proof could be found that copulation has taken place in the emergency boxes. The average of copulation however is very variable. The number of populations sampled and the variability are respectively too low and too high to give any average reliable enough to be published. Certainly the average of copulation was never higher than 10%, and also of the populations sampled in the field none exceeded this average.

Kaufman (4) states that she was able to get consecutive generations of african *Forcipomyia* species in a round cage, covered with fine mesh cloth. She states nothing about moisture and light conditions in the cages. In our experience we are inclined to believe that copulation is favoured by dark,

moist conditions and can take place immediatly after emerging. The average of copulation could be improved by looking for means to synchronize the emergence, in order to increase the chance of encounter of the adult midges.

The suggestion in literature, that swarming is necessary for copulation (6) can be rejected.

CONCLUSIONS

1. Feeding adult females with a combination of cacao flowers and a sugar solution, has a beneficial effect on the reproduction, resulting in a higher percentage of females ovipositing and a larger number of eggs per egg batch.

2. Feeding of adult females with a sugar solution has a beneficial effect on the longevity of the females, more than any other diet tried in this study.

3. The beneficial effect of feeding with both flowers and sugar on reproduction cannot be imitated by combining sugar with a protein or sterol, so that emphasis sould be put now on the role of water soluble vitamins.

4. The larvae do not feed on the biomass of the decaying material which forms their habitat, but on the biomass of the microorganisms living in this habitat.

5. Bacteria such as *Pseudomonas* sp. and *Xanthomonas* sp. provide the best food for the larvae, whereas they are unable to feed on fungi such as *Penicillium* sp. *Aspergillus niger* and *Bothryodiplodia theobromae*, which inhibit the growth if present in the same medium as bacteria. The fungus *Mucor* sp. as a food source causes a lower larval growth rate than bacteria.

6. There is a clear bending point, in the case of this study 0.43 larvae/cm^2, above which density inhibits larval growth, causes larval mortality and increases the length of larval stage.

7. Peak temperatures affect adult longevity in single rearing cages.

8. *Forcipomyia* midges are able to copulate in moist, dark conditions, shortly after emergence.

ACKNOWLEDGEMENTS

We wish to thank Drs. Forbes Benton, Max de Menezes and Kamal El-Kadi for revision of the manuscript, Agr.-Eng. Pedrito Silva for translation of the abstract to Portuguese, Dr. H. C. Evans and M. Sci, J. M. Figueiredo for identi-

fication of the fungi and bacteria, and Mrs. Nadja Montalvão, Mr. Arlindo Nepomuceno and Mr. Waldeck Oliveira for help in the laboratory. We are specially grateful to Dr. J.B.M. Van Dinther, Agricultural University of Wageningen (Holland) and to Dr. Paulo de T. Alvim, CEPLAC (Brazil) for their personal efforts in arranging for the senior author to visit CEPLAC.

LITERATURE CITED

1. COOKE, J. and SANG, J. S. Utilisation of sterols by larvae of *Drosophila melanogaster*. Journal of Insect Physiology 16: 801-812. 1970.

2. DESSART, P. Contribution à l'étude des Ceratopogonidae (Diptera). Les *Forcipomyia* pollinisateurs du cacaoyer. Bulletin Agricole du Congo 3: 525-540. 1961.

3. HERNANDEZ, J. Insect pollination of cacao (*Theobroma cacao* L.) in Costa Rica. Ph.D. Thesis. Madison, University of Wisconsin, 1965. 167p.

4. KAUFMAN, T. Pollinators of *Theobroma cacao* in Ghana. *In* Conference of Cacao Entomologist, 4th, Legon, Ghana, 1974. Proceedings. Legon, University of Ghana, 1975. pp. 77-80.

5. SAUNDERS, L. G. Methods for studying *Forcipomyia* midges, with special reference to cacao pollinating species (Diptera, Ceratopogonidae). Canadian Journal of Zoology 37: 33-51. 1959.

6. SORIA, S. de J. and WIRTH, W. W. Ciclos de vida dos polinizadores do cacaueiro *Forcipomyia* spp. (Diptera, Ceratopogonidae) e algumas anotações sobre o comportamento das larvas no laboratório. Revista Theobroma (Brasil) 5(4): 3-22. 1975.

7. VANDERZANT, E. S. Growth and reproduction of the pink boll worm on an aminoacid medium. Journal of Economic Entomology 50: 219-221. 1957.

8. WINDER, J. A. and SILVA, P. Pesquisa sobre a polinização do cacaueiro por insetos na Bahia. Revista Theobroma (Brasil) 2(3): 36-46. 1972.

RESUMO

Criação de Mosquinhas *Forcipomyia* spp. (Diptera, Ceratopogonidae) em Laboratório: 1. Alimentação de Adultos e Larvas e Ensaios de Cópula; Uma Revisão do Método "Saunders" de Criação

A criação massal de *Forcipomyia*, em condições de laboratório, poderá ser importante para aumentar a polinização do cacaueiro (*Theobroma cacao* L.). O método de criação de Saunders permite somente a obtenção de uma geração das mosquinhas em laboratório sendo, portanto, insatisfatório.

No presente trabalho, vários compostos foram testados como alimento para o adulto, a fim de incrementar sua capacidade reprodutiva. Da mesma forma, vários microorganismos foram testados como alimento larval e o efeito da densidade larval sobre a taxa de crescimento das mesmas foi determinado. O efeito da temperatura e da umidade sobre a longevidade do adulto foi determinado e o seu grau de fertilização nas caixas de emergência foi comparado ao de campo.

A alimentação constituída de uma combinação de açúcar e flor de cacaueiro aumentou a percentagem de oviposição de 33,3 para 71,4% e o tamanho da postura de 55,5 para 117,3 ovos, quando comparada a alimentação exclusivamente constituída de açúcar. Esse efeito não pôde ser simulado pela adição da caseína, colesterol ou oxalato de calcio à dieta do adulto.

A larva alimenta-se principalmente de bacterias e cresce mais rapidamente nas placas de malteagar com *Pseudomonas* sp. e *Xanthomonas* sp. A presença de fungos saprófitos parece inibir o crescimento das larvas.

Há uma densidade de larvas/cm^2 acima da qual o crescimento é retardado; no entanto, não ocorrem diferenças significativas entre as taxas de crescimento de larvas criadas a densidades inferiores àquela, o mesmo acontecendo para as densidades superiores a ela.

Nenhuma correlação foi encontrada entre a longevidade das formas imaturas e adultas e as temperaturas médias e máximas e mínimas diárias e umidade. Entretanto, houve alta correlação entre o número de dias decorridos antes que o pico máximo de temperatura fosse atingido durante o ciclo da mosquinha e a sua longevidade. Os picos de temperatura são, pois, fatores importantes na determinação da longevidade do adulto.

A cópula pode ocorrer no escuro, em condições úmidas, logo após a emergência, pois foi encontrado esperma na espermateca de fêmeas oriundas das caixas de emergência. Tanto no laboratório quanto no campo, a percentagem de fêmeas fertilizadas foi baixa, menor que 10%.

Face ao exposto, chegou-se à conclusão de que devem ser enfatizadas as pesquisas que visam determinar o possível papel desempenhadopelasvitaminas obtidas pela alimentação em flores sobre a capacidade reprodutiva das mosquinhas.

◈ ◈ ◈

LABORATORY REARING OF *Forcipomyia* spp. MIDGES (DIPTERA, CERATOPOGONIDAE): 2. DETERMINATION OF THE REPRODUCTIVE AND BIOTIC POTENTIALS, PRELIMINARY TESTS

*Saulo de J. Soria**

ABSTRACT

The purpose of this work was to determine the reproductive and biotic potentials of the following species: *Forcipomyia (Euprojoannisia) spatulifera* Saunders, *F. (E.) blantoni* Soria and Bystrak, *F. (F.) genualis* (Loew), *F. (F.) poulaineae* Macfie, *F.* vic. *Lepidohelea* sp. 1 and *F. (Microhelea) fuliginosa* (Meigen) in laboratory conditions. Saunders' method for rearing midges was adapted to the present work. The methods to measure the reproductive and biotic potentials were similar to those used for other insects according to literature.

Results indicated high values of reproductive potential for the non-pollinating species *F. genualis, F. poulaineae* and *F.* vic. *Lepidohelea* sp. 1. The reproductive potential values for the pollinating species *F. (E.) blantoni* (34.87×10^8 midges/year) and *F. (E.) spatulifera* (22.43×10^7) were considered sufficient to forecast good possibilities of mass rearing for colonization and/or inundation purposes in field conditions. Numeric values of biotic potential were not yet determined because of the lack of quantification of the levels of population suppression caused by the predators parasites and pathogens in natural and laboratory conditions.

