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THE INFLUENCE OF BLANKET AND SELECTIVE SPRAYING ON ANT DISTRIBUTION IN A WEST AFRICAN COCOA FARM

J. D. Majer *

ABSTRACT

Observations on the effect of insecticides on the beneficial ants, *Oecophylla longinoda* (F.) and *Macromischoides aculeatus* (Mayr), and the less desirable *Crematogaster* spp. are reviewed. A field trial was conducted to investigate the effect of blanket spraying trees with Baygon/Gammalin formulation and of selective spraying of all trees except for those with *O. longinoda* nests.

By upsetting the competitive balance, blanket spraying encourages *Crematogaster* at the expense of reduced *O. longinoda* and *M. aculeatus* distribution and abundance. Selective spraying depresses the *O. longinoda* population less and leads to localized replacement of *Crematogaster* by the more beneficial ants.

Anti-capsid spraying of all trees, apart from those supporting *O. longinoda,* is suggested as part of an integrated control scheme for cocoa.

INTRODUCTION

The benefits of the ant *Oecophylla longinoda* (F.) to the welfare of West African cocoa have been pointed out by a number of workers as reviewed by Leston (6). Additional investigation by Aryeetey (1) revealed that another ant, *Macromischoides aculeatus* (Mayr) was also of value. *Crematogaster,* the other main ant genus present, has been indited in the spread of cocoa swollen shoot virus disease (11) and in blackpod, *Phytophthora palmivora* (Butl.) Butl. epidemiology (3).

In view of the value of certain ant species it is important to know what effect anti-capsid spraying has on their abundance and distribution. Gerard (4) found that carbaryl and fenitrothion

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destroyed colonies of *O. longinoda* and *M. aculeatus* and Marchant (10) found that repeated high doses of Arprocarb (Baygon) could depress *O. longinoda* by 75% during a season while having no effect on *Crematogaster africana* Mayr. Collingwood (2), on the other hand, found that only gamma BHC (Gammalin) had a significantly depressing effect on *O. longinoda.*

Using a different approach, Leston (5) scored the percentage of trees with *O. longinoda* nests in randomly selected farms in different regions of Ghana. He found low occurrences of this ant in areas which had a history of heavy spraying but up to 50% occupation of trees in high yielding areas where little spraying had been undertaken. It remains uncertain whether spraying caused the observed ant distributions.

In view of these uncertain findings, a field trial was performed to investigate the effects of the Ghana Government's 1972 mass spraying scheme on the ant fauna. Also, since Leston (5) and Majer (8) have suggested integrated control schemes in which trees supporting *O. longinoda* are omitted from anti-capsid spraying, blanket and selective spraying regimes were compared.

METHOD

The trial was performed at the Agricultural Research Station of the University of Ghana at Kade (0° 55' W, 6° 09' N). Three plots were selected for their similarity of tree spacing, canopy density, topshade, tree age and variety, and similar ratios of *O. longinoda, M. aculeatus* and *Crematogaster (Atopogyne)* species tree occupancy. The plots measured 63 x 30m (20 x 10 tree rows) and were surrounded on all sides by two rows of trees which were subjected to the same treatment to avoid edge effects. The cocoa was Amelonado variety planted in 1959 - 1960 under moderate topshade on a 3 x 3m spacing system.

Plots 1 (blanket sprayed) and 2 (selectively sprayed) contained colonies of. *C. depressa* (Latr.) while Plot 3 (unsprayed control) contained *C. clariventris* (Mayr). It was not possible to select plots with the same *Crematogaster* species.

The trees in each plot were scored for presence or absence of *O. longinoda* and *Crematogaster* foragers and number of *O. longinoda* and *M. aculeatus* leaf nests.

The initial survey was performed between 15th and 20th March 1972. All Plot 2 trees supporting colonies of *O. longinoda* were marked by tying a palm frond around the trunk.

Selective anti-capsid sprayings were carried out as suggested by Leston (5) and Majer (8); that is omitting those trees with *O. longinoda* nests.

Spraying of Plots 1 and 2 was performed on 22nd March using two

Solo knapsack motorised mistblowers to apply a formulation of 30 ml Baygon and 60 ml Gammalin in 50 l water/ha. This formulation was used in the Government mass spraying scheme although it has since been abandoned. The treatment was completed between 0930-1030 hours.

Each plot was resurveyed at approximately 15, 50 and 100 days after spraying in order to monitor changes in ant distribution and abundance. Only leaf nests found to contain ants were recorded during the nest counts.

RESULTS

The trees foraged by *O. longinoda*, nested by *O. longinoda*, by *M. aculeatus* and foraged by *Crematogaster* species are shown in Figure 1 and are expressed as percentages of pre-spray date values.

There was an immediate and large drop in *O. longinoda* foraged and nested trees in the blanket sprayed plot. This drop continued for about 50 days after which there was a certain amount of recovery. Similar trends were observed in the selectively sprayed plot although the initial reductions were not as great.

The reduction of trees nested by *M. aculeatus* was greater than the corresponding value for *O. longinoda* although numbers started returning to pre-spray levels within 15 days of spraying. A similar effect was observed in the blanket and selectively sprayed plots.

The number of *C. depressa* foraged trees increased in the blanket sprayed plot as the ant spread on to trees where *M. aculeatus* had been eliminated by the insecticide. The trend reversed as *M. aculeatus* recovered after spraying. Similar reductions were noted in the selectively sprayed plot although in contrast to events in the blanket sprayed plot, the number of trees foraged continued to drop during the entire observation period.

It should be noted that the ant distribution and abundance dropped slightly in the control plot during the course of the observation period. This was probably a seasonal effect.

DISCUSSION

The results should be viewed in the terms of an ant mosaic in which the blocks, or ant territories are partially maintained by interspecific competition (9). Reduction in competitive ability by factors such as spraying could lead to the spread of adjacent species into the weakened ants territory.

Both spraying regimes would tend to act selectively against species of ants nesting in the lower regions of the cocoa forest since there is a limit to the spray height of the mistblowers. The observed changes following blanket spraying tie in with

