BioAssay

Received: 10/V/2006
Accepted: 16/IX/2006
Published: 04/X/2006
Available online at: www.bioassay.org.br/articles/1.8

SEMIOCHEMICALS

Efficacy of Mating Disruption for Control of the Coffee Leaf Miner *Leucoptera coffeella* (Guérin-Méneville) (Lepidoptera: Lyonetiidae)

BIANCA G. AMBROGI[1], ERALDO R. LIMA[1] E LEANDRO SOUSA-SOUTO[1]

[1] *Universidade Federal de Viçosa, Departamento de Biologia Animal, Viçosa – MG, Brasil.*
Email: bianca@quimica.ufpr.br

BioAssay 1:8 (2006)

Eficácia da Confusão Sexual de Machos no Controle do Bicho-mineiro do Café *Leucoptera coffeella* (Guérin-Méneville) (Lepidoptera: Lyonetiidae)

RESUMO – O bicho-mineiro do café *Leucoptera coffeella* (Guérin-Méneville) (Lepidoptera: Lyonetiidae) é considerado atualmente a principal praga desta cultura no Brasil. O controle por meio de inseticidas tem sido o mais utilizado, causando problemas para o homem e o meio ambiente. Para amenizar estes problemas, têm-se desenvolvido novas técnicas de manejo de pragas. A técnica denominada confusão sexual de machos objetiva interferir na comunicação entre os parceiros sexuais. A viabilidade desta técnica foi avaliada em uma lavoura de café onde foram instaladas três unidades experimentais de 20 ha, sendo uma área tratada com feromônio sexual sintético, outra com aplicações de inseticidas e por fim uma área controle. Como agente de confusão sexual de machos foi utilizada a mistura racêmica de 5,9-dimetilpentadecano, na concentração de 1 g do feromônio por liberador. Para liberação do feromônio no campo foram utilizados 20 liberadores por hectare. A eficiência desta técnica foi avaliada por meio da comparação de machos capturados em armadilhas tipo delta (20 armadilhas por unidade experimental) contendo 0,5 mg de 5,9-dimetilpentadecano, em septos de borracha, entre as unidades experimentais. O número de folhas minadas também foi avaliado. A análise dos resultados permite concluir que a presença do feromônio não diminuiu o número de machos capturados, bem como não reduziu o número de folhas minadas. Diversos fatores podem ter contribuído para o insucesso na interrupção do acasalamento dessa espécie, como diferenças na composição química, dosagem ou na formulação empregada do feromônio dos liberadores, o momento de aplicação na lavoura, densidade populacional da praga no início do experimento e fatores climáticos.

PALAVRAS-CHAVE – feromônio sexual, manejo integrado de pragas, comunicação química

ABSTRACT – The coffee leaf miner, *Leucoptera coffeella* (Guérin-Méneville) (Lepidoptera: Lyonetiidae), is the main pest of coffee plantations in Brazil. Indiscriminate chemical control has been used frequently to control the attack of *L. coffeella*, causing serious problems to the environment. To avoid such problems, new techniques have been developed to control the attack of this pest. A technique to control lepidopteran pests, called mating disruption, aims to obstruct the communication among sexual partners. The potential of pheromone-mediated mating disruption for control of leaf miner population was evaluated in a coffee plantation in Patrocínio-MG, Brazil. Three experimental areas were installed: 20 ha plot treated with synthetic sex pheromone; another 20 ha plot with insecticide applications and 20 ha plot maintained as control. The pheromone plot was treated with 400 pheromone dispensers with 1g of 5,9-dimethylpentadecane per dispenser. The efficacy of mating disruption was evaluated by the comparison of number of males caught in delta traps (20 traps per plot) baited with 0.5 mg of 5,9 -dimethylpentadecane. The number of mined leaves was also recorded in each plot. The presence of pheromone did not reduce the number of males caught nor decreased the number of mined leaves in the plot. The failure of the mating disruption technique may be attributed to a combination of several factors, such as composition and dose of the pheromone and its formulation, the moment of application in the crop, the population density at the begin of the experiment and climatic factors.

