MAYARA CRISTINA LOPES

DECISION-MAKING SYSTEM AND NATURAL FACTORS DETERMINING THE INTENSITY OF *Liriomyza huidobrensis* ATTACK TO TOMATO CROPS

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fitotecnia, para obtenção do título de *Doctor Scientiae.*

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BIOGRAFIA

MAYARA CRISTINA LOPES, filha de Wilson Antônio Lopes e Maria Célia de Souza Pimentel Lopes, nasceu em Viçosa, Minas Gerais, no dia 10 de janeiro de 1989.

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SUMMARY

RESUMO

LOPES, Mayara Cristina, D.Sc., Universidade Federal de Viçosa, fevereiro de 2019. **Sistemas de tomada de decisão e fatores naturais determinantes da intensidade de ataque de *Liriomyza huidobrensis* em lavouras de tomate.** Orientador: Marcelo Coutinho Picanço.

A avaliação dos agroecossistemas e dos sistemas de tomada de decisão são essenciais em programas de manejo integrado de pragas. *Liriomyza huidobrensis* (Diptera: Agromyzidae) é uma importante praga do tomate. Apesar da importância desta praga*,* até o momento não existem pesquisas sobre a sua dinâmica espaço temporal e sobre sistemas de tomada de decisão em cultivos de tomate. Assim, os objetivos desta pesquisa foram determinar: (i) os fatores que regulam a população de *L. huidobrensis* em cultivos de tomate, (ii) plano de amostragem convencional, (iii) nível de dano econômico (NDE) e (iv) plano de amostragem sequencial para *L. huidobrensis* em cultivos de tomate. Os principais fatores que regularam as populações da praga foram os parasitóides *Diglyphus* sp. (Hymenoptera: Eulophidae) e *Opius* sp. (Hymenoptera: Braconidae). A folha mais basal do terço mediano do dossel da planta foi a amostra ideal para a avaliação de *L. huidobrensis*. As densidades de *L. huidobrensis* ajustaram a distribuição binomial negativa e tiveram parâmetro de agregação comum (K_{commom} = 0,7289). O plano de amostragem convencional foi composto por 73 amostras por talhão. Este plano de amostragem foi realizado em 47min, 1h:09 min e 1h:25 min em talhões de 1, 5 e 10 hectares, respectivamente. O custo do plano de amostragem foi de US $1,74, 2,54 e 3,12 para talhões de 1, 5 e 10 ha, respectivamente. O NDE foi de 3,24 larvas por folha. O plano de amostragem sequencial tomou decisões corretas em 100% das situações, com economia de 87% do tempo e custo de amostragem. Portanto, os programas de manejo integrado de *L. huidobrensis* devem ter como objetivo preservar as populações de *Diglyphus* sp. e *Opius* sp. que são importantes na regulação populacional desta praga. A amostra ideal para a avaliação de *L. huidobrensis* é a folha mais basal do terço mediano do tomate. O plano de amostragem convencional é composto de 73 amostras e ele é preciso, rápido, representativo e barato. O NDE para *L. huidobrensis* em cultivos de tomate é de 3,24 larvas por folha. O plano de amostragem sequencial é adequado para ser usado em programas de manejo integrado de *L. huidobrensis* em cultivos de tomate por tomar decisões corretas com economia de tempo e custo.

ABSTRACT

LOPES, Mayara Cristina, D.Sc., Universidade Federal de Viçosa, February, 2019. **Decision-making system and natural factors determining the intensity of *Liriomyza huidobrensis* attack to tomato crops.** Adviser: Marcelo Coutinho Picanço.