It was concluded that mass rearing of *spatulifera, blantoni* and other *Forcipomyia* midges is a very promising technique due to the quick reproductive capacity observed in this research.

Received for publication November 11, 1977 and in revised form July 18, 1978.

* Ph. D., Divisão de Zoologia Agrícola, Centro de Pesquisas do Cacau, C.P. 7, 45.600 – Itabuna, Bahia, Brasil.

INTRODUCTION

Two practical approaches are proposed in literature (7, 12) for increasing natural levels of pollination in the cacao plantations: development of management techniques that help pollinator insects to reproduce under natural conditions, and mass rearing of these insects in the laboratory for release and further monitoring in field conditions.

The literature concerning the rearing of *Forcipomyia* midges in the laboratory is rather scarce. The most well known methods of rearing *Forcipomyia* midges in the laboratory (3, 5, 7, 12) fail to yield continuous generations. The numbers of midges obtained gradually decrease as mentioned by Hernandez (3), Kaufmann (5) and Soria and Wirth (12).

Determination of the biotic and reproductive potentials is necessary to predict the number of midges in field populations in both space and time. In order to obtain numerical estimates of the biotic potential, it is necessary to determine the sex ratio, the number of reproductive descendants of each generation, the number of generations per year and the reproductive potential. Then, based on these values, the biotic potential is calculated. Finally an approach to determine environmental resistance is established based on preliminary determinations of natural enemies, parasites, predators and pathogens.

The purpose of this work was to determine the reprodutive and biotic potentials of the following species: *Forcipomyia (Euprojoannisia) spatulifera* Saunders, *F. (E.) blantoni* Soria and Bystrack, *F. (F.) genuallis* (Loew), *F. (F.) poulaineae* Macfie, *F.* vic. *Lepidohelea* sp. 1 and *F. (Microhelea) fuliginosa* (Meigen) in laboratory conditions.

MATERIAL AND METHODS

In order to develop a rearing program the following conventional parameters were studied (2, 8):

Sex ratio (sr.) Is the proportion between the number of females and the number of females plus males per each generation (8). Therefore:

$$sr = \frac{\text{No. of females}}{\text{No. of females + No. of males}}$$

To determine sex ratios, each species was reared in the laboratory using the method of Saunders (7) adapted to this study. To determine seasonal variations of sex ratios, systematic sampling of adults with an automatic Johnson & Taylor suction trap was used (4). Finally sex determination of adults of the different species was made using characteristics of the genitalia.

The number of generations. The number of generations per year was calculated based on the life span per generation in the laboratory (8, 9).

$$\text{No. of generations/year} = \frac{365 \text{ days}}{\text{life span (days/generation)}}$$

The number of descendants per female. The relationship between birth rates and death rates determined in the past (9) indicated the egg and adult stages to be the most seriously menaced in mortality. From experience in recent years, it seems that this mortality occurs as a consequence of defective techniques in the laboratory rearing of midges. In nature and when rearing of midges starts from larvae obtained in the field, the recovery of the adults is almost total. The number of larvae per generation, used for the calculation of the number of descendants, gives a safe estimate.

Reproductive potential (Rp). Is the speed at which an individual is able to reproduce itself (8). It is dependent on the sex ratio (sr), on the number of descendants (d), and the number of generations (n). Therefore:

$$Rp = (sr \times d)^n .$$

Biotic potential (Bp). Is the inherent capacity of the individual to reproduce and survive itself, in other words, capacity to increase in numbers (8). This depends on the Reproductive Potential (Rp) and the Environmental Resistance (Er).

Therefore, the biotic potential (Bp) of an insect can be calculated with the following equation:

$$Bp = \left(\left(\frac{\text{No. of females}}{\text{No. of females} + \text{No. of males}} \right) \times d \right)^n - Er$$

The environmental resistance (Er). Is the integration of physical and biotic factors which limit the growth of the insect population (8). In the equation it represents the number of individuals dead in the time considered. This number divided by the number of insects initially available, gives an indication of the mortality rate of the species under study.

In the present work, determination of environmental resistance was limited to the listing for organisms found causing mortalities of midges in laboratory and field conditions.

RESULTS AND DISCUSSION

Sex ratio (sr). Results on the number of female and male adults obtained in the laboratory, and their specific sex ratios (Table 1), indicates that *F.* vic. *Lepidohelea* sp. 1 (0.86), *spatulifera* (0.77), *blantoni* (0.75), *fuliginosa* (0.71), *poulaineae* (0.67) and *genualis* (0.56), showed sex ratio values that usually occurred a little bit above the expected 0.50 sex ratio for any animal species.

Table 1 – Sex ratio of *Forcipomyia* adults captured for biological studies.

Insect	Females	Males	Sex ratio
Forcipomyia (Euprojoannisia) spatulifera Saunders	1090	318	0.77
F. (Euprojoannisia) blantoni Soria and Bystrak	62	21	0.75
F. (Forcipomyia) genualis (Loew)	29	23	0.56
F. (Forcipomyia) near *poulaineae* Macfie	2	1	0.67
F. (Microhelea) fuliginosa (Meigen)	113	47	0.71
F. vic. *(Lepidohelea)* sp. 1	6	1	0.86

Results of the variations of adult sex ratios throughout the year (Table 2) indicate a variability that changes from species to species. A striking example of seasonal variation in the sex ratios occured in *F. (Euprojoannisia)* sensu strito. This species at the beginning of the year (January) had a sex ratio of 1.00, whereas at the end of the year (November) it was 0.38. The males outnumbered the females at the end of the year. This sort of variation also occurs in closely related species of *Culicoides* as indicated by Linley and Hinds (6).

Table 2 – Seasonal variation in the sex ratios of *Forcipomyia (Euprojoannisia)* and *Forcipomyia (Forcipomyia)* under field conditions.

Data	*Euprojoannisia*			*Forcipomyia*		
	Female	Males	Sex ratio	Female	Males	Sex ratio
1972 Nov.	0	0	-	1	-	1.00
Dec.		0	-	0	0	-
1973 Jan.	3	0	1.00	11	1	0.92
Feb.	0	0	-	2	0	1.00
Mar.	5	0	1.00	19	2	0.90
Apr	12	2	0.86	53	3	0.95
May	115	4	0.97	110	23	0.83
Jun.	171	81	0.68	190	105	0.64
Jul.	136	65	0.68	159	34	0.82
Aug.	40	28	0.59	10	3	0.77
Sep.	46	18	0.72	11	2	0.85
Oct.	32	22	0.59	47	3	0.94
Nov.	10	16	0.38	22	4	0.85
Dec.	0	0	-	35	10	0.78
1974 Jan.	0	0	-	20	42	0.48

There is a limitation in the conclusions related to sex ratios in Table 1. Calculations are based on the adults obtained from manual field collections where there is always the risk of biased collection favoring females of some species. A parallel systematic sample using a Johnson & Taylor automatic suction trap largely avoided the risk of bias (Table 2). This variation in records of sex ratios indicates that more information in this area should be accumulated to give accurate estimates of reproduction potentials.

The number of generations. Results (Table 3) of the potential number of generations per year indicated higher values for *F.* vic. *Lepidohelea* sp. 1 (13 generations/year) whereas the minimum values were observed for *genualis,* (8/year). Species that have particular economic value such as *blantoni* and *spatulifera* indicated 10 and 9 generations/year respectively. This is important from a practical stand point, because it favours quick reproduction of these insects in nature.

The number of descendents per female. Results (Table 4) indicated higher values (24, 22 and 17) for *genualis, F.* vic. *Lepidohelea* sp. 1 and *poulaineae.* The pollinating species *F. (E.) spatulifera* and *blantoni,* on the other hand, had a smaller number of descendants (11 and 12, respectively) per female in the laboratory. All these values have relevance in the final computation of the reproductive potential, as shown in the respective paragraph.

It was not possible to obtain descendants from *fuliginosa:* because eggs ovoposited never hatched. It was attributed to reproductive habits of these insects, different from the ones demonstrated in Table 4.

The reproductive potential. Results (Table 5) indicated that the reproductive potentials for *blantoni* (34.87

Table 3 – Estimate of the potential number of generations per year based on the life span egg-egg of the commonest *Forcipomyia* spp. midges. After Soria and Wirth, 1975.