KEY WORDS – sex pheromone, integrated pest management, chemical communication

The coffee leaf miner *Leucoptera coffeella* (Guérin-Méneville) (Lepidoptera: Lyonetiidae) is the main pest of coffee in Brazil, due to continuous presence in the crop and the economic damage to the culture (Reis & Souza 1996). Their larvae foraging inside the leaf, forming galleries (mines) that lead leaves to fall prematurely, reducing the photosynthetic capacity of the plant and causing severe damage to coffee crops, with losses that may reach 50% of the total production (Reis & Souza 1996).

Chemical control is the most used method to prevent the attack of this pest. Therefore, the applications of insecticides are increasing, leading to environmental pollution, higher production costs, reducing natural enemies and causing insecticide resistance (Guedes & Oliveira 2002, Fragoso *et al.* 2002, 2003). To avoid such problems, new techniques are being developed currently to control the attack of this pest.

The main sexual pheromone of *L. coffeella* was identified as 5,9-dimethylpentadecane, (Francke *et al.* 1988) and its efficiency was confirmed through the mass capture of males using delta traps baited with 0.5 mg of this pheromone in a coffee crop (Lima 2001). This formulation (0.5 mg of the main compound) was established as the best combination to capture males during a period of 30 days (Lima 2001). Sex pheromones have been used to control several lepidopteran pests on different crops through mating disruption technique (Cardé & Minks 1995a). This is obtained with the release of high doses of synthetic pheromone, saturating the atmosphere, thereby decreasing the ability of mates to locate each other, reducing the mating, decreasing egg deposition and consequently dropping the new generation. (Agosta 1990, Cardé & Minks 1995b). Some successful examples are the control of *Pectinophora gossypiella* (Lepidoptera: Gelechiidae), one important pest in cotton (Cardé & Minks 1995a); this technique is also an important component of pest management in Australia, to control *Grapholita molesta* (Lepidoptera: Tortricidae) (Il'ichev *et al.* 2004), the major pest in commercial crops of peaches and nectarines. Also, there are some cases where the mating disruption has been failure. For example, *Lobesia botrana* (Lepidoptera: Tortricidae) in Sardinian vineyards (Nannini & Delrio 1993) and *Tuta absoluta* (Lepidoptera: Gelechiidae), in Brazilian tomato crops (Michereff Filho *et al.* 2000). In this paper, we report the results of initial field studies to evaluate the effectiveness of mating disruption technique to control coffee leaf miner in a commercial coffee plantation in Brazil.

Material and Methods

Location and characteristics of experimental areas. This study was carried out between September and November 2003, at the farm of Daterra Atividades Rurais Ltda, in Patrocínio, Minas Gerais State, Brazil (18º17' S, 46º59' N) and altitude of 870 m. The region presents annual average temperature of 21^{o}C, 45% of relative humidity in the dry season (May to September) and 70% RH in wet season (October to April); annual average precipitation of 1,500 mm concentrated mainly from November to March.

The period when the experiment was done was characterized by monthly average temperature of 24^{o}C and mean rainfall between 42 mm in September, mean rainfall of 216 mm and average temperature of 24ºC in November. The coffee variety used in the experimental area was Mundo Novo spacing of 4.0 × 1.20 m, with 29 years old, with approximately 4,170 plants/ha.

Experimental Design. The experiment was done in three plots of 20 ha, with a minimum distance of 300 m from each other. The treatments were: (i) 20 ha treated with pheromone; (ii) 20 ha with insecticide applications and (iii) 20 ha maintained as control. The plot treated with insecticide was sprayed with cartap (800 g a.i./ha) on August 10 and September 23, using a tractor-mounted sprayer. This insecticide has a residual time of approximately 40 days.

These three plots were monitored a week before the application of synthetic pheromone for mating disruption, to verify the pest population density and the quantity of mined leaves.