The evaluation of agroecosystems and decision-making systems are essential in integrated pest management programs. *Liriomyza huidobrensis* (Diptera: Agromyzidae) is one of the major pests of tomato crops. Despite the importance of this pest, to date, there is no research on its spatiotemporal dynamics and on decision-making systems in tomato crops. Thus, the aims of this study were to determine: (i) the natural factors that regulate *L. huidobrensis* populations in tomato crops, (ii) conventional sampling plan, (iii) economic injury level (EIL) and (iv) sequential sampling plan for *L. huidobrensis* in tomato crops. The main natural enemies of *L. huidobrensis* were the parasitoids *Diglyphus* sp. (Hymenoptera: Eulophidae) and *Opius* sp. (Hymenoptera: Braconidae). The basal leaf of the middle section of the plant canopy was the best plant part for sampling. Leafminer densities were fitted to the negative binomial distribution with a common aggregation parameter (Kcommon = 0.7289). The sampling plan consists of 73 samples per field, irrespective of field size (1, 5 or 10 ha). Evaluations using this sampling plan were performed in 47 min, 1 h 9 min and 1 h 25 min with a cost of US $1.74, 2.54 and 3.12 per sampling in fields of 1, 5 and 10 ha, respectively. The EIL was 3.24 larvae per leaf. The sequential sampling plans made correct decisions in 100% of the situations, with sampling time and cost savings of 87%. Therefore, integrated management programs for *L. huidobrensis* should aim to preserve populations of *Diglyphus* sp. and *Opius* sp., which are important in the population regulation of this pest. The ideal sample for the evaluation of *L. huidobrensis* is the most basal leaf of the middle section of the tomato. The conventional sampling plan consists of 73 samples and is accurate, fast, representative and inexpensive. The EIL for *L. huidobrensis* in tomato crops is 3.24 larvae per leaf. The sequential *L. huidobrensis* sampling plan is suitable for use in integrated management programs in tomato crops for making correct decisions with time and costs savings.

INTRODUÇÃO GERAL

Os programas de manejo integrado de pragas (MIP) tem por objetivo preservar ou incrementar os fatores de mortalidade natural da praga, pelo uso dos métodos de controle de forma compatível. Nestes programas os métodos de controle são selecionados com base em parâmetros técnicos, econômicos, ecológicos e sociológicos. Os componentes dos programas de MIP são a avaliação do agroecossistema, tomada de decisão, estratégias e táticas de controle (Pedigo & Rice 2006). Na avaliação do agroecossistema são determinados os fatores que regulam a dinâmica populacional das pragas. Entre os principais fatores que regulam as populações de insetos estão as características da planta hospedeira, elementos climáticos e inimigos naturais (Power, 1992; Pereira et al., 2007; Silva et al., 2017).

A planta hospedeira pode afetar os insetos herbívoros de forma direta ou indireta (Rosado et al. 2015). Diretamente ela pode afetar o desenvolvimento, sobrevivência e reprodução dos herbívoros devido a sua qualidade nutricional, barreiras físicas e produção de compostos tóxicos (Power 1992; Awmack & Leather 2002; Johnson et al. 2016). Já o efeito indireto das plantas sobre os insetos herbívoros está relacionado a sua influência sobre os inimigos naturais (Johnson et al. 2016). Os principais elementos climáticos que afetam as populações de insetos pragas são temperatura, precipitação pluviométrica, velocidade dos ventos e fotoperíodo (Bacca et al. 2006; Rosado et al. 2015; Johnson et al. 2016). Já os inimigos naturais podem causar alta mortalidade às populações de pragas influenciando o tamanho de suas populações (Semeão et al. 2012; da Silva et al. 2017).

Os sistemas de tomada de decisão são compostos por planos de amostragem e índices de tomada de decisão (Pedigo & Rice 2014; Lima et al.

2018). Os planos de amostragem podem ser convencionais ou sequenciais (Pinto et al. 2017).

Os planos de amostragem convencionais são compostos por um número fixo de amostras (Pinto et al. 2017). Esses planos são o ponto de partida para a geração de sistemas de tomada de decisão. Os planos de amostragem convencionais são usados para determinar os níveis de dano econômico e para validar os planos de amostragem sequenciais (Bacci et al. 2008; Rosado et al. 2014). Nesses planos a unidade amostral, técnica de amostragem, distribuição de frequência das densidades da praga e o número de amostras por área são determinados (Rosado et al. 2014; Pinto et al. 2017; Lima et al. 2018). Para que um plano de amostragem convencional seja usado em cultivos agrícolas ele deve ser representativo, preciso, rápido, de baixo custo e praticável (Rosado et al. 2014; Pinto et al. 2017; Lima et al. 2018).