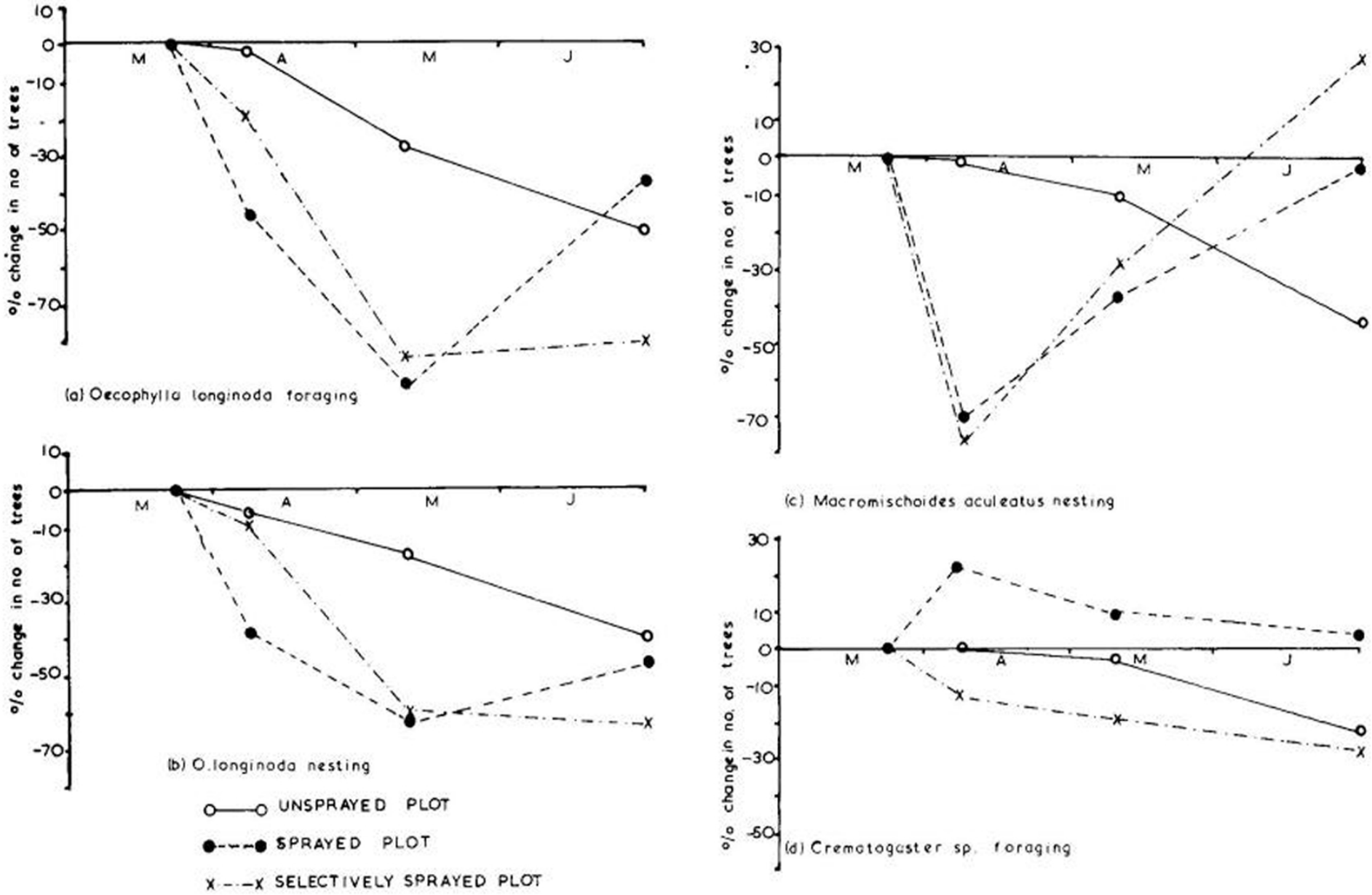

Figure 1 – Changes in the numbers of trees nested or foraged by *O. longinoda, M. aculeatus* and *Crematogaster* spp. following spray treatments on 22nd March 1972.

this factor. *O. longinoda* and *M. aculeatus*, which both nest in the cocoa canopy, were reduced in both distribution and abundance. *C. depressa*, which tends to nest high up on shade trees (7) was less affected by the spray and gained territory previously occupied by the two former species.

The observed reduction of *O. longinoda* in the selectively sprayed plot probably resulted from spray drifting into its territory. Nevertheless, this spray regime seemed to be less detrimental to *O. longinoda* and may have altered the competitive balance so that *C. depressa* was locally replaced by *O. longinoda* and *M. aculeatus*. These changes resulted from a single spray application. Repeated sprayings would probably enhance such effects and might account for the low occurrence of *O. longinoda* which Leston (5) observed in heavily sprayed cocoa growing areas.

The long term reduction of *C. depressa* following a selective insecticide application suggests that Leston's (6) and Majer's (8) integrated control schemes, which incorporate this type of treatment, would be a feasible method of encouraging beneficial ants to replace the less useful *Crematogaster* species.

ACKNOWLEDGEMENT

The author is grateful to Dr. A. Opoku and the Kade Management Board for assistance with the spraying operations.

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RESUMO

INFLUÊNCIA DE PULVERIZAÇÃO TOTAL E SELETIVA SOBRE A DISTRIBUIÇÃO DE FORMIGAS EM UMA FAZENDA DE CACAU NO OESTE AFRICANO

Um ensaio de campo foi realizado com a finalidade de avaliar o efeito de inseticidas sobre as formigas benéficas *Oecophyla longinoda* (F.), *Macromischoides aculeatus* (Mayr), e a menos desejável *Crematogaster* spp. As pulverizações das parcelas foram executadas com formulação de Baygon/Gammalin. Uma das parcelas foi totalmente pulverizada, a outra recebeu um tratamento seletivo em que todas as árvores foram pulverizadas exceto aquelas com *O. longinoda*, e a terceira não foi pulverizada.

Ao alterar o balanço competitivo, a pulverização total favorece *Crematogaster* pela redução na distribuição e abundância de *O. longinoda* e *M. aculeatus*. A re-

dução na população de *O. longinoda* é menor quando é utilizada a pulverização seletiva, promovendo consequentemente a substituição localizada de *Crematogaster* por formigas mais benéficas.

A pulverização de todas as árvores para combater os capsídeos, exceto daquelas que abrigam *O. longinoda*, é sugerida como parte de um esquema de controle integrado para cacau.

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EFEITO DE UM ANTITRANSPIRANTE (MOBILEAF) SOBRE A TRANSPIRAÇÃO DE MUDAS DO CACAUEIRO (*Theobroma cacao* L.)

José Moacir Pinheiro Lima Filho *
Ronald Alvim **

ABSTRACT

Effect of an Antitranspirant (Mobileaf) on the Transpiration of Cacao (*Theobroma cacao* L.) Seedlings

A greenhouse study was conducted in the area of Centro de Pesquisas do Cacau, Itabuna, Bahia, from June to July, 1976, in order to estimate the efficiency of an antitranspirant (MOBILEAF) on the control of transpiration of young cacao plants (*Theobroma cacao* L.). Five concentrations were applied on leaves of approximately 6 months old cacao plants. This age was chosen because it has been recommended as a suitable one for transplanting seedlings. It was found that concentrations of 10% and 20% reduced the rate of transpiration in 32.0% and 45.9%, respectively, although symptoms of toxicity were observed on leaves of the seedlings treated with the higher concentration.

INTRODUÇÃO

Os efeitos adversos de períodos de desequilíbrio hídrico são particularmente sérios por ocasião do transplante de mudas, nas espécies em que esta prática é recomendada.

A possibilidade de se reduzir temporariamente a transpiração das plântulas – com vários objetivos ou especificamente a fim de que o seu sistema radicular tenha, após o transplante, condições mais propícias de se adaptar ao novo ambiente e assim compensar

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adequadamente as perdas transpiratórias da folhagem – tem motivado muitos investigadores. Alguns têm realizado estudos básicos sobre os efeitos de inibidores metabólicos do mecanismo estomático como o acetato de fenil mercúrio e ácido abscísico (3, 9, 10, 11, 16, 17, 18, 19, 20). Outros têm avaliado a eficiência de substâncias capazes de formar películas sobre as folhas que diminuem as transpirações cuticular e estomática, seja por reflexão da energia radiante incidente (2, 5, 7, 15) ou simplesmente pela impermeabilização da folhagem e conseqüente proteção contra outros agentes externos que promovem a perda de água para a atmosfera (8, 11). Reduções da taxa de transpiração da ordem de 40 a 50% têm sido encontradas (8, 12, 14).

Poucos resultados têm sido obtidos para o cacaueiro *(Theobroma cacao* L.), planta reconhecidamente sensível à desidratação (1) e que é normalmente transplantada após 4 a 6 meses de enviveiramento.