Pheromone application. The application of synthetic sex pheromone started in September 2003. The pheromone was released using 400 dispensers with 1g of sex pheromone (racemic mixture of 5,9-dimethylpentadecane, synthesized by ChemTica Co., Costa Rica) per dispenser. The dispensers were applied according to recommendations at 20 dispensers/ha, which were placed in the lower third of the coffee plants being one dispenser for 100 plants, at a minimum distance of 12 m far from each other.

Evaluation of the mating disruption efficacy.
(a) Field trapping - The efficacy of the mating disruption was verified by comparing male captures in pheromone-baited traps (delta) placed in the three plots at a rate of 20 traps per plot. These traps were placed in the plots seven days prior to pheromone application to check the initial number of males in each area. Each trap was baited with a lure loaded with 0.5 mg of 5,9-dimethylpentadecane and was placed at 5 cm from the soil between the rows of plants. After pheromone release the number of males caught by the traps was checked once per week during 7 weeks in the 3 plots. The pheromone lures were replaced every 3 weeks according to Lima (2001) and the sticky bottoms were replaced when it was needed.
(b) Evaluation of mined leaves. Another way to measure the efficacy of mating disruption was by verifying the number of mined leaves in the plots. In each plot, 144 coffee plants were randomly sampled to check the damage caused by *L. coffeella.* The sampling was made from the edge to the middle of the plot. Mined leaves were collected during one minute from these chosen plants and, after sampling, these leaves

were counted and the mean number of mined leaves/plot was assessed. This sampling was done once per week, simultaneously with the number of males caught in traps evaluation. At each week 144 different plants were chosen for sampling, totalizing 1,008 sampled plants per plot.

Statistical analysis. All analyses were performed using ANOVA with generalized linear models with R statistical package (R Development Core Team 2005), using analysis of residues to check for the suitability of error distribution and for model adjustment (Crawley 2002). To avoid pseudoreplication in nested designs, mixed effect models were used in data transformed to either Poisson or binomial errors (Crawley 2002).

Results

There was no significant difference among treatments in relation to the mean number of males caught in the traps on the week before the placement of pheromone dispensers (F = 0.0835; d.f. = 2, 57; P = 0.92), indicating that the quantity of males inside the 3 plots was the same prior pheromone releasing.

The number of mined leaves on the week before the placement of dispensers was significantly different among treatments (F = 156.21; d.f. = 2, 24; P < 0.0001). In this previous sampling, the number of mined leaves in the plot submitted to insecticide control was lower than other plots (Fig. 1). After the experiment, this trend was maintained (F = 323.90; d.f. = 2, 14; P < 0.0001) with the smallest number of mined leaves in the insecticide-plot although there was a small increase in another two plots (Fig. 1). During the experiment the mean number of mined leaves sampled weekly did not differ during the sampling period in all plots. For this reason, the data of mined leaves sampled weekly are presented together (Fig. 1). The number of mined leaves collected in plants localized in the edge and at the middle of the pheromone-treated area did not differ (F = 0.057; d.f. = 1, 7; P = 0.8180). Similar results were found for the other two plots.

However, it was found a significant difference on the number of males caught among treatments. The insecticide-plot presented the lowest values whereas the number of males in the pheromone-treated plot was higher compared to the other plots (F = 116.03; d.f. = 2, 57; P < 0.0001) (Fig. 2).

Discussion

The three areas presented the same number of males caught in traps before the pheromone application at the mating disruption area. This result indicates that the population levels inside the plots were similar at the beginning of the experiment. However, the number of mined leaves was different among treatments. The plot treated with insecticide presented lower number of mined leaves since the beginning of the experiment.

The presence of higher number of males caught in traps during the experiment in the mating disruption area, in contrast to the two other plots, suggests that there was not disruption of communication between the sexual partners. The fact that high number of males was caught in the mating disruption area may indicate that the pheromone formulation had lower concentration than necessary for mate disruption. In this case, the low concentration could act as an attractive, similar to those formulations used for monitoring and mass trapping.