Species	Life span egg-egg (No. days)	Number of generations per year
Forcipomyia (Euprojoannisia) spatulifera Saunders	37	9
F. (Euprojoannisia) blantoni Soria and Bystrak	34	10
F. (Forcipomyia) genualis (Loew)	41	8
F. (Forcipomyia) near *poulaineae* Macfie	38	9
F. vic *(Lepidohelea)* sp. 1	26	13

Table 4 – The number of descendents: fourth instar *Forcipomyia* larvae obtained from one fertile female under laboratory conditions.

Midges	Average number descendents	Size of sample (No. of females)
F. (Euprojoannisia) spatulifera Saunders	11	35
F. (Euprojoannisia) blantoni Soria and Bystrak	12	7
F. (Forcipomyia) genualis (Loew)	24	18
F. (Forcipomyia) near *poulaineae* Macfie	17	1
F. vic. *Lepidohelea* sp. 1	22	1
F. (Microhelea) fuliginosa (Meigen)	0	20

x 10^8 midges/year) and for *spatulifera* (22.43 x 10^7 midges/year) are high enough to guarantee plenty of midges for pollination, if secondary limiting factors do not appear in the environment. In reality, limiting factors for reproduction are always present as will be demonstrated in the next paragraph.

The reproductive potentials for *F.* vic. *Lepidohelea* sp. 1 was 39.81 x 10^{14}, for *poulaineae* was 32,26 x 10^{10} and for *genualis* was 10.89 x 10^8. These species are non pollinating ones.

The biotic potential and environmental resistance. The mites *Tyrophagus putrescentiae,* the nematodes *Aphelenchoides* sp. and several entomophagous fungi were found damaging immature stages of *Forcipomyia* midges in laboratory conditions (12). Under field conditions, on the other hand, the ants *Solenopsis* sp. (Formicidae, Hymenoptera) and some spiders

Table 5 – The reproductive potential: expected numbers of midges per year starting from one fertile female after continuous generation under laboratory conditions.

Midges	Amount of midges expected
Forcipomyia (Euprojoannisia) spatulifera Saunders	22.43 x 10^7
F. (Euprojoannisia) blantoni Soria and Bystrak	34.87 x 10^8
F. (Forcipomyia) genualis (Loew)	10.89 x 10^8
F. (Forcipomyia) near *poulaineae* Macfie	32.26 x 10^{10}
F. vic. *Lepidohelea* sp. 1	39.81 x 10^{14}
F. (Microhelea) fuliginosa (Meigen)	- -

(Arachnidae) were observed to prey on all stages of *Forcipomyia* (Table 6). The suppression and degree of damage of a midge population, caused by the combined action of these organisms, has not been quantified. Field records give an estimated damage of more than 30% of the population per generation.

Due to the fact that midge population growth is controlled by the relationship between birth and death rates (1, 2), a mass rearing program will be able to succeed if death rates are reduced to minimum levels.

Precise information of midge death rates is necessary however to determine how much of the population is being lost because of the action of natural enemies. Numerical values for the biotic potential can then be safely calculated..

Since biotic factors are classically considered density dependent factors, biotic limiting factors may be active in diverse forms depending on particular circumstances of space and time. The action of contaminant mites on aging laboratory cultures, for example, is a clear example of density depending factors affecting reproductive potential of midges, as indicated in Table 7.

Determination of variation of populations of *Tyrophagus putrescentiae,* according to the material used as source of inoculum for cultures for *Forcipomyia* feeding (Table 7) indicated for example, that cultures using rotten pods facilited more the contamination of acari ($6/cm^2$), than inoculation with moss material ($4/cm^2$). More research is therefore needed to obtain a more precise determination of the biotic potentials of the midges.

Physical factors, such as climate, among other density independent factors, also deserve paramount consideration. Information on population dynamics of *Forcipomyia* midges indicate that water budget and heat budget factors interact most closely with midge populations in field conditions (10, 11).

Table 6 – Organisms found causing mortality of *Forcipomyia* midges by predation and/or parasitism in laboratory and field conditions.

Organism	Stage of host			
	Egg	Larva	Pupa	Adult
Tyrophagus putrescentiae (Acarina, Arachnida)	x			
Solenopsis sp. (Formicidae, Hymenoptera)	x	x	x	
Spiders (Arachnidae)				x
Entomofagous fungi	x	x	x	
Aphelenchoides sp. (Aphelinchoididae, Nematoda)	x	x	x	

Tale 7 – Populations of mites *Tyrophagus putrecentiae* per cm^2 occuring on the surface of malt-agar media inoculated with several microorganisms for feeding immatures of *Forcipomyia* midges in the laboratory.

Petri dish	Sources of inoculum		
	Moss	Rotten pods	Leaf mat
1st	9	6	9
2nd	4	6	6
3rd	4	4	3
4th	3	11	3
5th	3	1	3
6th	3	10	7
Mean	4	6	5

CONCLUSIONS

1. The reproductive potentials of the pollinating species *blantoni* and *spatulifera* are high, due mainly to the number of generations per year.

2. The reproductive potential values corresponding to the non pollinating species such as *poulaineae* and sp. 1 near *Lepidohelea* were as high as the values observed for the pollinating species.

3. The number of descendents per female/generation seems to contribute a great deal for the high values of reproductive potential in sp. 1 near *Lepidohelea, poulaineae, genualis* and other species.

4. The biotic potentials for the species studied were not yet determined, because of the lack of quantification of mortality values due mainly to predation and parasitism. From field observations, however, it was estimated to be not less than a 30% of the values observed in the reproductive potentials.

5. For *fuliginosa* it was not possible to determine the values of reproductive potential because of the impossibility of getting descendents in laboratory conditions.

6. Mass rearing techniques of pollinating species seem to be highly promising to program colonization and/or inundation of midges in the field for practical purposes.

7. Programming insect release for colonization and/or inundation purpose in the field should be based on the exact measurement of the population decrease due to the occurrence of parasites, pathogens and predators, which will finally determine the biotic potentials of these midges.

ACKNOWLEDGEMENTS

I wish to thank Agr.-Eng. Pedrito Silva, Drs. Forbes Benton, Max de Menezes and Kamal El-Kadi and M. Sci. G. Smith Figueroa for kind revision of the manuscript. Thanks are given also to Mr. Florisvaldo Andrade Galvão, Mr. Waldeck M. de Olveira and Mr. Arlindo Nepomuceno for field and laboratory help.

LITERATURE CITED

1. ALLEE, W. C. et al. Principles of animal ecology. Philadelphia, W. B. Saunders. 1949. 837 p.

2. ANDREWARTHA, H. G. and BIRCH, L. C. The distribution and abundance of animals. Chicago, The University of Chicago Press, 1954. 782 p.

3. HERNANDEZ, J. Insect pollination of cocoa (*Theobroma cacao* L.) in Costa Rica. Ph.D. Thesis. Madison, University of Wisconsin, 1965. 167 p.

4. JOHNSON, C. G. and TAYLOR, L. R. The development of large suction traps for airborn insects. Annals of Applied Biology 43: 51-62. 1955.

5. KAUFMANN, T. Behavioral biology of a cocoa pollinator, *Forcipomyia inornatipennis* (Diptera, Ceratopogonidae) in Ghana. Journal of the Kansas Entomological Society 47 (4): 541-548. 1974.

6. LINLEY, J. R. and HINDS, M. J. Seasonal changes in size, female fecundity and male potency in *Culicoides melleus* (Diptera, Ceratopogonidae). Journal of Medical Entomology 13 (2): 151-156. 1976.

7. SAUNDERS, L. G. Methods for studying *Forcipomyia* midges (Diptera, Ceratopogonidae) with special reference to cocoa pollinating species. Canadian Journal of Zoology 37: 33-51. 1959.

8. SILVEIRA NETO, S. et al. Manual de ecologia dos insetos. Piracicaba, Ceres, 1976. 419 p.

9. SORIA, S. de J. Tabelas etárias dos polinizadores do cacaueiro *Forcipomyia* spp. (Diptera, Ceratopogonidae) em condições de laboratório. Revista Theobroma (Brasil) 6: 5-13. 1976.

10. ————————. Dinâmica populacional de *Forcipomyia* spp. (Diptera, Ceratopogonidae na Bahia, Brasil. 2. Variáveis bióticas relacionadas com a polinização do cacaueiro. Revista Theobroma (Brasil) 7: 19-33. 1977.