Alvim et al. (2), estudando o efeito do emprego de tinta branca refletora sobre a folhagem de plântulas, em condições de casa de vegetação, demonstraram uma redução da taxa transpiratória da ordem de 12,6%, quando a face ventral, não estomática, das folhas, era pintada. Neste trabalho foi ainda observada uma queda de temperatura na face estomática das plantas tratadas, em relação à testemunha.

O antitranspirante "SPRUCE SEAL" foi testado em plântulas de cacau por Olofinboba e Fawole (12), que concluiram ser a concentração de 0,5% a mais adequada. Reduções da taxa transpiratória da ordem de 38% foram obtidas nestas condições.

O presente trabalho teve por objetivo investigar o efeito do antitranspirante "MOBILEAF"* sobre a abertura estomática e a transpiração de plântulas de cacau, procurando-se escolher a concentração mais conveniente no sentido de diminuir a taxa de mortalidade de mudas por ocasião do transplante sob condições adversas.

MATERIAIS E MÉTODOS

O ensaio foi realizado no Centro de Pesquisas do Cacau, Itabuna, Bahia, sob condições de casa de vegetação, no período de junho a julho de 1976.

Utilizaram-se 120 plântulas de uma mistura de híbridos de cacau *(Theobroma cacao* L.), originária de cruzamentos entre tipos Alto Amazônico, Amelonado e Trinitário, com aproximadamente 6 meses de idade (idade recomendada como mais propícia ao transplante), cultivadas em sacos de polietileno com capacidade para 3 quilos de terriço. Essas mudas foram irrigadas previamente durante 5 dias, nos períodos da manhã e da tarde, e divididas em seis grupos de 20 plântulas.

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* Fabricado pela "Mobil Oil" do Brasil.

As plantas de cinco desses grupos tiveram sua parte aérea tratada por imersão, durante 30 segundos, em soluções aquosas do antitranspirante, nas concentrações de 1%, 2%, 5%, 10% e 20%. A parte aérea das plantas do sexto grupo foi imersa em água. Foram então estudados os seguintes parâmetros fisiológicos:

1. **Abertura relativa dos estômatos.** Estimada através de um porômetro ventilado de resistência difusiva, modelo VP–1, fabricado por "Cayuga Development" (Ithaca, New York, USA), utilizando-se folhas da mesma idade, tomadas ao acaso nos diferentes tratamentos.

Preliminarmente foram efetuadas medições horárias, com a finalidade de se determinar o período de máxima abertura estomática. Esta foi encontrada entre as 9 e 10 horas, horário em que as leituras passaram a ser tomadas, em dias alternados, até o décimo dia.

Para se avaliar o tempo de ação do antitranspirante, efetuaram-se ainda mensurações da resistência estomática 30 dias após o início dos trabalhos.

2. **Transpiração relativa.** Estimada através de método gravimétrico. Foram usadas cinco plantas para cada tratamento, envolvendo-se previamente o seu sistema radicular em sacos de polietileno, com as extremidades firmemente amarradas à base do caule, para evitar perda de água por evaporação na superfície do solo.

Determinou-se o peso inicial de cada planta. Em seguida, a intervalos de 48 horas, durante dez dias, as plantas foram pesadas, adicionando-se aos vasos, a cada 2 dias, a quantidade de água transpirada no período.

Ao final deste ensaio as mudas foram desfolhadas, aferindo-se, para o cálculo das taxas de transpiração, a área foliar total de cada tratamento, com o auxílio de um aparelho "Automatic Area Meter", modelo AAM–5, fabricado por "Hayashi Denko Co. Ltd", Japão.

RESULTADOS E DISCUSSÃO

As Figuras 1 e 2 mostram as variações da resistência estomática e da transpiração, no período de 10 dias após a aplicação das diversas concentrações de MOBILEAF. Observa-se que todas elas exerceram influência sobre esses dois parâmetros.

As resistências mais altas foram observadas nas concentrações de 20% e 10% (Figura 3), correspondendo às maiores reduções na transpiração (Figura 4). Analisando-se a Figura 5, verifica-se que essas concentrações reduziram a transpiração em 45,9% e 32,0%, respectivamente. Este fato demonstra a eficiência do antitranspirante MOBILEAF para plântulas de cacau cultivadas em casa de vegetação. A sua eficácia, entretanto, torna-se menor à medida que diminuem as concentrações. Observa-se, assim, que as resistências mínimas

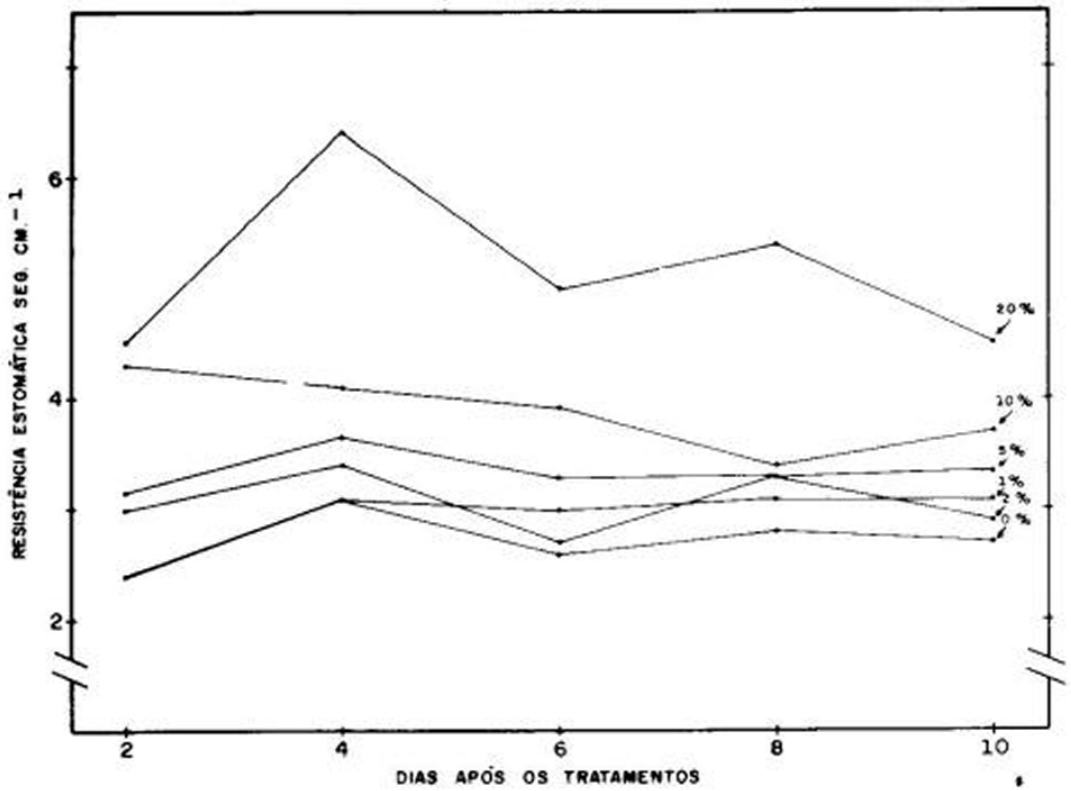

Figura 1 – Resistência estomática observada em cacaueiros sob influencia da aplicação de várias concentrações de MOBILEAF, durante os 10 primeiros dias posteriores ao início dos trabalhos.