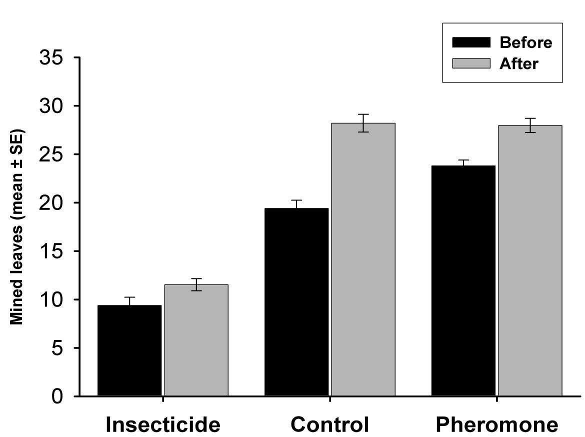

Figure 1. Number of mined leaves (mean ± SE) by *Leucoptera coffeella* one week before the pheromone liberation and during seven weeks after (values grouped) (n = 37). Insecticide – conventional insecticide control; Control – untreated area; Pheromone – saturated atmosphere with 20 g/ha of synthetic sex pheromone (5,9-dimethylpentadecane).

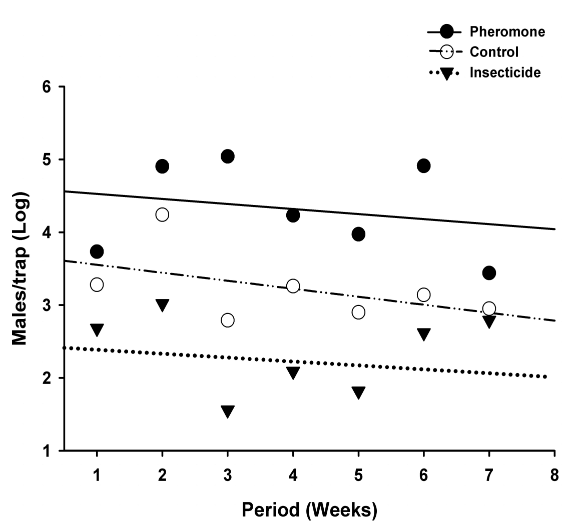

Figure 2. Mean number of *Leucoptera coffeella* males caught (log + 1) in monitoring traps during 7 weeks of experiment (n = 20). ● = Treated area with mating disruption, ○ = Control area, ▼= Insecticide area. (F = 116.03; gl = 2, 57; P < 0.0001).

Similar results were observed in a mating disruption study done for *Cydia pomonella* (Lepidoptera: Tortricidae), important pest in apple (Witzgall *et al.* 1997). In this study, these authors verified that, after the application of 400 and 1,000 dispensers/ha with 250 mg of the main sex pheromone component (*E*8,*E*10-12OH; codlemone) in plots with 2 to 15 ha, males of *C. pomonella* were attracted upwind to codlemone treatments from nearby untreated orchards, over at least 50 m. High number of males of *Lymantria dispar* (Lepidoptera: Lymantriidae), in oak areas treated with sex pheromone in comparison to control plots was also verified (Schwalbe & Mastro 1988).

The efficacy of mating disruption, however, is not a guarantee of reduction of plant damage. For *T. absoluta*, the releasing of 30-50 g/ha of its sex pheromone 3*E*,8*Z*,11*Z*-14:Ac in a tomato crop was efficient to interrupt male orientation (60-90%) but the number of mined leaflets or bored fruits was similar to control plots (Michereff Filho *et al.* 2000).

In the present study, the similarity in the number of mined leaves between the pheromone and control plots demonstrates that application of synthetic sex pheromone did not reduce the attack of this insect on the crop (Fig. 1). There was a lower number of mined leaves both before and after the pheromone application only in the plot treated with conventional insecticides. It is probably due to a residual effect of the insecticides applied some days before the week of experimental monitoring.

Failure in mating disruption for other moth pests has also been attributed to migration of mated females within the areas treated with sex pheromone (Sanders 1989). In the present study, female migration was tested through a scheme of sampling from the edge to the middle of the plot. This scheme allows verifying the female entrance in the treated area. The fact that attack intensity was similar throughout the plot treated with sex pheromone suggests that the increase in the number of mined leaves was not due to mated female immigration of untreated adjacent areas. Instead, a similar number of mined leaves in all area treated with pheromone, suggests a homogenous distribution of the insect. Moreover, the size of the plot treated with pheromone in the present work was large (20 ha), and the treatment of larger areas could prevent female immigration (Albajes *et al.* 2002).