11. ————————. Dinâmica populacional de *Forcipomyia* spp. (Diptera, Ceratopogonidae) na Bahia, Brasil. 3. Variáveis climáticas relacionadas com a polinização do cacaueiro. Revista Theobroma (Brasil) 7: 69-84. 1977.

12. ————————. and WIRTH, W. W. Ciclos de vida dos polinizadores do cacaueiro *Forcipomyia* spp. (Diptera, Ceratopogonidae) e algumas anotações sobre o comportamento das larvas no laboratório. Revista Theobroma (Brasil) 5(4): 3-22. 1975.

RESUMO

Criação de Mosquinhas *Forcipomyia* spp. (Diptera, Ceratopogonidae) em Laboratório: 2 . Determinação dos Potenciais Biótico e Reprodutivo, Ensaios Preliminares.

A finalidade deste trabalho foi determinar os potenciais biótico e reprodutivo das seguintes espécies: *Forcipomyia (Euprojoannisia) spatulifera* Saunders, *F. (E.) blantoni* Soria e Bystrak, *F. (Forcipomyia) genualis* (Loew), *F. (F.) poulaineae* Macfie, *F.* vic. *Lepidohelea* sp. 1 e *F. (Microhelea) fuliginosa* (Meigen), sob condições de laboratório. Para criar as mosquinhas foi utilizado o **método de Saunders,** adaptado para o presente trabalho. Para medir os potenciais biótico e

reprodutivo, foram utilizados os métodos já consagrados para outros insetos, de acordo com a literatura.

Os resultados indicaram valores altos de potencial reprodutivo para as espécies não polinizadores *F. genualis, poulaineae* e *F.* vic. *Lepidohelea* sp. 1. Os valores do potencial reprodutivo para as espécies polinizadoras *blantoni e spatulifera* (34.87×10^8 e 22.43×10^7, respectivamente) foram considerados suficientes para prever boas possibilidades de criação em massa das mesmas objetivando as finalidades de colonização e/ou inundação sob condições de campo. Os valores numéricos de potencial biótico não foram, todavia, determinados em virtude da falta de quantificação dos níveis populacionais eliminados pela ação de predadores parasitas e patógenos sob condições naturais e laboratoriais.

Conclui-se que a criação em massa de *spatulifera, blantoni* e outras mosquinhas *Forcipomyia* é uma técnica bastante promissora, devido à sua rápida reprodução observada nesta pesquisa.

◈ ◈ ◈

EFEITO DA APLICAÇÃO DE CALCÁRIO E FÓSFORO NO CRESCIMENTO DE PLÂNTULAS DE CACAU EM CASA DE VEGETAÇÃO

Francisco I. Morais *
Charles J. L. Santana **
M. Bernadeth M. Santana **

ABSTRACT

Effect of Lime and Phosphorus Application on the Growth of Cacao Seedlings

Cacao seedlings grown under greenhouse conditions on an acid soil (Vargito – Typic Tropudult) were submited to five levels of lime (0, 15, 25, 80 and 100% of the amount required to reach pH 6.4) and three levels of phosphorus (0, 20 and 100 ppm). All the plants received an uniform dosage of nitrogen, potassium, magnesium, sulfur and micronutrients (Fe, Cu, Mn, Zn and Bo).

Liming increased yield of cacao when the Al saturation was greater than 50%, this effect being enhanced by P fertilization. On soil samples with Al less than 50%, P application was more effective than lime in increasing growth of cacao seedlings.

Growth response to liming took place up to the point of elimination of exchangeable Al (25% of the amount of lime required to reach pH 6.4) after which a significant reduction occurred due to micronutrient deficiencies, especially zinc.

Recebido para publicação em 1º de março, 1978, e em forma revisada em 8 de novembro, 1978.

* PhD., Divisão de Geociências (DIGEO), Centro de Pesquisas do Cacau (CEPEC), Caixa Postal 7, 45.600 - Itabuna, Bahia, Brasil.

** M.S., DIGEO, CEPEC.

INTRODUÇÃO

A acidez do solo é um problema associado com a presença de hidrogênio e alumínio em forma trocável. O alumínio, em concentração tóxica, afeta o crescimento das plantas cultivadas, limitando o desenvolvimento do sistema radicular e, em consequência, a absorção de água e nutrientes do solo (5, 8, 10, 19). Os efeitos deprimentes do alumínio solúvel no crescimento do cacaueiro foram demonstrados por Miranda e Dias (12) e Santana, Cabala e Miranda (15).

A calagem, prática recomendada para o controle da acidez do solo, tem sido tradicionalmente efetuada com a finalidade de elevar o pH do solo para uma faixa próxima à neutralidade, geralmente 6,5 a 7,0 (3). Admite-se que, desta forma, fatores tais como a baixa saturação de bases, a fixação de fósforo e a toxicidade devida ao alumínio e manganês sejam eliminados ou favoravelmente influenciados. No entanto, diversos investigadores mostraram que a utilização deste critério em solos de regiões tropicais provoca efeitos negativos na produção dos cultivos agrícolas, consistentes com uma deficiência de micronutrientes (14, 20) ou diminuição na disponibilidade de P (6, 9).

A recomendação da calagem baseada na quantidade de alumínio extraído por sal não tamponado, sugerida por Coleman, Weed e McCraken (4), aparece, deste modo, como uma alternativa mais racional para o controle da acidez de solos de regiões tropicais. O alumínio é o cation predominante e principal responsável pela toxicidade de solos ácidos (13, 19). Ademais, a precipitação do alumínio trocável se processa quando o pH do solo atinge a faixa entre 5,0 e 5,5 (10), situando-se este limite entre as respostas positivas e negativas das culturas à incorporação de calcário.

Embora a acidez do solo e a deficiência de fósforo constituam os principais fatores que limitam o crescimento e produção do cacaueiro na Bahia (1, 12), nenhum estudo foi realizado sobre a influência da calagem na disponibilidade de P e outros nutrientes em solos regionais. Não são conhecidos, também, resultados experimentais que comprovem qual o método mais adequado para estimar a necessidade de calagem para o cacaueiro.

Este trabalho teve por objetivos: i) determinar o efeito da aplicação de calcário e fósforo no crescimento de plântulas de cacau; e ii) selecionar um método adequado para estimar a necessidade de calagem do cacaueiro.

MATERIAIS E MÉTODOS

O experimento foi realizado em casa de vegetação, utilizando-se solo da unidade Vargito, pobre em fósforo, classificado como Typic Tropodult (18) de acordo com a 7ª aproximação americana. As amostras do solo foram selecionadas para abranger valores va-

riáveis de capacidade de troca de cations (CTC), alumínio trocável e índice de saturação de alumínio (Quadro 1).

Plântulas de cacau da variedade **Catongo,** cultivadas em vasos plásticos contendo 5 kg de solo, foram submetidas a cinco doses de calcário e três de fósforo. O esquema experimental foi em parcelas subdivididas, com cinco repetições. A calagem foi efetuada pelo método SMP (17) nas dosagens de 0; 15; 25; 80 e 100% da quantidade necessária de calcário para elevar o pH do solo para 6,4 (Quadro 1). Os níveis de 15 e 25% de calagem correspondem a 1,0 e 1,5 vezes a quantidade de alumínio trocável do solo. A aplicação de fósforo foi determinada em função das características de fixação de P, empregando-se quantidades que elevaram o P da solução do solo para 0,14 (20 ppm P) e 0,90 ppm (100 ppm P) e uma testemunha sem fósforo (Figura 1).

O solo, antes de cultivado, foi homogeneizado com calcário e incubado durante 4 semanas, recebendo, em seguida, os tratamentos à base de fósforo e outros elementos. Todas as parcelas foram adubadas com 100 ppm de N, 100 ppm de K, 27 ppm de S, 30 ppm de Mg, 5 ppm de Fe e 1 ppm de Zn, Mn, Cu e Bo.

Seis meses após a semeadura, as plântulas de cacau foram colhidas, oportunidade em que foi realizada uma amostragem de solo, raízes e folhas para análise de laboratório. Amostras do solo foram também coletadas ao término do período de incubação.

