SCIENTIFIC NOTE

Method for Maintenance of Coffee Leaves *In Vitro* for Mass Rearing of *Leucoptera coffeellum* (Guérin-Méneville) (Lepidoptera: Lyonetiidae)

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Método para Manutenção de Folhas de Café *In Vitro* para Criação Massal de *Leucoptera coffeellum* (Guérin-Méneville) (Lepidoptera: Lyonetiidae)

RESUMO – Para realizar estudos sistemáticos com o bicho-mineiro-do-cafeeiro, é necessário estabelecer uma criação massal em condições artificiais. É possível criar esta espécie, de ovo até adulto, em condições de laboratório, sem usar mudas de café, utilizando-se folhas destacadas mantidas *in vitro*. Citocininas sintéticas são habitualmente usadas para manutenção de células e tecidos vegetais *in vitro*. Devido a essa propriedade, foram escolhidos dois reguladores de crescimento de planta, benziladenina e cinetina, em concentrações 10^{-6} e 10^{-7} M, para a manutenção das folhas. Folhas coletadas em campo foram mantidas nas diferentes soluções a serem testadas. Utilizou-se água destilada como controle. O experimento durou 30 dias, período suficiente para o desenvolvimento completo do inseto. Observou-se que ambas as citocininas artificiais em ambas as concentrações aumentaram o tempo de vida das folhas de café mantendo-as verdes e em condições de criar o inseto. Folhas colocadas nas gaiolas atraíram os adultos, verificando-se oviposições nas mesmas. Estes ovos resultaram em indivíduos que completaram todo o ciclo de desenvolvimento. Os testes com os reguladores em diferentes concentrações com folhas saudáveis mostraram eficiência, porém, há evidências de que as concentrações do hormônio a ser usado com folhas minadas devem ser maiores, porque quando estas foram submetidas à concentração escolhida de 10^{-7} M, o tempo de vida não foi satisfatório. Portanto, devem ser executados testes com concentrações de hormônio diferentes, utilizando-se folhas minadas, para se descobrir a concentração ideal à sobrevivência destas folhas. Em nosso laboratório estamos usando benziladenina na concentração de 10^{-6} M com sucesso para a manutenção de folhas minadas.

PALAVRAS-CHAVE: Insecta, citocininas, bicho-mineiro-do-café.

ABSTRACT – To accomplish systematic studies with coffee leafminer, it is necessary to establish a mass rearing system under artificial conditions. It is

possible to rear this species, from egg to adult, under laboratory conditions, without using coffee seedlings but detached leaves maintained *in vitro*. Synthetic cytokinins are routinely used for maintenance of plant cell and plant tissues *in vitro*. Two plant growth regulators, benzyladenin and kinetin, in concentrations 10^{-6} and 10^{-7} M were used to mantain the leaves. Green leaves collected in the field were maintained in the solution to be tested. Distilled water served as control. The experiment lasted 30 days, a period longer than the necessary for the complete development of the insect. Both artificial cytokinines indeed increased the lifetime of the coffee leaves, maintaining them green and healthy. Leaves placed in the cages for oviposition were attractive to the insect, with significant number of eggs per leaf. In most cases, eggs resulted in individuals that completed the whole developmental cycle. Tests with regulator in different concentrations with healthy leaves showed efficiency. However, we believe that hormone concentrations to be used with mined leaves should be larger, because these when maintained at 10^{-7} M leaves did not present a satisfactory lifetime. Therefore, tests with mined leaves with different hormone concentrations should be made to find out the ideal concentration for leaf survival. In our laboratory we are successfully using 10^{-6} M benzyladenin for the maintenance of mined leaves.

KEY WORDS: Insecta, cytokinine, leafminer.

The coffee leafminer, *Leucoptera coffeellum* (Guérin-Méneville) (Lepidoptera: Lyonetiidae) is a serious pest of coffee plantations in Brazil, mainly in Minas Gerais State, where it is widely spread and causes great economic losses (Parra 1985). Improvements in the quality of the productive process, as well as decrease in the production costs and in the pollution caused by agro-chemicals, will demand the development and the adoption of new technologies in pest management.