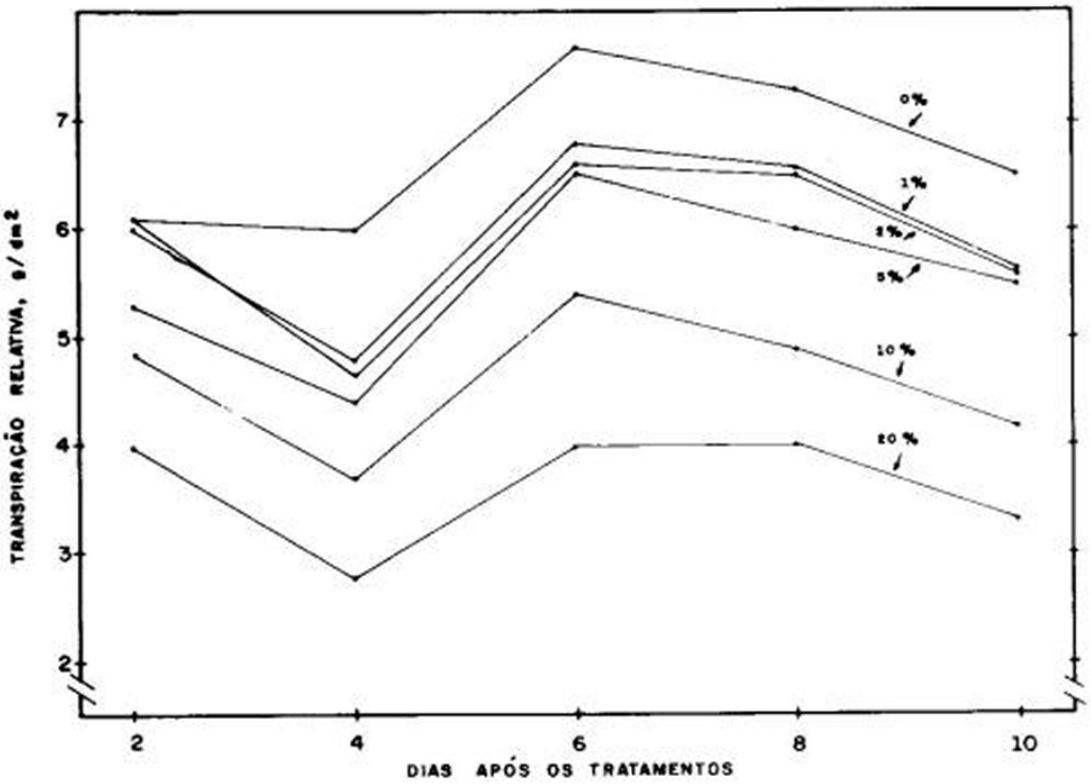

Figura 2 – Transpiração relativa observada em cacaueiros sob influência da aplicação de várias concentrações de MOBILEAF, durante os 10 primeiros dias após o início dos trabalhos.

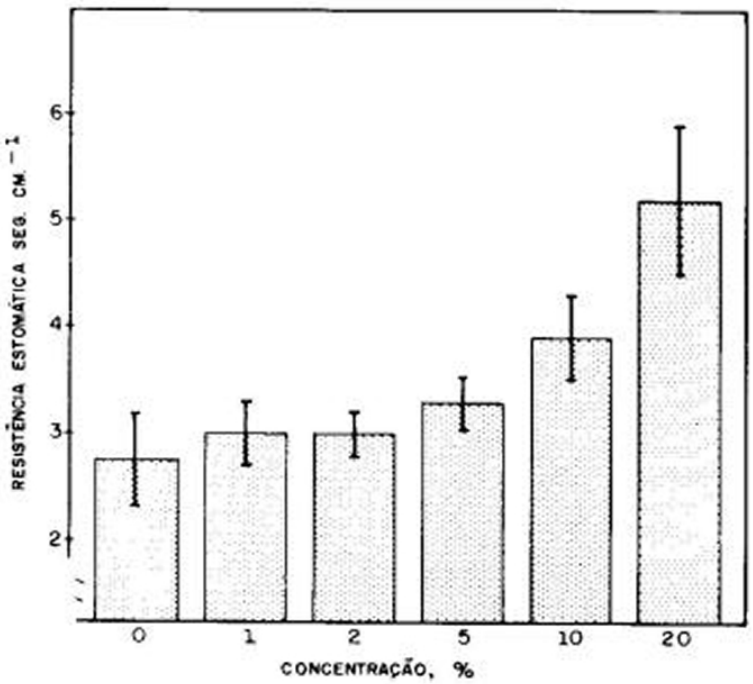

Figura 3 – Valores médios de resistência estomática, obtidos em função da concentração de MOBILEAF. As barras verticais sobre as colunas indicam os desvios das médias.

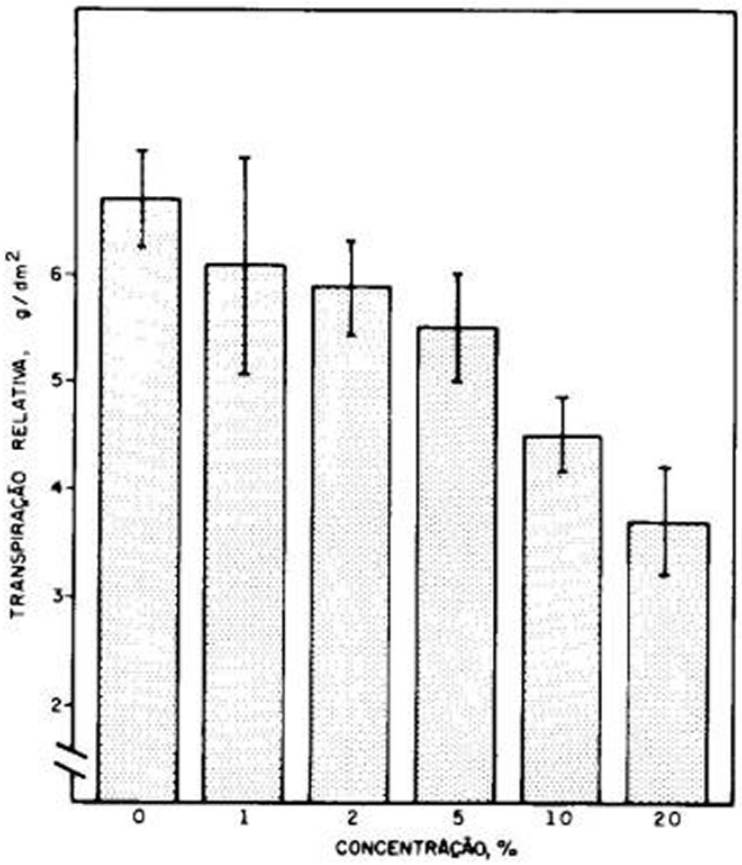

Figura 4 – Valores médios de transpiração relativa, obtidos em função da concentração de MOBILEAF. As barras verticais sobre as colunas indicam os desvios das médias.