Já os planos de amostragem sequenciais são compostos por um número variável de amostras (Pereira et al. 2016, Pinto et al. 2017). Além disto, geralmente planos sequenciais necessitam de um menor número de amostras, o que faz com que eles sejam mais rápido e de menor custo do que os planos convencionais (Wald 1945; Severtson et al. 2016; Moura et al. 2017). Para que os planos sequenciais sejam usados pelos agricultores é necessário que eles sejam validados. Esta validação pode ser de duas formas. A primeira é feita por simulação e nelas são geradas as curvas operacional (que indica a probabilidade de não controlar a praga) e do número de amostras necessárias para se tomar decisão em cada densidade da praga. Já na segunda validação as decisões e o tempo de amostragem do plano sequencial são comparadas com o plano convencional pela amostragem da praga em lavouras comerciais. O plano de amostragem sequencial só deve ser usado quando ele tomar decisões corretas (iguais ao plano convencional) e reduzir o tempo e o custo de amostragem (Cocco et al. 2015, Pereira et al. 2016, Moura et al. 2017).

Dentre os índices de tomada de decisão o nível de dano econômico é o mais adotado. O nível de dano econômico corresponde a menor densidade populacional da praga, em que medidas de controle devem ser adotadas para que não haja danos econômicos aos cultivos (Stern et al. 1959; Pedigo & Rice 2014; Alves et al. 2017). Na tomada de decisão, a densidade populacional da praga é comparada com o nível de dano econômico. Quando a densidade da praga é igual ou superior a este nível deve-se tomar a decisão de controle da praga (Pedigo et al. 1986, Gusmão et al. 2006). O nível de dano econômico é influenciado pelo preço dos insumos, preço do produto agrícola, rendimento da cultura, eficiência de controle, capacidade da praga de danificar a cultura e a suscetibilidade da cultura a praga (Higley & Pedigo 1996; Wang & Shipp 2001; Moura et al. 2017).

As moscas minadoras do gênero *Liriomyza* (Diptera: Agromyzidae) constituem importantes pragas agrícolas. Este gênero de insetos possui cerca de 300 espécies distribuídas mundialmente, incluindo 23 espécies que são pragas importantes em vários cultivos (Burgio et al. 2005; Alves et al. 2014). A identificação das espécies de moscas minadoras pode ser realizada usando características morfológicas ou por marcadores moleculares. A identificação morfológica das moscas minadoras é feita pela análise da genitália dos machos (Masetti et al. 2006). Entretanto, muitas vezes esta identificação não é possível, visto que, algumas espécies de moscas minadoras são morfologicamente semelhantes, falta taxonomistas capacitados para realizar esta identificação e devido aos insetos estarem danificados. Neste contexto, uma alternativa para a identificação destas espécies é o uso de marcadores moleculares (Parish et al. 2017).

Uma das principais espécies de mosca minadora é *Liriomyza huidobrensis* (Blanchard) (Spencer 1973; López et al. 2010; Alves et al. 2014). Esta espécie possui mais de 365 espécies de plantas hospedeiras dente elas plantas das famílias Asteraceae, Cucurbitaceae, Fabaceae e Solanaceae (Spencer 1973; López et al. 2010; Gao et al. 2012; Alves et al. 2014). *L. huidobrensis* está distribuída nas Américas, África, Ásia e Europa (Araujo et al. 2013; Alves et al. 2014; Weintraub et al. 2017).