To accomplish systematic studies on coffee leafminer, it is necessary to establish a mass rearing system under artificial conditions. The dependence on field infestations can either affect or make unfeasible planned studies because of seasonal occurrence of the insect. *L. coffeellum* has been reared on coffee seedlings infested by adults obtained on the field (Katiyar 1968). This method presents serious inconveniences such as: (i) coffee seedlings for insect infestation need to be maintained in sunny places under greenhouse environment, with considerable expenditure of money and labor; (ii) coffee seedlings need large areas and provide few leaves; (iii) new leaves produced under these conditions are small, what can be an inconvenience, because the viability of the coffee leafminer's caterpillars are much greater in older leaves (Walker & Quintana 1969), and the capacity of caterpillars maintained by these little leaves decreases (Bigger 1969); and (iv) coffee seedlings are more frequently under attack by fungi and insects, like coccids and aphids, demanding larger maintenance effort and causing decrease in production.

After the penetration into a leaf, the leafminer caterpillars are not able to move to another leaf to continue feeding. Its fragility practically makes unfeasible its rearing in artificial diet. Therefore, it was explored the possibility of rearing this species, from egg to adult, under laboratory conditions, using detached leaves maintained *in vitro* instead of coffee seedlings. However, coffee leaves do not survive for sufficient time in water to allow the coffee leafminer

cycle to be completed.

This work aimed to develop a methodology for mass rearing the coffee leafminer, optimizing the production of insects and minimizing the inconveniences of the rearing using a methodology for maintenance of green detached coffee leaves for long periods.

Maintenance of Leaves. In order to maintain coffee leaves healthy and green, for a period of time so that the insect could develop its life cycle, two plant growth regulators, benzyladenin and kinetin, in concentrations 10^{-6} and 10^{-7} M were used. These synthetic cytokinins are routinely used for *in vitro* maintenance of plant cell and plant tissues (Jourdain *et al.* 1997 Kreslavsky *et al.* 1997, Zarrabeitia *et al.* 1997).

The experiment was conducted under laboratory conditions (25±1ºC and 70±5% RU), using leaves collected in the field and standardized according to size and age. Large and perfect leaves were placed in a plastic bag and mixed for randomization. Leaf petioles were inserted and fixed on a polyethyline foam, density 28, and 3 cm thickness with eight sections, and placed in germination boxes (Gerbox), measuring 11 x 11 x 3,5cm (width x length x height), containing the solution to be tested. Distilled water was used as control. For each treatment three replication were used, with 25 coffee leaves per box. The experiment lasted 30 days, a period longer than the necessary for the complete development of the coffee leafminer. Test solution level was completed whenever necessary.

Obtaining *L. coffeellum* Colonizers. Initially, adults of *L. coffeellum* were obtained from mined leaves brought to the laboratory to obtain the chrysalides. Collected leaves were placed in Gerbox containing the hormone solution. These Gerbox were placed in an incubator (BOD) at 25ºC, in order to accelerate the development of the insect. A BOD with five shelves allowed keeping around 1800 mined leaves for the formation of the chrysalides. The leaves were observed each two days for the retreat of the chrysalides. The decrease in time of maintenance was not a problem, because the leaves were kept in the plates according to the size of the mine, guaranteeing that, when the leaf was cut for the retreat of the chrysalides, other caterpillars were already in the pupation phase. All chrysalidescollected were taken for the oviposition cages so that the adults that emerged made the postures.

Mass Rearing of Coffee Leafminer. Large and perfect leaves (without any stain or deformity) were collected weekly from the lower part of coffee plants in the field. These leaves were, then, taken to the laboratory, placed in trays with water and washed in order to remove dust and small organisms (eggs, mites, etc.). A new selection eliminated undesirable leaves. After the cleaning, leaves were placed in sectioned foams and again placed in 26 x 41cm trays, containing the hormone solution. This tray was maintained in a separate room apart from the insects. Leaves that, for any reason showed problems (yellowness or diseases), were immediately discarded.

For oviposition, six to 10 leaves were put in a Gerbox, with the hormone solution. These boxes were placed in cages with the chrysalides prepared for the emergence. The cages were 30 x 30 x 30cm, made of wood and covered with a screen of white organza. The front of the cages was covered with the same organza preached in the upper part and closed with Velcro in the inferior and lateral parts, to facilitate the maintenance. Insects were fed with a 10% sucrose solution, because it significantly increases adults' longevity, number and viability of the eggs produced (Nantes & Parra 1978). For feeding, cut foams soaked in the solution of sucrose 10% were placed in the upper part of the cage, on the outside. The contact of the foam with the screen allowed the insects to feed. These foams were replaced daily to avoid food fermentation. Leaves with postures were

collected according to the adult density in the cage. In cages with many individuals, leaves were inspected daily. In cages with smaller number of insects, every two or three days. A 10X glass magnifying with a mobile arm was used to examine the postures. Leaves with eggs were transferred to another Gerbox and kept in a BOD until the formation of the chrysalides. The volume of the solution in each Gerbox was observed and the level completed whenever necessary. Each leaf with eggs retreat was replaced by a new leaf.