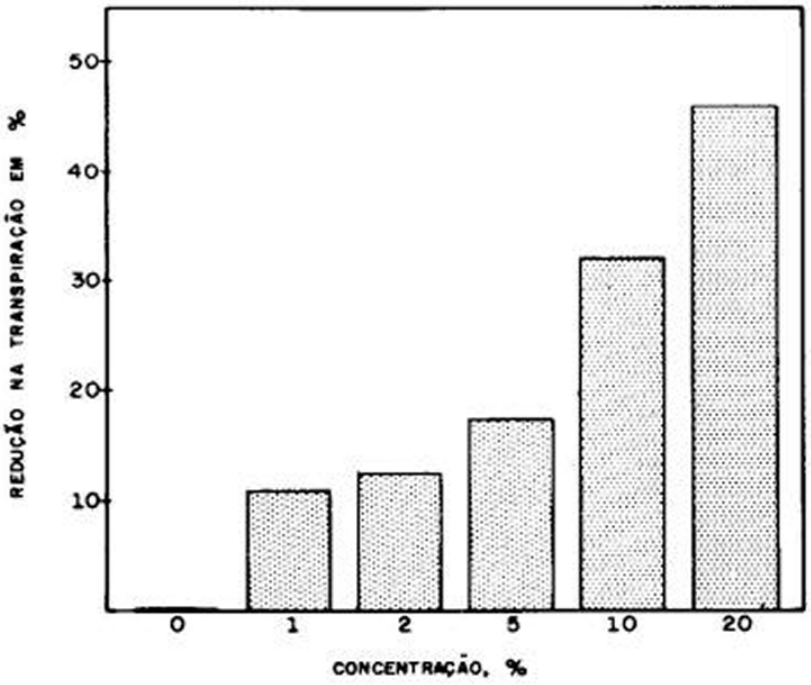

Figura 5 – Efeito de várias concentrações do MOBILEAF na redução da transpiração de cacaueiros.

corresponderam às concentrações de 1%, 2% e 5%, quase igualando-se aos valores obtidos nas mudas não tratadas (Figura 3).

Infelizmente não se têm notícias do modo de ação nem das características físico-químicas do produto. Parece, entretanto, que seu efeito sobre o controle da transpiração se deve mais a ações mecânicas que metabólicas.

Sabe-se que os antitranspirantes que agem metabolicamente sobre o mecanismo estomático têm um período de ação bastante reduzido (5). Observa-se, na Figura 6, que o MOBILEAF ainda exercia influência sobre a resistência estomática, trinta dias após o início dos trabalhos. Isso leva a crer que esse antitranspirante age mecanicamente, bloqueando os estômatos pela formação de filmes sobre a superfície foliar ou penetrando nas folhas através do poro estomático.

Os principais problemas envolvendo o uso de tais produtos residem na dificuldade de se encontrarem substâncias que tenham permeabilidade seletiva aos gases (13). A não seletividade dos antitranspirantes conhecidos e o uso de concentrações elevadas têm sido freqüentemente associados ao aparecimento de sintomas de toxidez (4). No presente trabalho, observaram-se manchas amareladas nas folhas apenas nas mudas tratadas com a emulsão de MOBILEAF a 20%. Lee e Kozlowsky (6) observaram sintomas semelhantes em quatro espécies de plantas lenhosas, após a aplicação de um antitranspirante à base de silicone, em concentrações elevadas.

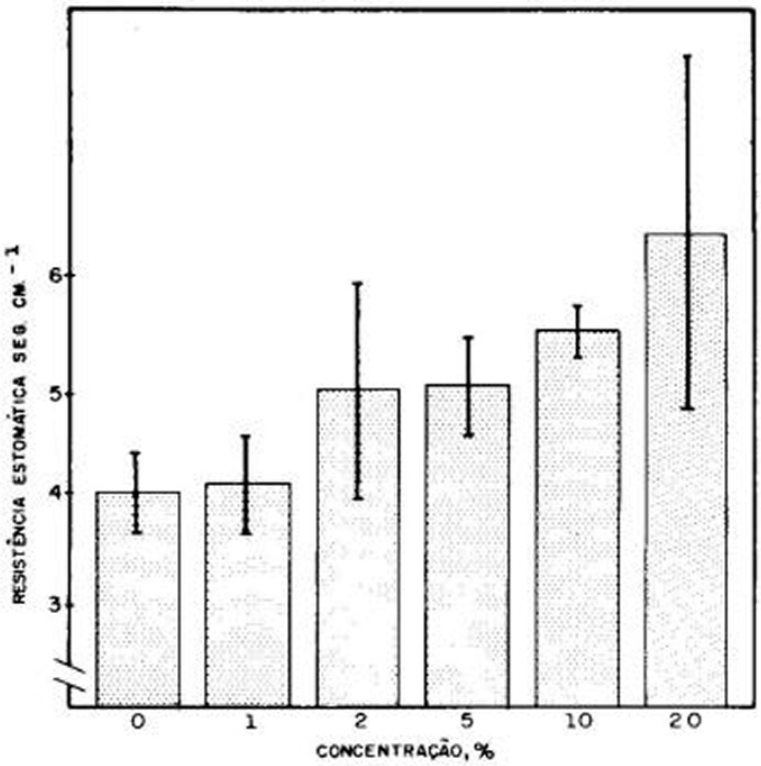

Figura 6 – Resistência estomática observada 30 dias após a aplicação de várias concentrações de MOBILEAF. As barras verticais sobre as colunas indicam os desvios das médias.

Devido à falta de informações sobre a formulação do MOBILEAF, torna-se difícil concluir sobre os danos causados às folhas nas mudas tratadas pela concentração de 20%. Parece provável, entretanto, que tais danos provenham da ação de alguma substância fitotóxica de sua composição.

Os dados obtidos demonstram a viabilidade da utilização do produto testado a fim de se reduzir a mortalidade de mudas por ocasião do transplante, principalmente quando esta prática for realizada em épocas menos propícias. Sugerem ainda a possibilidade do seu emprego para o transplante de mudas com raízes lavadas, prática que poderia reduzir os gastos com vasos no enviveiramento, pela utilização de canteiros, e facilitar o transporte das mudas do viveiro para o local de plantio definitivo.

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RESUMO

Um estudo foi realizado na área do Centro de Pesquisas do Cacau, Itabuna, Bahia, no período de junho a julho de 1976, sob condições de casa de vegetação, com a finalidade de avaliar a eficácia de um antitranspirante (MOBILEAF) sobre o controle da transpiração de plântulas do cacaueiro (*Theobroma cacao* L.). Foram aplicadas cinco concentrações às folhas de mudas de aproximadamente 6 meses de idade, que é recomendada como a mais adequada ao transplante.

Verificou-se que as concentrações de 10% e 20% reduziram a transpiração em 32,0% e 45,9%, respectivamente, embora se observassem sintomas de toxidez nas folhas das mudas tratadas pela concentração mais alta (20%).

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OPTIMUM TEMPERATURE AND pH OF INVERTASE OF THE SEEDS OF *Theobroma cacao* L.

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ABSTRACT

The location of invertase in the cacao seed was confirmed and its pH and temperature optima determined.

Acetone powders prepared from the pulp, pulp with testa and the cotyledons of cacao seeds were tested for enzyme activity by assessing their ability to invert sucrose. The reducing sugars formed after incubation of the powders with sucrose were determined by T.L.C., G.L.C. and by spectrophotometry.

Only preparations which included the testa were found to be active. Maximum inversion occurred at pH 4 and 5.25, at 52°C and 37°C respectively. This suggests the presence of more than one enzyme.

Solutions of alcohol and acetic acid below 7.5% and 2% respectively, did not inhibit enzyme activity.

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INTRODUCTION

The study of invertase of the cacao bean is of great importance as its activity during fermentation furnishes the reducing sugars which, together with the proteins and peptides are the precursors for the Strecker type reactions that are responsible for the flavour of chocolate. Therefore any changes in the methods to improve fermentation should take into account the effect on invertase activity.

The first studies of the enzymes of cacao were those of Brill and of Ciferri cited by Knapp (5). Both found invertase as well as other enzymes in cacao seeds, however, their results are in disagreement with respects to the location of the enzyme and the effects of fermentation. Thus, Brill reported invertase to be present in fresh pulp juice and in cotyledons of four-day fermented beans but did not find it in cotyledons of unfermented fresh beans. Ciferri on the other hand, found it in pulp and cotyledons of partially fermented beans (5). Neither author described the optimum pH and temperature for the enzyme. Roelofson (7) suggests that differences in the results are due to discrepancies in methods.