The efficacy of mating disruption is related with the initial population level of the pest as the optimal moment of pheromone application (Cardé & Minks 1995a, Michereff Filho *et al.* 2000). Most studies with mating disruption pointed out that the pheromone should be applied strategically on the first generation of the target-specie, which are normally more susceptible to the control. High population densities may favor mating through increase in competition among calling females and synthetic pheromone dispensers, by reducing the distance among adults, increasing the likelihood of casual mating and consequently, less time spent searching for females (Molinari & Cravedi 1990). Application of mating disruption for *L. dispar* did not reduce mating under high-density conditions. On the other hand, the experiments that were done under low moth density conditions proved that this technique can reduce substantially this pest population (Leonhardt *et al.* 1996). These factors must be considerate in this study. Owing to operational problems, the pheromone application was done late, when *L. coffeella* adults were established in the crop with high number of both moths and caterpillars on the foliage. The appearance of the first *L. coffeella* adults in coffee plantations would be the best moment to apply the sex pheromone.

Climate is another important factor that interferes on the density of pest attack at the crops. The biggest coffee leaf miner infestations can be observed under dry weather conditions (Reis & Souza 1996). It means that a prolonged dry period increases this pest damage level and consequently increase leaves fall, which can be a physiologic strategy of the plant for water economy. As pheromone dispensers were applied in the field at the late dry season, the insect population would be high due to low humidity. In addition, high temperatures make the life cycle of coffee leaf miner short (Reis & Souza 1996), consequently increasing the number of generations. These climatic conditions can also affect the pheromone release rate and its dispersion in the environment, leading to areas with low pheromone concentration and heterogeneous distribution through the crop, thus favoring mating (Flint *et al.* 1993).

In this study, the use of mating disruption method for control of *L. coffeella* did not reduce the number of males caught in monitoring traps nor decreased the number of mined leaves in comparison with the control plot or insecticide plot. In this context the failure of this technique may be attributed to a combination of several factors, such as differences on composition or dose of the pheromone used in the dispensers and its formulation; the moment of application in the crop, the population density, the mating strategy of the pest, and climatic factors.

In spite of the main compound of the coffee leaf miner sex pheromone, 5,9-dimethylpentadecane, has been efficient for insect monitoring, its application as mating disruptive was not confirmed. Further studies considering the factors cited above are needed to confirm the efficacy of this method.

Acknowledgements

We thank DATERRA farm for providing access to their fields for the experiments. We also thank to Miryan Coracini, Og de Souza, Ronaldo Reis, José Maurício Bento and José H. Schoereder for their valuable suggestions. This research was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil), PNP&D-Café – Programa Nacional de Pesquisa e Desenvolvimento do Café – and Bio Controle - Métodos de Controle de Pragas LTDA.

References

Agosta, W.C. 1990. (ed.) Chemical communication: the language of pheromones. Scientific American Library, New York, 179p.

Albajes, R., M. Konstantopoulou, O. Etchepare, M. Eizaguirre, B. Frérot, A. Sans, F. Krokos, A. Améline & B. Mazomenos. 2002. Mating disruption of the corn borer *Sesamia nonagrioides* (Lepidoptera: Noctuidae) using sprayable formulations of pheromone. Crop Prot. 21: 217-225.

Cardé, R.T. & A.K. Minks. 1995a. Control of moth pests by mating disruption: successes and constrains. Annu. Rev. Entomol. 40: 559-585.

Cardé, R.T. & A.K. Minks. 1995b. Insect pheromone research: new directions. Chapman and Hall, New York, 684p.

Crawley, M.J. 2002. (ed.) Statistical Computing: An Introduction to Data Analysis using S-Plus. John Wiley and Sons, LTD, 761p.