Durante o ciclo de vida, *L. huidobrensis* passa pelos estádios de ovo, larva, pupa e adulto. Suas fêmeas perfuram a superfície da folha com o ovipositor confeccionando puncturas. A maioria destas puncturas são usadas para extravasar o conteúdo celular, o qual é usado para sua alimentação. Cerca de 15% destas puncturas são usadas para inserção dos ovos no tecido foliar (Parrella et al. 1985). As fêmeas depositam um ovo por punctura. Ao eclodirem, as larvas confeccionam minas serpenteadas nas folhas e elas passam por quatro instares. No final do último instar, as larvas saem das minas para empupar na superfície das folhas ou solo (Parrella et al. 1983, 1985). A mosca minadora causa danos às plantas principalmente devido à alimentação de suas larvas que confeccionam minas nas folhas. Estas minas reduzem a fotossintesse das plantas e são porta de entrada para patógenos, sobretudo de bactérias (Parrella et al., 1985). Além disto, esta praga em altas infestações, pode provocar queda prematura de folhas, resultando na exposição dos frutos aos raios solares (Zehnder and Trumble 1984).

Apesar da importância de *L. huidobrensis* como praga em cultivos de tomate, até o momento não existem pesquisas sobre a sua dinâmica espaço temporal em regiões tropicais e sobre sistemas de tomada de decisão. Neste sentindo, essa tese foi dividida em três capítulos. No primeiro capítulo foi determinado os fatores naturais que regulam as populações de *L. huidobrensis* em lavouras de tomate. No segundo, foi determinado plano de amostragem convencional para *L. huidobrensis* em lavouras de tomate nos estágios vegetativo e reprodutivo. Já no terceiro foi determinado o nível de dano econômico e plano de amostragem sequencial para *L. huidobrensis* em cultivos de tomate.

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CHAPTER I: PARASITOIDS AS REGULATORS OF RECENT POPULATION OUTBREAKS OF *Liriomyza huidobrensis* IN TOMATO CROPS

ABSTRACT- Studying the natural factors that affect pest populations is important to predict the occurrence of pest outbreaks and to the development of integrated pest management programs. Among these factors, the most important are the natural enemies, climatic elements and the host plant. The tomato crop (*Solanum lycopersicum*) is the second most consumed vegetable in the world. The leafminer *Liriomyza huidobrensis* (Diptera: Agromyzidae) is an important tomato pest in several regions of the world. Thus, the aim of this study was to determine the natural factors that regulate *L. huidobrensis* populations in tomato crops. For this, the densities of *L. huidobrensis* and its natural enemies were evaluated in eight commercial tomato crops during two years. The temperature, photoperiod and rainfall were daily monitored. The intensity of *L. huidobrensis* attack on the eight tomato crops showed a population peak between the middle part at the end of the crop. The main natural enemies of *L. huidobrensis* were the parasitoids *Diglyphus* sp. (Hymenoptera: Eulophidae) and *Opius* sp. (Hymenoptera: Braconidae). Our results suggest that *Diglyphus* sp. and *Opius* sp. are the main determinant factors that influence the population dynamics of *L. huidobrensis* on tomato crops. Therefore, integrated pest management programs in tropical regions should aim to preserve populations of the parasitoids *Diglyphus* sp. and *Opius* sp., which are important to the regulation of *L. huidobrensis*.

Keywords: biological control, *Diglyphus* sp., leafminer, *Opius* sp.

1. INTRODUCTION

The pea leafminer *Liriomyza huidobrensis* (Blanch) (Diptera: Agromyzidae) is an polyphagous pest, with the capacity to develop in more than 365 species of host plants belonging to 49 families [1, 2, 3; 4]. Among the hosts of this pest are families of great world importance such as: Cucurbitaceae, Fabaceae, Liliaceae, Malvaceae, Rosaceae and Solanaceae [4]. *L. huidobrensis* is widely distributed in the Americas, Africa, Europe, Asia and the Middle East [4, 5]. This leafminer causes damage to the plants due to the feeding of their larvae that make mines in the leaves. These mines reduce the photosynthetic area of plants and are gateways for pathogens, especially bacteria [6, 7]. Consequently, the economically viable production of these crops depends on the proper management of these insects.