Although the occurrence of invertase has not been confirmed, its presence is implied by the studies of Bracco et al (2), Reineccius et al (6), and Berbert (unpublished results).

This study describes the location of invertase in the unfermented beans of cacao and its pH and temperature optima.

MATERIAL AND METHODS

Cacao fruits (Comum variety) at full maturity as well as at various stages during ripening were obtained from the Cacao Research Center's experimental fields in Bahia, Brazil.

The ripe fruits were broken open and the seeds were removed and immediately freeze-dried. The brittle testa with adhering pulp parenchyma of the freeze-dried seeds was broken and separated from the cotyledons and each was stored separately in dessicators. In immature fruits up to the age of 5 months, the pulp cells form a solid mass in which the seeds are imbedded. In this case the fruit wall was removed and the pulp with imbedded seeds was sliced into thin sections from which the cotyledons were scooped out. The remaining tissue, consisting of pulp with testa, and the cotyledons, were freeze-dried separately and stored in dessicators.

Enzyme powders were prepared essentially according to the method of Forsyth et al (4). The freeze-dried material (10g) was finely ground in a cold mortar and pestle with a little cold dry acetone (−20°C)

and filtered on a buchner funnel under vacuum. The residue was successively washed with cold 80% aqueous acetone (400 ml), cold 50% aqueous acetone, (1000 ml), and with cold dry acetone (500 ml). The resulting white to buff coloured powder was dried under vacuum and sifted through a 35 mesh screen. Coarse particles remaining on the screen were ground, resifted and added to the powder. This was stored in an air-tight container in the refrigerator until used.

The enzyme powders were tested for activity by observing their ability to invert sucrose to glucose and fructose. Dried enzyme preparation (0.03g) in a test-tube was suspended in 0.1M, pH 6 phosphate-citrate buffer (2 ml) containing sucrose (0.04g) and incubated at room temperature (24–27°C) for a period up to 72 hours. A blank containing sucrose and no enzyme and another containing enzyme and no sucrose were included in each test. After incubation, the enzyme was inhibited by placing the test-tubes in a boiling water-bath for 2 minutes. The mixtures were then freeze dried and the sugars extracted into 80% aqueous ethanol (2 ml) and the reducing sugars formed were assayed by one of the following methods.

a) **Thin layer chromatography (T.L.C.).** T.L.C. was used as a quick qualitative and a semi-quantitative method for investigating enzyme activity. Silica gel G layers of 250 μ thickness were prepared using 0.03M H_3BO_4 instead of water. The layers were air-dried for 16 hours and used without activation. A small quantity (3–5 μl) of the alcoholic extract of the freeze-dried reaction mixture was spotted on to the layer and developed twice in a solvent system consisting of chloroform, acetic acid and water (30:35:5 v.v.v.) (3). The developed plates were dried briefly in an oven at 110°C and the sugars revealed by spraying with a solution of diphenylaniline phosphoric acid reagent for qualitative identification or with 2, 3, 5 – triphenyltetralone for quantitative determinations of reducing sugars (1). The former reagent revealed sucrose and fructose as brown spots going to grey and glucose as a blue spot going to blue/grey. The latter reagent showed glucose and fructose as crimson spots on a pale pink background. These were scraped off the plates, eluted with chloroform and the colour measured at 510mμ in a spectrophotometer to give semi-quantitative determinations of reducing sugars.

b) **Spectrophotometric determinations.** Direct quantitative determinations of total reducing sugars were made using the arsenomolybdic method of Nelson (1). A portion of the 80% alcoholic extract of the freeze-dried incubated material (0.2 ml) was diluted with water (20 ml) and an aliquot of this

(1 ml) was reacted with a arsenomolybdic reagent. The resulting blue colour was diluted with water (10 ml) and read in a spectrophotometer at 520 m μ. A linear relationship was observed between optical density and glucose concentration within the range of 10 to 80 μg.

c) **Gas-liquid chromatography (G.L.C.).** Quantitative determinations of sugars were made by chromatographing the trimethylsilyl-derivatives. To an aliquot of the 80% ethanolic extract of the dried incubated material (0.5 ml), a 10% solution of rhamnose (0.2 ml) was added as internal standard. The mixture was freeze-dried and the sugars redissolved in pyridine (1 ml). Trisil 'Z' from Pierce Chemical Co., P.O. Box 117, Rock, Illinois, (0.5 ml), and a few granules of dehydrating agent (Na_2SO_4) were added to the solution and the mixture was allowed to react for one hour at room temperature. A portion (3–5 μl) of the clear supernatant was chromatographed directly on a 6' x 1/8" I.D., stainless steel column packed with 3% OV 17 on AWDMCS Chromosorb W. The carrier gas was nitrogen flowing at 65 ml per minute. Detector and injector temperatures were 250 and 200°C, respectively, and the oven temperature was programmed from 180 to 190°C at 2°C per minute and from 190 to 260°C at 6°C per minute. Rhamnose (internal standard) eluted at 1.8 minutes, fructose at 6.8 minutes, α – and β – glucose at 8.8 and 10 minutes, respectively, and sucrose at 18.2 minutes. Quantitative determinations were made by measuring peak areas of the sugars.

RESULTS AND DISCUSSION

Only powders prepared from the testa with some adhering pulp parenchyma showed enzime activity. This is in accordance with the finding of Brill (5). These powders were highly active and inverted over 50% of the added sucrose in an hour under the test conditions.

It was suspected at first, that the absence of enzyme activity from powders prepared from cotyledons was due to inactivation by polyphenols of the bean during the preparation procedure; especially as a considerable amount of browning occurred in storage. However, subsequent preparations made with solvents containing cysteine, EDTA, ascorbic acid, or thioglycollic acid to stabilize the enzyme also showed no enzyme activity even after incubation for 72 hours. Powders made in this way were much more stable to browning than before.

Enzyme inactivation due to the method itself was ruled out because when fresh cotyledon fragments were infiltrated with a sucrose solution no changes in the concentrations of the sugar constituents could be detected

by thin layer chromatography. Furthermore, preparations made from whole beans (pulp, testa and cotyledon) were as active as those from the testa alone.

In cacao pods up to about 3 months old, the cotyledon tissue exists in undifferentiated state as a jelly. Consequently this study was limited to fruits in which differentiation had already occurred.

In order to purify the enzyme, its extraction into water and buffers of various pHs was attempted. In each case all activity remained in the powder which suggests that the enzyme is probably bound to the testa. Dislodging the enzyme by mild hydrolisis or enzyme digestion was not attempted and all determinations of temperature and pH optima were done on crude powders.

Two pH and temperature optima were found. At room temperature minimum inversion occurred below pH 2.5 and above pH 6.5, and a maximum occurred at pH 4.0 – 4.25 with another peak at pH 5–5.25. (Figure 1). Similarly, under test conditions in pH 6 phosphate-citrate buffer, optima were observed at 37°C and at 52°C. At pH 4.25, maximum inversion occured at 52°C, whereas at pH 5, the maximum shifted to 37°C. This suggests that the powders are probably a complex of more than one carbohydrate hydrolase. Maltase, raffinase and galactosidase as well as invertase, have been reported to be present (5, 8) and it is quite probable that some of these are non-specific and under certain conditions will invert sucrose as well. The enzyme powders were not inhibited by ethanol up to 7.5% nor by 2% acetic acid.