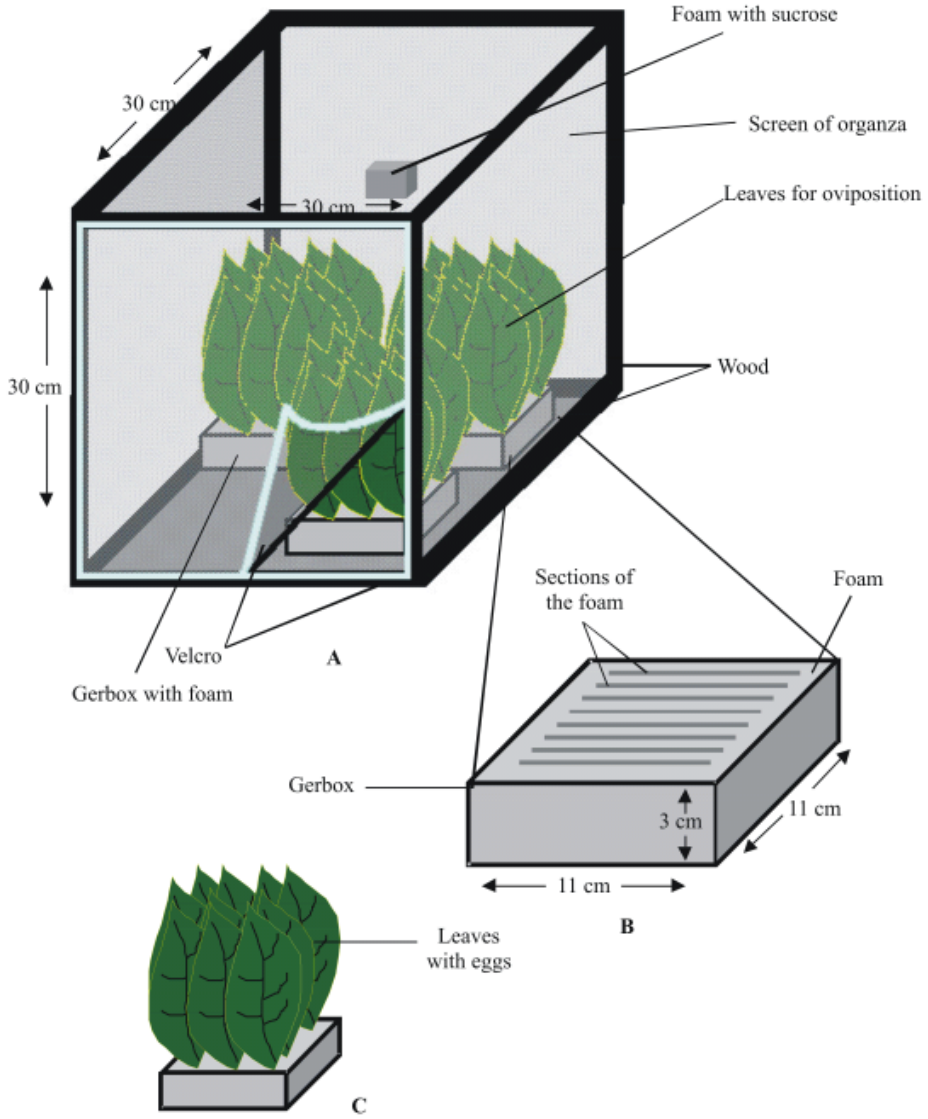

Figure 1. Schematic representation of the rearing system. A) cage for oviposition; B) Gerbox with foam in detail; C) leaves with eggs retired of the cage for oviposition.

The whole system can be visualized in Fig. 1.