A análise química de raízes e folhas do cacaueiro foi realizada de acordo com a metodologia descrita por Chapman e Pratt (2). A caracterização do solo quanto a textura, carbono orgânico, bases, pH e micronutrientes (Zn, Bo, Fe e Mn) foi efetuada por métodos utilizados no laboratório da Divisão de Geociências do CEPEC (16). A acidez trocável (H + Al) foi determinada pela técnica desenvolvida por Yuan e Fiskell (21). A CTC efetiva do solo foi obtida pela soma de bases trocáveis com a acidez extraída por solução de KCl (4). O incremento no teor de cálcio trocável do solo (ΔCa), após a calagem, foi considerado como acidez total neutralizada, sendo a neutralização da acidez trocável avaliada pela diferença entre alumínio existente antes e depois da calagem (ΔAl). A fixação de P no solo foi determinada segundo o método de Fox e Kamprath (7).

RESULTADOS

A Figura 2 mostra os efeitos da calagem e da aplicação de fósforo no crescimento do cacaueiro. A adição de calcário provocou incrementos significativos ($P < 0,01$) na produção de biomassa de plântulas de cacau quando o índice de saturação de alumínio (Al%) do solo foi maior do que 50% (solos 4 e 5), sendo este efeito intensificado em presença de fósforo. Nas amostras com Al inferior a 50%, a adubação fosfata-

Quadro 1 – Características gerais do solo[+].

Solo*	pH(H_2O) (1:2,5)	Acidez Trocável Al^{+++}	H^+	Ca	Mg	K	CTC efet.	C	N	Sat. Al	Fe_2O_3 Livre	P	Calagem§	Micronutrientes Bo	Mn	Fe	Zn	Granulometria Areia	Silte	Argila
		mEq/100g						%				(ppm)	(t/ha)	ppm				%		
1	4,3	1,4	1,0	1,2	0,9	0,18	4,68	1,7	0,16	29,9	0,7	4	16,0	2,3	8,4	24,3	2,5	29,1	54,8	16,1
2	4,5	2,0	1,3	1,9	1,4	0,15	6,75	1,3	0,11	29,6	0,8	2	18,5	0,6	24,4	25,2	2,2	23,7	36,6	19,7
3	4,7	5,0	1,5	2,5	1,8	0,52	11,32	1,9	0,20	44,2	2,6	2	25,5	0,3	19,4	26,1	2,1	1,7	60,3	38,0
4	4,1	3,0	1,0	1,0	0,5	0,10	5,60	1,8	0,15	53,6	0,4	4	24,0	0,5	12,0	28,8	1,9	33,9	53,3	12,8
5	3,9	8,0	2,5	1,4	0,9	0,34	13,14	2,4	0,27	60,9	1,1	2	35,0	0,3	10,3	27,9	2,1	2,4	62,3	35,3
6	4,0	2,3	1,7	0,7	1,0	0,17	5,87	1,3	0,14	39,2	0,4	3	23,0	tr	39,2	18,9	2,1	2,8	73,9	17,7

* Amostras coletadas de 0 a 20cm de profundidade;

* 1 - Fzda. D. Eduardo (Camacã); 2 - Fzda. 2 Amigos (Mascote); 3 - Fzda. Bela Vista (Camacã); 4 - Fzda. São Jorge (Camacã); 5 - Fzda. Aparecida (Camacã); 6 - Fzda. Brasil (Mascote);

§ Necessidade de calagem baseada no método SMP pH 6,4.

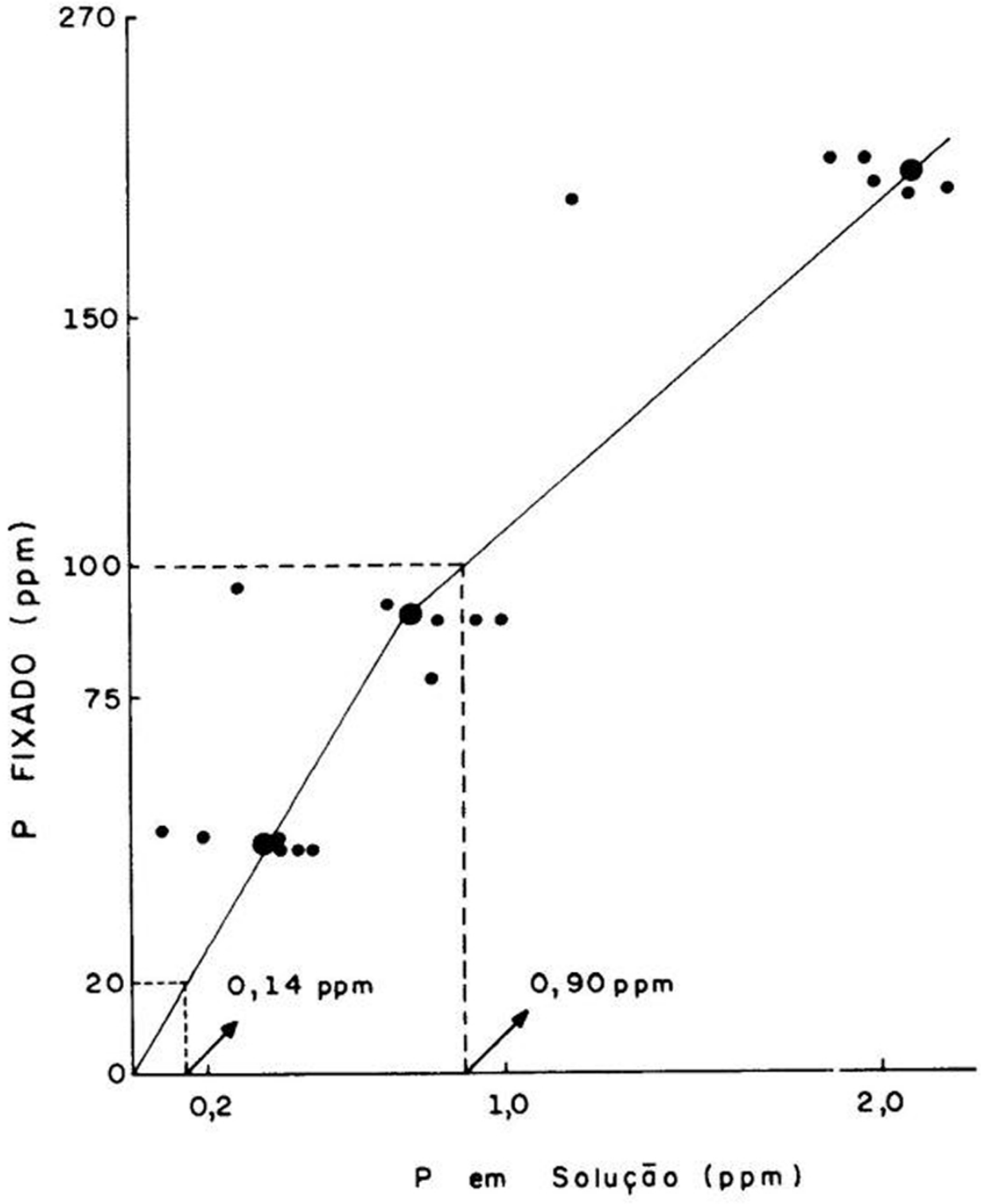


Figura 1 – Fixação de P no solo.