Hydrolysis of sucrose in the cacao bean cotyledon is known to occur during fermentation of the beans (2, 6, 8). Assuming that invertase is not present in the bean cotyledon as our results indicate, two hypotheses for the mechanism of sucrose hydrolysis can be postulated. Firstly, the solvents entering into the bean during fermentation dissolve the sucrose in the cotyledon and transport it to sites of enzyme activity on the inner surface of the testa where hydrolysis occurs. Secondly, fermentation may produce changes in the testa which render the testa-bound enzyme soluble so that both enzyme and substrate are found in solution.

The rate of sucrose hydrolysis during fermentation appears to depend on the method. Rohan and Stewart (8) found that the rate of hydrolysis in a 500 lb Nigerian heap fermentation was faster than in a 150 lb Ghanian heap. The same sort of difference in rate of sucrose hydrolysis as influenced by the quantity of cacao fermented is also observed with box fermentations when the results of Reineccius et al (6), and Bracco et al (2) are compared. In a normal size box and heap fermentation, hydrolysis of sucrose is virtually complete after 72 hours (2, 3, 8). During this time

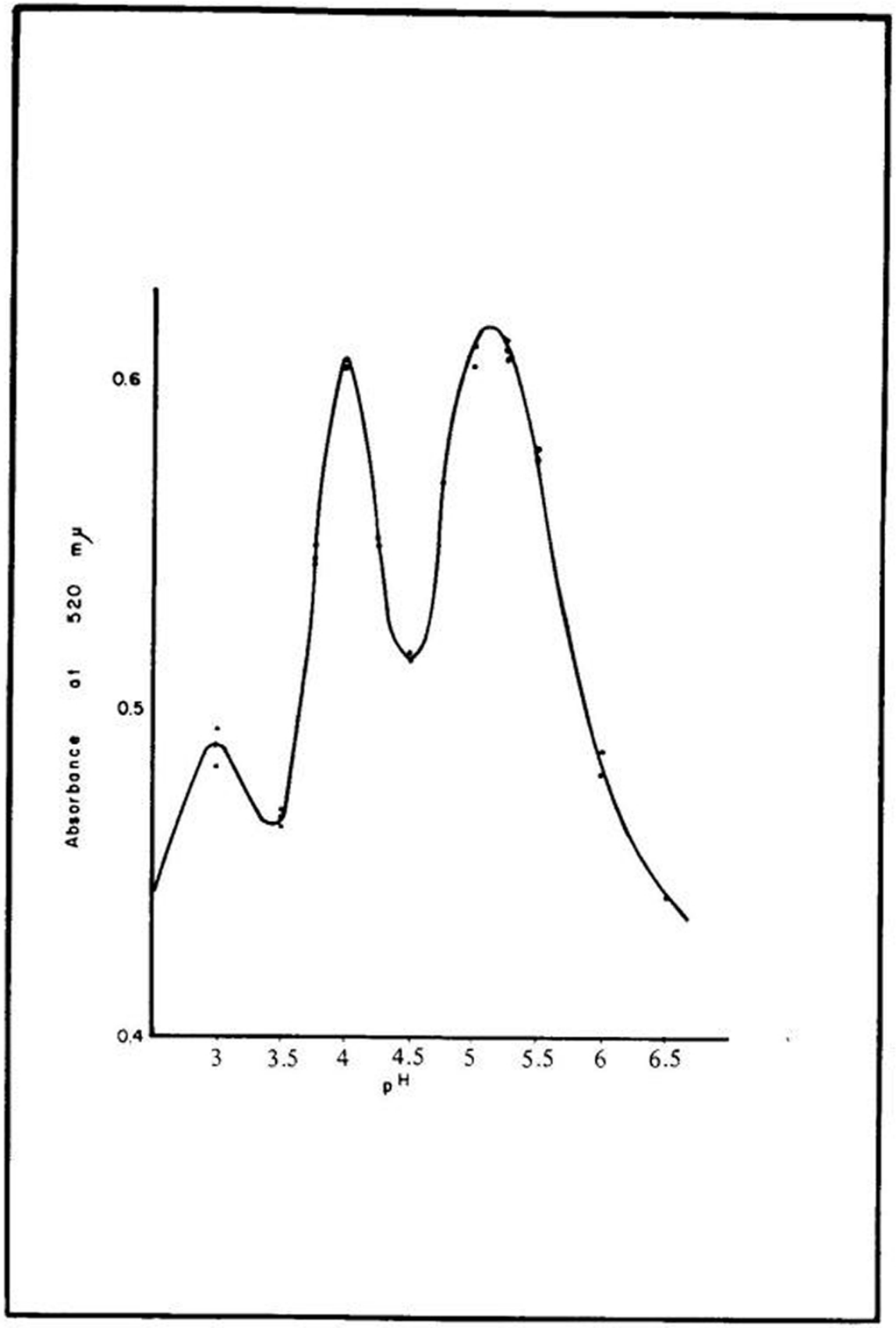

Figure 1 – Invertase activity of the testa preparations of the cacao bean under test conditions, (phosphate–citrate buffer at room temperature, 24–27°C).

the bean temperature rises to between 40 and 47°C and the pH of the cotyledon falls from 6.9–6.6 to about 5.2. The falling cotyledon pH and moderate rise in temperature during this early stage in fermentation provides conditions best suited for the enzyme having optima of pH 5 and 37°C. As fermentation progresses these conditions change to favour the second enzyme with optima of pH 4.2 and 52°C. In large scale fermentations where the initial rise in temperature is slow, optimum conditions are soon reached for the enzyme operating best as 37°C and consequently most of the hydrolysis of sucrose will occur early in fermentation under the influence of this enzyme. In small scale fermentations, where temperature rise is more rapid because of the increase in the rate of oxidation resulting from the larger surface to volume ratio, these optima are soon passed. Hence, most of the hydrolysis can be expected to occur under the influence of the enzyme system with optima of pH 4.2 and 52°C. In this case hydrolysis would be less rapid and would peak later. Other physical or chemical factors which affect the rate of temperature increase of a fermentation or the rate or entry of solvents will also affect the rate of sucrose hydrolysis.

Invertase activity was not found in the cotyledon of fresh beans in this study. However, there is the possibility that invertase is present in a latent from and is activated during fermentation or during the incipient germination that is believed to occur during the start of fermentation. Brill (5) could only find the enzyme in cotyledon after fermentation, which supports the above argument as well as the hypothesis that fermentation results in the solubility of the testa-bound enzyme.

The investigation of the behavior of invertase of cacao bean during fermentation is the topic for a study under consideration.

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RESUMO

Temperatura e pH Ótimos de Invertase das Sementes do *Theobroma cacao* L.

A presença de invertase na semente de cacau foi confirmada e determinado pH e temperatura ótimos para a sua atividade.

"Pós de acetona" preparados a partir da polpa, polpa com testa, e dos cotilédones das sementes de cacau, foram examinados com relação à atividade da invertase, pela avaliação de sua capacidade para inverter sacarose. Os açúcares redutores, formados após a incubação dos "pós" com sacarose foram determinados por T.L.C., G.L.C. e espetrofotometria.

Apenas as preparações que incluiam a testa da semente se apresentaram ativas. A inversão máxima verificou-se no pH 4 e pH 5.25 correspondentes às temperaturas de 52°C e 37°C respectivamente. Este fato sugere a presença de mais de um enzima na preparação.

Soluções de etanol e de ácido acético de concentração inferior a 7.5% e 2% respectivamente, não inibiram a atividade do enzima.