Both artificial cytokinines indeed increased the lifetime of the coffee leaves, maintaining them green and healthy (Fig. 2). Leaves harvested from field trees and maintained in the regulator solutions survived enough time for *L. coffeellum* to complete its cycle in good conditions. Leaves placed in the cages for oviposition were attractive to the insect, with significant number of eggs per leaf. These eggs resulted in individuals which completed the whole developmental cycle, without apparent damage to their biology. Diseases or an exaggerate number of eggs per leaf resulted in the interruption of the cycle of the insect, because the leaf deteriorates very fast. It is very important to maintain the leaves with eggs in separated room from the leafminer adults collected in the field, in order to avoid contact with parasitoids that can emerge from them.

Mass rearing of the coffee leafminer using coffee leaves *in vitro* is feasible as shown here and the efficiency of this method will be proven following complete studies of the biology of the insect in these conditions. Tests with regulator in different concentrations with healthy leaves showed efficiency; however, we believe that hormone concentrations to be used with mined leaves should be larger, because these when maintained at 10^{-7} *M* leaves did not present a satisfactory lifetime. Therefore, tests with mined leaves with different hormone concentrations should be made to find out the ideal concentration for leaf survival. In

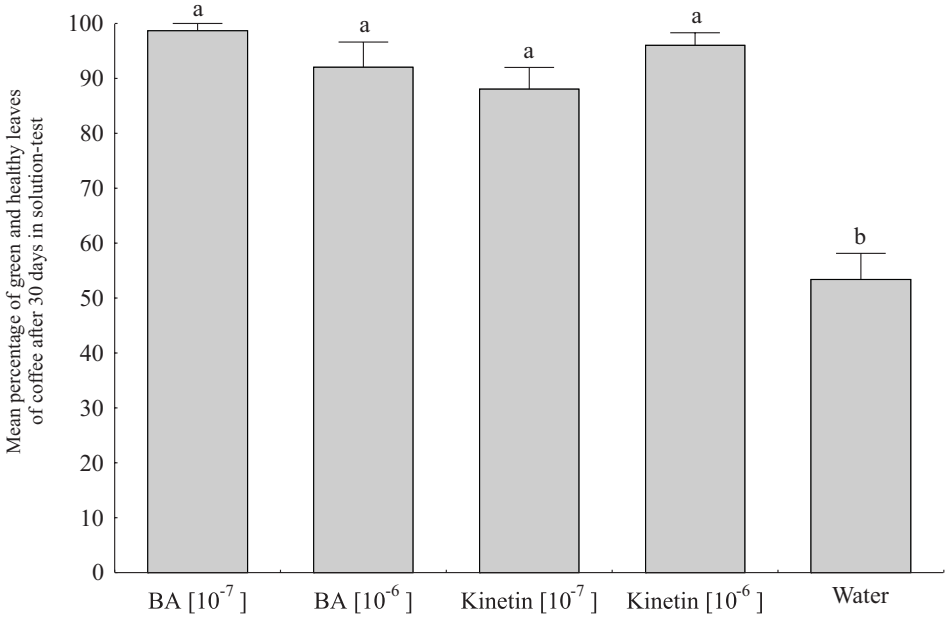

Figure 2. Maintenance of green and healthy coffee leaves in plant hormone solution. Each treatment consisted of 75 leaves, with three replications. ANOVA $F_{(4,10)} = 25{,}29$; $P < 0{,}01$. Treatments followed by the same letter do not statistically differ from each other by the Tukey's test at 1% level.

our laboratory we use 10^{-6} *M* benzyladenin for the maintenance of mined leaves.

The following advantages over the method of mass rearing in coffee seedlings are: (i) the leaves can be collected and maintained in laboratory until its use, without the requirement of solar light or special facilities; (ii) the leaves do not need to be specially produced for this aim, they can simply be collected directly from field trees; (iii) the leaves occupy a very small area, and can be placed in any cage, BOD or small rooms; (iv) as leaves are collected in the field, large and mature leaves can be chosen. These leaves are capable of supporting large amount of eggs, are preferred for oviposition and provide high viability of offspring (Bigger 1969, Walker & Quintana 1969); (v) control of diseases and undesirable pests in detached leaves are much easier of being accomplished, therefore more resistant leaves can be used; (vi) as they can be maintained in small rooms, they can be divided in small units, decreasing the risk of total losses. This method can supply a great quantity of insects per BOD, as each Gerbox can support up to 24 leaves and a BOD can support up to 75 Gerbox units, producing a minimum of 1800 chrysalides per BOD.

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