Flint, H.M., A.K. Yamamoto, N.J. Parks & K. Nyomura. 1993. Aerial concentrations of gossyplure, the sex pheromone of the pink bollworm (Lepidoptera: Gelechiidae), within and above cotton fields treated with long-lasting dispensers. Environ. Entomol. 22: 43-48.

Fragoso, D.B., R.N.C. Guedes & J.A. Ladeira. 2003. Seleção na evolução de resistência a organofosforados em *Leucoptera coffeella* (Guérin-Mèneville) (Lepidoptera: Lyonetiidae). Neotrop. Entomol. 32: 329-334.

Fragoso, D.B., R.N.C. Guedes, M.C. Picanço & L. Zambolim. 2002. Insecticide use and organophosphate resistance in the coffee leaf miner *Leucoptera coffeella* (Lepidoptera: Lyonetidae). Bull. Entomol. Res. 92: 203-212.

Francke, W., M. Tóth, G. Szocs, W. Krieg, H. Ernest & E. Buschmanm. 1988. Identifizierung and Synthese von Dimethylalkanen als Sexuallockstoffe weiblicher Miniermotten (Lyonetiidae). Zeitschrift fur Naturforschung 43: 787-789.

Guedes, R.N.C. & E.E. Oliveira. 2002. Resistência a inseticidas-pragas do cafeeiro: Situação e perspectivas, p. 471-497. In L. Zambolim. (ed.), O estado da arte de tecnologias na produção de café, Viçosa, UFV, 568p.

Il'ichev, A.L., D.G. Willians & A.D. Milner. 2004. Mating disruption barriers in pome fruit for improved control of oriental fruit moth *Grapholita molest*a Busck (Lep., Tortricidae) in stone fruit under mating disruption. J. Appl. Entomol. 128: 126-132.

Leonhardt, B.A., V.C. Mastro, D.S. Leonard, W. McLane, R.C. Reardon and K.W. Thorpe. 1996. Control of low-density gypsy moth (Lepidoptera: Lymantriidae) populations by mating disruption with pheromone. J. Chem. Ecol. 22: 1255-1272.

Lima, E.R. 2001. Feromônio sexual do bicho-mineiro do café *Leucoptera coffeella:* Avaliação para uso em programas de manejo integrado. Tese de Doutorado, UFV, Viçosa, 71p.

Michereff Filho, M., E.F. Vilela, G.N. Jham, A. Attygalle, A. Svatos & J. Meinwald. 2000. Initial studies of mating disruption of the tomato moth, *Tuta absoluta* (Lepidoptera: Gelechiidae) using synthetic sex pheromone. J. Braz. Chem. Soc. 11: 621-628.

Molinari, F. & P. Cravedi. 1990. Application of pheromones in the control of *Cydia molesta* (Busck) (Lepidoptera, Tortricidae) by mating disruption in Piedmont. Redia. 73: 381-395.

Nannini, M. & G. Delrio. 1993. Experiments on mating disruption of grape vine moth, *Lobesia botrana* in Sardinian vineyards. IOBC/WPRS Bulletin 16: 163-168.

R Development Core Team. 2005. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

Reis, P.R. & J.C. Souza. 1996. Manejo integrado do bicho-mineiro *Perileucoptera coffeella* (Guérin-Menevelli, 1842) (Lepidoptera: Lyonetiidae), e seus reflexos na produção de café. An. Soc. Entomol. Brasil 25: 77-82.

Sanders, C.J. 1989. The further understanding of pheromones: Biological and chemical research for the future. p. 325-351. In A.R. Jutsum & Gordon R.F.S. (eds.), Insect pheromone in plant protection. New York, John Wiley & Sons, 369p.

Schwalbe, C.P. & V.C. Mastro. 1988. Gypsy moth mating disruption: dosage effects. J. Chem. Ecol. 14: 581-588.

Witzgall, P., C.R. Unelius, F. Rama, J-P. Chambon & M. Bengtsson. 1997. Mating disruption of pea moth, *Cydia nigricana*, and codling moth, *C. pomonella*, using blends of sex pheromone and attraction antagonists. IOBC/WPRS Bulletin 20: 207-215.