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NOTA

LIMITAÇÕES AO USO DO MÉTODO COLORIMÉTRICO PARA DETERMINAÇÃO DA INTENSIDADE DE CONTAMINAÇÃO DE FUMAÇA EM AMÊNDOAS DE CACAU

Paulo Romeu F. Berbert *
José Marcondes Borges **

A presença de fumaça em amêndoas de cacau, adquirida durante a secagem e/ou armazenagem, é considerada defeito grave para o produto. Por esse motivo, tem-se procurado desenvolver um método de detecção de fumaça, eficiente porém suficientemente simples, para poder ser amplamente utilizado em postos de classificação ou, mesmo, a nível do agricultor.

Publicou-se a descrição de um "método colorimétrico simples para determinação de fumaça em amêndoas de cacau" (1, 4). O citado método baseia-se na extração com etanol, a 96%, de compostos fenólicos componentes da fumaça, presentes na amêndoa de cacau contaminado e que são responsáveis pela coloração amarelada que aparece na solução alcoólica. A comparação da intensidade da coloração do extrato problema com a de extratos padronizados dá uma idéia bastante aproximada do grau de contaminação das amêndoas.

Nos testes para desenvolver esse método, usaram-se como padrão amêndoas das áreas do Centro de Pesquisas do Cacau (CEPEC), devidamente fermentadas, secas em barcaças e indubitavelmente livres de fumaça. Lotes de cacau, artificialmente enfumaçados ou reconhecidamente contaminados com fumaça, foram comparados com o cacau do CEPEC que apresentou, invariavelmente, extratos incolores. Os extratos alcoólicos de amêndoas contaminadas sempre se apresenta-

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ram com coloração amarelada, sendo esta tanto mais intensa quanto mais fumaça continham os extratos.

O método é muito sensível e muito simples; daí o pensamento de sua utilização generalizada, mesmo em condições de campo. Com tal objetivo, e antes de sua recomendação definitiva, resolveu-se testá-lo intensivamente em laboratório, simultaneamente com o método tradicional de detecção de fumaça (cortar e cheirar a amêndoa), em lotes de cacau provenientes de diferentes fazendas da Região Cacaueira da Bahia.

Durante o uso intensivo do método, notou-se que algumas amostras, apesar de não ser detectado nenhum contacto destas com fumaça, também apresentavam extratos com colorações levemente amareladas. Suspeitou-se então que estas colorações estivessem relacionadas com o grau de fermentação das amêndoas. Isto foi posteriormente confirmado, utilizando-se o citado método em amêndoas com 0 a 7 dias de fermentação realizado segundo as recomendações da CEPLAC (3), e não contaminadas com fumaça. Constatou-se então que extratos de amêndoas com 1 e 2 dias de fermentação são incolores. Amêndoas com 3 a 5 dias de fermentação dão extratos com colorações amareladas cujas intensidades diminuem a partir do 5º dia.

Em condições normais, após o segundo dia de fermentação, substâncias polifenólicas são liberadas do interior das células, difundindo-se na amêndoa. Em torno do terceiro dia, inicia-se a perda dessas substâncias, por exudação, através da testa. Os polifenóis e os primeiros produtos de transformação, que exudam através da testa no terceiro e quarto dias de fermentação, são solúveis em etanol, 96%, e podem também, igualmente à fumaça, proporcionar uma coloração amarelada à solução. Ao final da fermentação, em torno do 6º dia, os polifenóis, que exudaram através da testa, são transformados por reações de oxidação e hidrolíticas. As novas substâncias formadas não mais dão coloração amarelada à solução etanólica. Os polifenóis que permanecem no interior da amêndoa também sofrem reações de oxidação e hidrólise, combinando-se com proteínas e formando substâncias complexas (2).

Assim, embora o método colorimétrico desenvolvido guarde uma boa correlação entre intensidade de cor e contaminação por fumaça, no caso de amêndoas bem fermentadas, o mesmo não acontece quando se trata de amêndoas de fermentação incompleta. Conseqüentemente, o método não deve ser utilizado para detecção de fumaça em amêndoas de cacau, a menos que se tenha conhecimento prévio do histórico da fermentação do lote a ser analisado. Alternativamente, caso se tenha certeza da ausência de contaminação com fumaça, o método colorimétrico pode, também, juntamente com uma análise visual

das amêndoas, ser utilizado como um teste para se ter uma indicação do grau de fermentação de um lote de cacau.

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LIMITATIONS ON THE USE OF THE COLORIMETRIC METHOD FOR DETERMINING SMOKE CONTAMINATION IN CACAO BEANS

The presence of smoke in cacao beans, whether acquired during drying or storage, is considered a defect of marketable cacao. Because of this, efforts have been made to develop an efficient but simple smoke determination method which could be used in quality inspection stations or by the farmer.

A description of a simple colorimetric method for determining smoke contamination in cacao beans was published (1, 4). In the cited method the colour obtained on extracting the phenolic smoke components from contaminated cacao beans with 96% ethanol is compared with that from a graded series of extracts prepared from mixtures of artificially contaminated beans and uncontaminated beans. The yellowish color that appears in the alcoholic solution of contaminated beans is caused by phenolic compounds and its intensity has been found to have a positive correlation with the smoke content of the extracts.

Beans, thoroughly fermented, dried on platforms and definitely free of smoke, from the growing plots of the Cacao Research Center (CEPEC) were

used as a standard in the tests for the development of the above mentioned method. The lots of cacao artificially smoked or known to be contaminated with smoke were tested against the cacao of CEPEC which invariably gave colorless extracts. The alcohol extracts of contaminated beans give a yellowish coloration. The coloration is more intense as the extracts contain more smoke.

This method is not only sensitive, but also simple, and gave rise to the idea of its general use under field conditions. With this objective in mind, and before making definitive recommendations, it was decided to conduct intensive tests in the laboratory using cacao obtained from different farms in the cacao region of Bahia. These tests were made simultaneously with the traditional method (cut and smell test) for detecting smoke.

During the intensive use of the method it was found that, althought some samples had no contact with smoke they gave extracts with a yellow coloration. It was suspected that the coloration was related to the degree of fermentation of the beans. This was later confirmed using the cited method with beans fermented from 0 to 7 days. It was concluded that extracts from beans with 1 to 2 days of fermentation as recommended by CEPLAC (3), are colorless, while beans with 3 to 5 days of fermentation give yellowish extracts whose intensity diminish from the 5th day on.

Under normal conditions, after the second day of fermentation, the polyphenolic substance are liberated from the interior of the cells and diffuse throughout the bean. On the third day, there is a loss of those substances by exudation through the testa of the bean. The polyphenols and their first products of breakdown, which exudate through the testa, during the third and fourth days, are soluble in 96% ethanol, and they can also give, similar to smoke, a yellow coloration to the solution. At the end of fermentation, about the 6th day, the polyphenols that had exuded through the testa had been completely transformed by hydrolytic breakdown and oxidative reactions so that their final products no longer gave a yellow coloration. The polyphenols, that remains inside the bean, are also subjected to hydrolyses breakdown and oxidative reactions, combining with proteins to form polyphenol-protein complexes (2).

So, although in the case of well fermented beans the colorimetric method, referred to above, gives good correlation between color intensity and smoke contamination, it is unreliable in the case of improperly fermented beans. Consequently, this method should not be used to detect smoke in cacao beans, unless the history of the fermentation of the sample to be analyzed is known. Alternatively, in cases where one is certain that smoke contamination is absent, the colorimetric method may also, together with a visual analysis of the beans, be used to give an indication of the degree of fermentation of a cacao lot.

INFORMAÇÕES AOS COLABORADORES

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