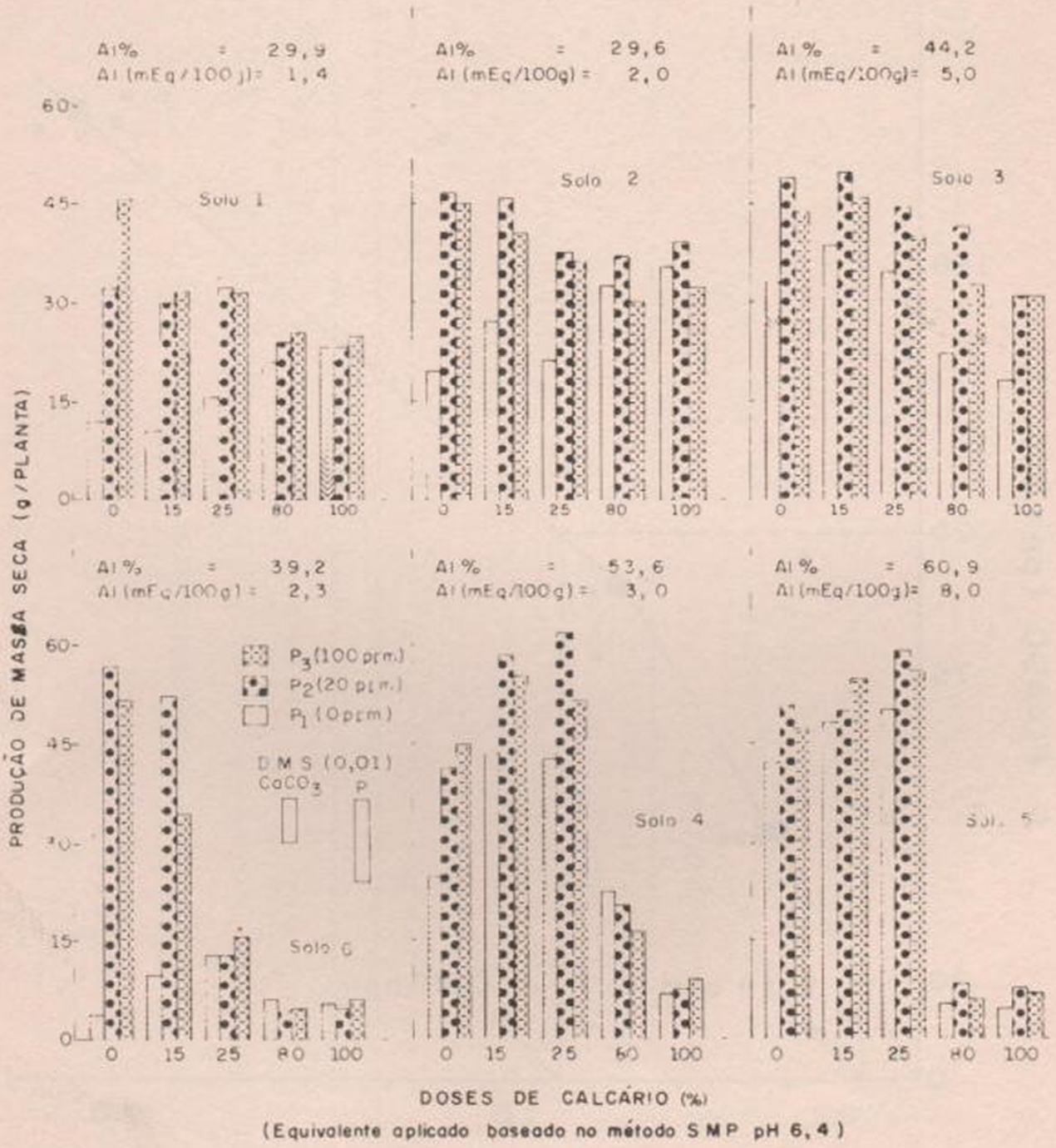


Figura 2 – Efeitos da calagem e da aplicação do fósforo no desenvolvimento de plântulas de cacau em seis ultisols da Região Cacaueira da Bahia.

da produziu maior desenvolvimento do cacaueiro do que a calagem, não se verificando diferenças significativas entre as doses de P empregadas.

Nas amostras do solo que apresentaram respostas do cacaueiro à aplicação de calcário a melhor dose do corretivo situou-se em torno de 25% da necessidade determinada pelo método SMP (Figura 2). Doses superiores a essa quantidade retardaram o crescimento das plântulas de cacau, provocando deficiências de micronutrientes, especialmente zinco, em quase todos os solos utilizados no experimento (Figuras 2 e 3).

O efeito da calagem nas propriedades químicas do solo e na absorção de nutrientes pelo cacaueiro está sumarizado no Quadro 2 e Figura 4. Durante o período experimental, a calagem não ocasionou modificações nos teores de carbono, nitrogênio, potássio, magnésio e micronutrientes (Fe, Cu e Bo) do solo e, portanto, os dados obtidos não são apresentados.

A aplicação de doses crescentes do calcário neutralizou progressivamente o alumínio trocável e elevou o pH do solo para os valores esperados de 6,3 e 6,5. Deve-se notar que as menores quantidades do corretivo reagiram principalmente com o alumínio e as doses mais altas com a acidez não trocável do solo. A dosagem equivalente a 25% da necessidade determinada pelo método SMP neutralizou quantidades aproximadamente iguais ($\Delta Al / \Delta Ca$ = 0,58) de alumínio e acidez não trocável (Quadro 2).

A calagem aumentou o teor de cálcio e reduziu os níveis de zinco e manganês do solo e de folhas e raízes do cacaueiro, em função das doses aplicadas. O maior acréscimo no nível de cálcio ocorreu entre as doses de 25 e 80% da necessidade de calagem determinada pelo método SMP, que correspondeu aos maiores decréscimos nos teores de zinco e manganês (Figura 4a, c e d). O fósforo, no solo e nas raízes das plântulas, apresentou pequenos decréscimos devidos à adição de doses crescentes de calcário, não se verificando influência desta prática no teor de P em folhas do cacaueiro. A adubação fosfatada incrementou o teor de P tanto no solo como em folhas e raízes das plântulas de cacau (Figura 4d).

DISCUSSÃO E CONCLUSÕES

Tanto a acidez quanto a deficiência de fósforo limitaram o desenvolvimento do cacaueiro no solo estudado, verificando-se uma interação calagem x fósforo em função do índice de saturação de alumínio (Al%). Nas amostras com Al maior do que 50%, o crescimento máximo do cacaueiro, medido em termos de produção de biomassa, foi obtido na dosagem de 25% da necessidade de calagem para elevar o pH do solo para 6,4 (SMP), especialmente quando em presença de P (Figura 2). A melhor dosagem do corretivo, quimicamente equivalente a 1,5 vezes o teor trocável de Al, neutralizou cerca

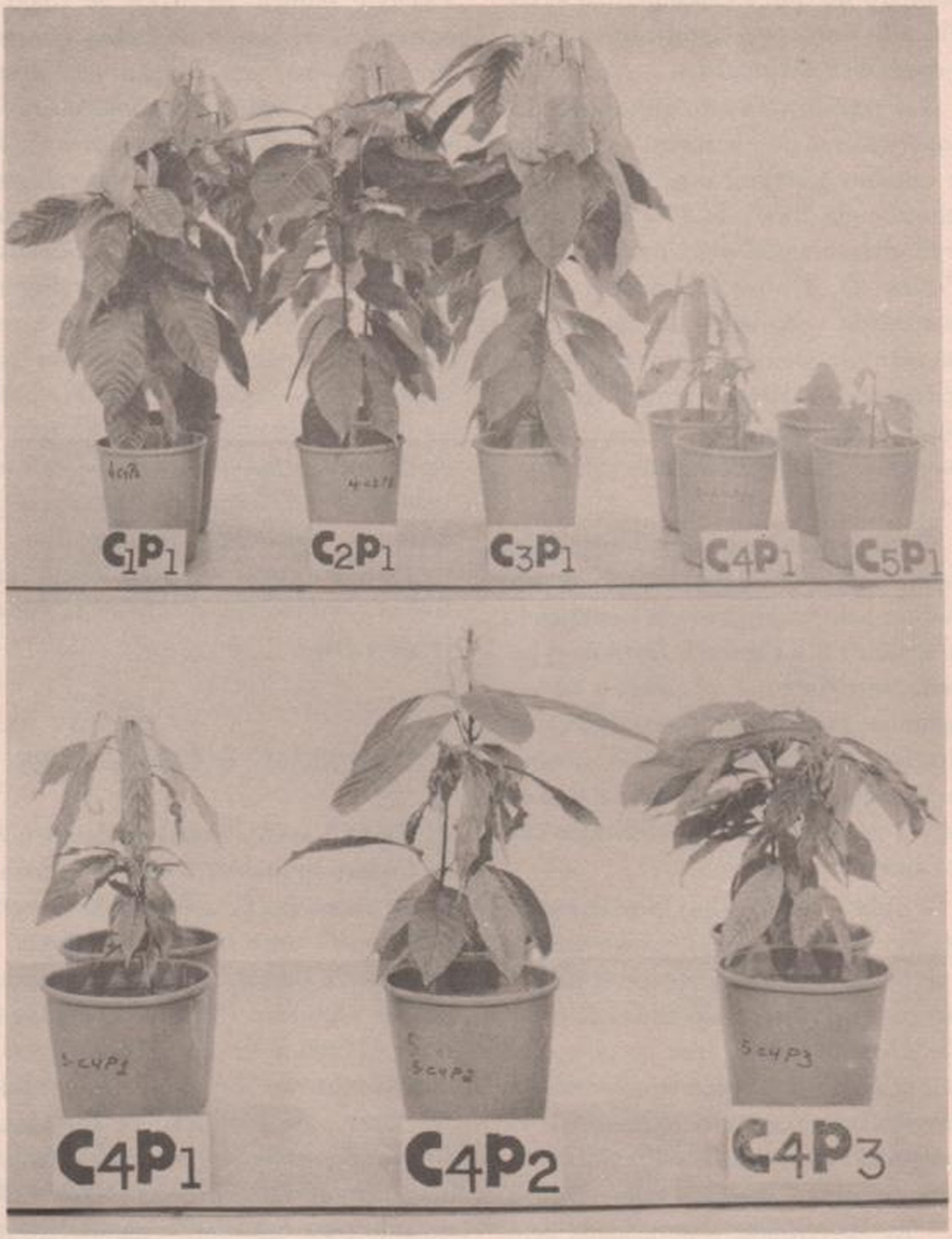


Figura 3 – Crescimento de plântulas de cacau e deficiências de micronutrientes em função de doses crescentes de calcário.C =doses do corretivo (0, 15, 25, 80 e 100% da necessidade de calagem pelo método SMP); P_1 = 0ppm P, P_2 =20 ppm P, P_3 =100 ppm P.

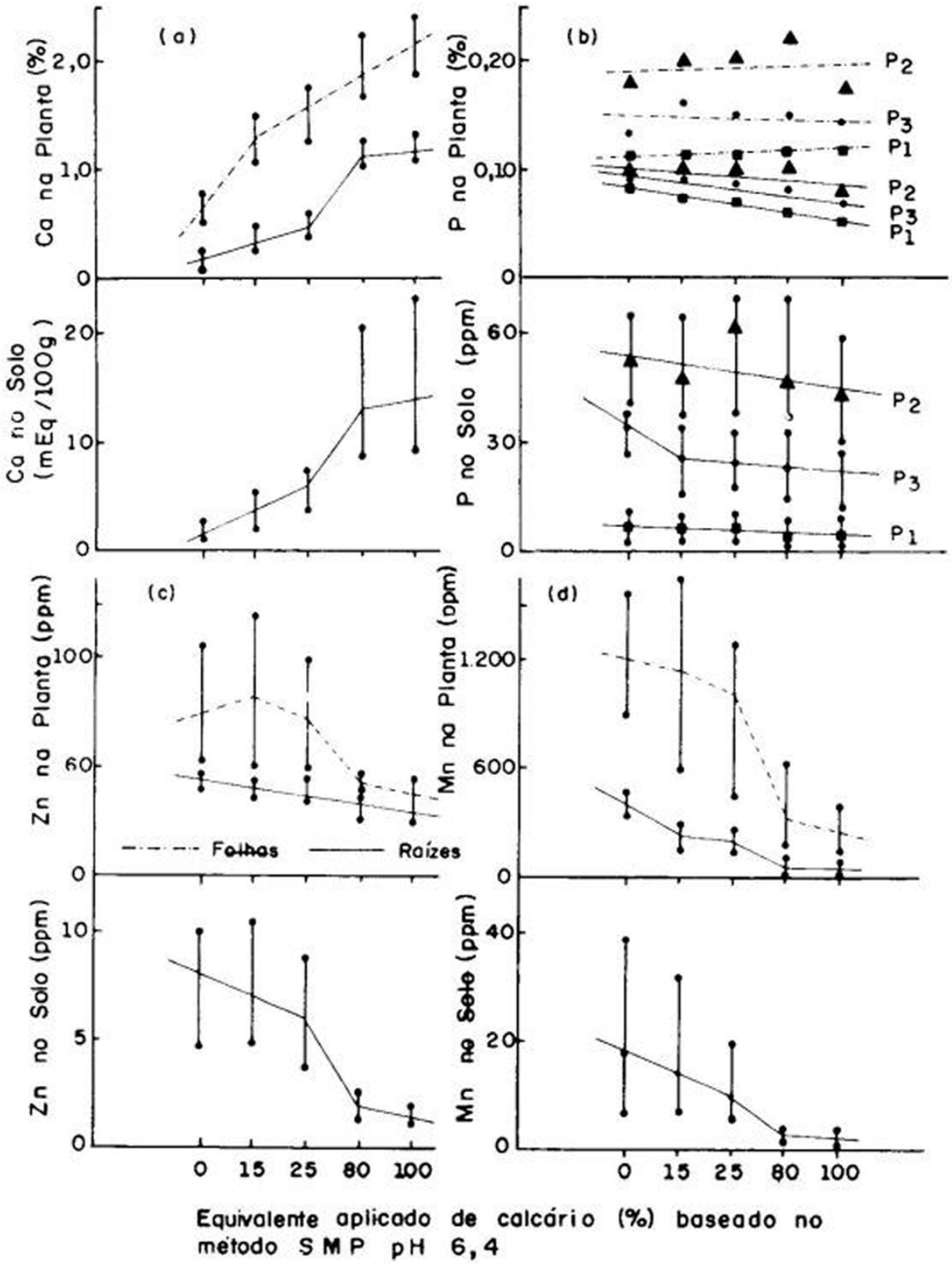


Figura 4 – Efeitos da calagem no teor de Ca, P, Zn e Mn do solo e em tecidos do cacaueiro (as barras verticais mostram a dispersão dos dados analíticos).

Quadro 2 – Efeitos da calagem no pH, alumínio trocável e na relação entre alumínio e acidez total neutralizada ($\Delta Al/\Delta Ca$)*.

Solo	Equivalente aplicado de calcário baseado no método SMP pH 6,4 (t)													
	0		15			25			80			100		
	pH	Al	pH	Al	ΔAl/ΔCa	pH	Al	ΔAl/ΔCa	pH	Al	ΔAl/ΔCa	pH	Al	ΔAl/ΔCa
1	4,3	1,4	5,4	0,6	0,89	5,6	0,1	0,63	6,3	0,0	0,02	6,5	0,0	0,0
2	4,5	2,0	5,2	0,5	0,79	5,3	0,1	0,45	6,2	0,0	0,02	6,4	0,0	0,0
3	4,7	5,0	5,2	2,1	0,83	5,3	1,5	0,67	6,0	0,0	0,17	6,3	0,0	0,0
4	4,1	3,0	5,2	1,2	0,75	5,5	0,6	0,55	6,3	0,0	0,15	6,5	0,0	0,0
5	3,0	8,0	5,0	4,8	0,91	5,2	2,8	0,71	6,0	0,0	0,28	6,4	0,0	0,0
6	4,0	2,3	5,4	0,7	0,67	5,7	0,0	0,44	6,3	0,0	0,00	6,5	0,0	0,0
Média	4,1	3,6	5,2	1,7	0,81	5,4	0,9	0,58	6,2	0,0	0,11	6,4	0,0	0,0

* Valores do alumínio estão expressos em mEq/100g do solo.

de 80% do alumínio do solo (Quadro 2). Kamprath (10) afirma que a aplicação de calcário baseada no alumínio trocável do solo é a melhor alternativa para solos de regiões tropicais. Nas amostras com Al% menor do que 50%, a adição de fósforo na dosagem de 20 ppm de P (90 kg/ha P_2O_5) ocasionou maior incremento de massa das plântulas de cacau do que a calagem (Figura 2). Embora os dados não tenham sido mostrados, a aplicação de P não modificou o teor de alumínio trocável do solo. Em ambos os casos, a adubação fosfatada aumentou o teor de P no solo e em tecidos do cacaueiro demonstrando que os resultados da interação calagem x fósforo se devem à neutralização do alumínio tóxico e maior suprimento de P, nos solos com Al superior a 50%, e apenas ao maior suprimento de P nos demais solos.

Na maioria das amostras do solo, a incorporação de calcário em quantidades superiores a 25% da necessidade determinada pelo método SMP (iguais ou superiores a 80% SMP) inibiu o desenvolvimento do cacaueiro. Comparação dos dados ilustrados nas Figuras 2 e 4 indica que o decréscimo observado na produção de biomassa das plântulas de cacau coincidiu com uma diminuição na absorção de manganês e, principalmente, zinco. A deficiência de Mn e Zn se manifestou quando o nível desses elementos nas folhas do cacaueiro decresceu para valores inferiores a 1000 e 70 ppm, respectivamente, que equivalem a uma quantidade menor do que 5 ppm no solo (Figura 2). Loué (11), na Costa do Marfim, encontrou teores variáveis de zinco entre 20 e 120 ppm e de manganês entre 192 e 528 ppm em folhas normais de plântulas de cacau. Sanchez e Kamprath (14) e Wear (20) também encontraram efeitos depressivos da sobrecalagem do solo na produção de alguns cultivos agrícolas devidos à deficiência de micronutrientes.

LITERATURA CITADA

1. CABALA, F. P. et al. Exigências nutricionais e fertilização do cacaueiro. Itabuna, Bahia, Brasil. Centro de Pesquisas do Cacau. Boletim Técnico nº 30. 1975. 59 p.

2. CHAPMAN, H.D. e PRATT, P.F. Métodos de analisis para suelos, plantas y agua. México, Trillas, 1973. 195 p.

3. COLEMAN, N.T. e THOMAS, G.W. The basic chemistry of soil acidity. *In* Person, R.W. e Adams, F., eds. Soil acidity and liming. Madison, Wisconsin, (Agronomy no. 12), American Society of Agronomy, 1967. pp. 1 - 41.

4. COLEMAN, N.T., WEED, S.B. e McCRAKEN, R.J. Cation exchange capacity and enchangeable cations in Piedmont soils of North Carolina. Soil Science Society America Proceedings 23:146 - 149. 1959.

5. EVANS, C.E. e KAMPRATH, E.J. Lime response as related to percent Al saturation, solution Al, and organic matter content. Soil Science Society America Proceedings 34:893 - 896. 1970.

6. FOX, R.L., De DATTA, S.K. e WANG, J.M. Phosphorus and aluminum uptake by plants from latosols in relation to liming. *In* International Congress Soil Science, 8th, Madison, Wisconsin, 1964, Transactions, Amsterdam, Elsevier, 1964. pp. 595 - 603.

7. ———— e KAMPRATH, E.J. Phosphate sorption isotherms for evaluating the phosphate requirements of soils. Soil Science Society America Proceedings 34:902 - 906. 1970.

8. FOY, C.D. e BROWN, J.C. Toxic factors of acid soils. I, Characterization of aluminum toxicity in cotton. Soil Science Society America Proceedings 27 : 403 - 407. 1963.

9. HOURIGAN, W.R. et al. Growth and calcium by plants as affected by rate and depth of liming. Soil Science Society America Proceedings 25:491 - 494. 1961.

10. KAMPRATH, E.J. Exchangeable aluminum as a criterion for liming leached mineral soils. Soil Science Society America Proceedings 34:252 - 254. 1970.

11. LOUÉ, A. A study of nutrient deprivation and deficiencies in the cacao tree. I. Study of deprivation of individual nutrients in cacao seedlings grown in artificial media. Fertilité 14:4 - 41. 1962.

12. MIRANDA, E.R. e DIAS, A.C.C.P. Efeito da saturação de alumínio no crescimento de plântulas de cacau. Revista Theobroma 1(3):33 - 42. 1971.

13. RAGLAND, J.L. e COLEMAN, N.T. The effect of soil solution aluminum and calcium on root growth. Soil Science Society America Proceedings 23:355 - 360. 1959.

14. SANCHEZ, C. e KAMPRATH, E.J. Effect of liming and organic matter content on the availability of native and applied manganese. Soil Science Society America Proceedings 23:302 - 304. 1959.

15. SANTANA, M.B.M., CABALA, F.P. e MIRANDA, E.R. Toxidez de alumínio em plântulas de cacau. Revista Theobroma 3(4):11 - 21. 1973.

16. SANTANA, M.B.M., PEREIRA, G.C. e MORAIS, F.I. Métodos de análise de solos, plantas e água utilizados no laboratório do Setor de Fertilidade do CEPEC. Itabuna, Bahia, CEPEC. 1977. 28 p.

17. SHOEMAKER, H.E., McLEAN, E.O. e PRATT, P.F. Buffer methods for determining lime requirements of soils with appreciable amounts of extractable aluminum. Soil Science Society America Proceedings 25:274 - 277. 1961.

18. SILVA, L.F. et al. Solos e aptidão agrícola. Itabuna, Bahia, CEPLAC/IICA, 1975. 179 p. (Diagnóstico Socioeconômico da Região Cacaueira da Bahia, V. 2).

19. VLAMIS, J. Acid soil infertility as related to soil solution and solid phase effects. Soil Science 75:383 - 394. 1953,

20. WEAR, J.I. Effect of soil pH and calcium on uptake of zinc by plants. Soil Science 81:311 - 315. 1956.

21. YUAN, T.L. e FISKELL, L.G.A. Al studies: soil and plant analyses of aluminum method. Journal of Agricultural and Food Chemistry 7: 115 - 117. 1959.

RESUMO

Plântulas de cacau da variedade **Catongo,** cultivadas em vasos plásticos contendo 5 kg de solo, foram submetidas a cinco doses de calcário e três de fósforo. O solo usado pertence à unidade Vargito, classificado como Typic Tropudult de acordo com a 7a. aproximação americana. A calagem foi efetuada com base na quantidade necessária para elevar o pH do solo para 6,4 (método SMP). Todas as parcelas foram adubadas com 100 ppm de N, 100 ppm de K, 27 ppm de S, 30 ppm de Mg, 5 ppm de Fe e 1 ppm de Zn, Mn, Cu e Bo.

A acidez do solo e a deficiência de fósforo limitaram o crescimento do cacaueiro no solo estudado, verificando-se uma interação entre estes fatores em função da saturação de aluminio. A calagem provocou acréscimos significativos ($P < 0,01$) na produção de biomassa do cacaueiro quando a Al foi maior do que 50%, sendo este efeito mais pronunciado na presença de fósforo. Nas amostras com Al menor do que 50%, a aplicação de fósforo ocasionou maior incremento de biomassa das plântulas de cacau do que a calagem.

Na maioria dos casos, a incorporação de calcário em quantidades superiores a 25% da necessidade determinada pelo método SMP ocasionou distúrbios nutricionais no cacaueiro, que se manifestaram por crescimento retardado e aparecimento de sintomas visuais de deficiências de micronutrientes, especialmente zinco.

◈ ◈ ◈

INFORMAÇÃO AOS COLABORADORES

Os conceitos e opiniões, emitidos nos artigos, são da exclusiva responsabilidade dos autores. São aceitos para publicação trabalhos que se constituam em real contribuição para um melhor conhecimento dos temas relacionados com problemas agronômicos e sócio-econômicos de áreas cacaueiras.

Os artigos devem ser datilografados em espaço duplo, com o máximo de 2.500 palavras ou 10 folhas tamanho carta (28,0 x 21,5 cm), em uma só face e com margens de 3 cm por todos os lados. Os originais devem ser acompanhados de duas cópias perfeitamente legíveis.

Desenhos e gráficos devem ser feitos com tinta nankin e não ultrapassar a medida de 18,0 x 20,0 cm; as fotografias devem ter 15,0 x 23,0 cm, em papel fotográfico brilhante com bom contraste. As ilustrações devem ser numeradas e com legendas escritas a máquina, em papel separado. Recomenda-se não dobrá-las para evitar dificuldades na reprodução.

As referências no texto devem ser feitas pelo nome do autor, acompanhado do número de ordem da citação bibliográfica. Ex.: Medeiros (5), ou simplesmente (5). A *Literatura Citada* deve ser organizada por ordem alfabética dos autores, com número de ordem, usando-se o seguinte sistema:

5. MEDEIROS, A.G. Método para estimular a esporulação do *Phytophthora palmivora* (Butl.) Butl. em placas de Petri. Phyton 22(1):73-77. 1965.

O resumo não deve exceder meia página datilografada, sendo acompanhado de versão em inglês. São aceitos artigos em português, espanhol, inglês e francês.

INFORMATION FOR CONTRIBUTORS

Concepts and opinions given in articles are the exclusive responsibility of the authors. Only articles concerned with agronomic and social-economic problems of cocoa growing areas, which represent a new contribution to the subject, will be accepted for publication.

Articles should be typed in double spacing with a maximum of 2,500 words or 10 letter sized pages (28.0 x 21.5 cm) with a 3 cm margin on all sides, together with two legible copies.

Drawings and graphs should be prepared with India ink not exceeding 18.0 x 20.0 cm; photographs should be 15.0 x 23.0 cm glossy prints with good contrast. Illustrations must be numbered, with the machine typed subtitles on separate paper. To avoid reproduction difficulties it is recommended that enclosures should not be foided.

Text references should appear with the name of the author and/or the order number in the literature citation. The *Literature Cited* should be numbered in alphabetical order employing the following system:

5. MEDEIROS, A.G. Método para estimular a esporulação do *Phytophthora palmivora* (Butl.) Butl. em placas de Petri. Phyton 22(1):73-77. 1965.

Articles are accepted in Portuguese, Spanish, English, and French.

CEPLAC
Divisão de Comunicação