Recently, the damage caused by *L. huidobrensis* due to the occurrence of population outbreaks of this pest in countries where it occurs has been increase. The leafminer is currently one of the main pests of potato, beans, fava, spinach, and tomato in Argentina [4, 8]. In Brazil, the outbreaks of *L. huidobrensis* increased in Asteraceae, Cucurbitaceae, Fabaceae and Solanaceae [9]. In Costa Rica from 1989 on, the leafminer reached the status of primary pest in lettuce and celery [4]. In Canada, leafminer flies had outbreaks and caused damage to lettuce, spinach, celery, ornamental, cucumber and onion [10]. In Kenya, leafminer flies show population outbreaks in different crops [11].

In the integrated pest management, it is important to know the main factors regulating herbivorous insect populations, such as host plant characteristics, climatic elements and natural enemies [12, 13, 14].

The host plant may affect herbivorous insects either directly or indirectly [15]. It can directly affect the development, survival and reproduction of herbivores due to their nutritional quality, physical barriers and production of toxic compounds [12, 16, 17]. The indirect effect of plants on herbivorous insects is related to their influence on natural enemies [17, 18]. The main climatic elements affecting insect pests' populations are temperature, rainfall, wind velocity and photoperiod [15, 17, 19].

However, the natural enemies can cause high mortality to the populations of pests, thus influencing the size of their populations [14, 20, 21]. Some studies report the influence of natural enemies on the regulation of *L. huidobrensis*. Among these natural enemies, predators such as spiders (Arachnida), larvae of *Crysoperla* sp. (Neuroptera: Chrysopidae), adults of *Condylostylus* sp. (Diptera: Dolichopodidae), predatory wasps (Hymenoptera: Vespidae) and parasitoids such as *Diglyphus* sp. (Hymenoptera: Eulophidae), *Chrysocharis* sp. (Hymenoptera: Eulophidae), *Opius* sp. (Hymenoptera: Braconidae) [4, 22].

Despite the importance of *L. huidobrensis* as a tomato pest and studies on factors on insect populations, researches about these subjects remain understudied. Thus, the aim of this work was to determine the natural factors that regulate *L. huidobrensis* populations in tomato crops.

2. MATERIAL AND METHODS

2.1. Study site

This study was conducted in eight commercial tomato crops in Coimbra, state of Minas Gerais, Brazil (20º51'24"S, 42º48'10"W, 720 m altitude and tropical climate), during two years (March 2015 to March 2017). The tomato crops cultivar Aguamiel were established in areas ranging from 4 to 6 ha, with a spacing of 0.5 m between plants and 1.0 m between rows. The plants were cultivated according to Silva & Vale (2007) [23].

2.2. Characteristics assessed

The densities of *L. huidobrensis* (percentage of leaves mined and number of mines per leaf) and their natural enemies (individuals per sample) were evaluated in 300 plants distributed throughout each crop. The crops were weekly monitored from the establishment until the end of harvest. The phenological stage of tomato plants (vegetative and reproductive) were noted during the evaluations.

The densities of *L. huidobrensis* were evaluated by counting the mines that contained larvae in each leaf inserted between the median and basal section of the plant canopy. The densities of natural enemies were evaluated using the apical leaf beat technique against a white plastic tray (40 × 25 × 6 cm). Then the insects that fall into the tray were counted. These techniques and sample units were used because they are ideal for evaluation of leafminer and natural enemy populations in tomato crops [24, 25]. Specimens of *L. huidobrensis* and natural enemies were collected and stored in 10 mL glass bottles containing 90% ethanol solution for further identification. The specimens of leafminer and parasitoids were identified by Dr. Maria Dolores Alcazár Alba, from the laboratory of Producción y Sanidad Vegetal em La Mojonera (Almeira), Spain. The other natural enemies were identified using taxonomic keys available in the literature [26].

Data of mean air temperature (°C), total rainfall (mm day^{-1}) and photoperiod (light hours) were daily obtained from the Meteorological Station local.

2.3. Statistical Analysis

We calculated the means and standard errors for *L. huidobrensis* densities and natural enemies during the experimental period.

Curves of variation were made regarding the densities of *L. huidobrensis* and climatic elements during the experimental period. Also, was represented the phenogical stages of tomato plants (vegetative or reproductive). The densities of *L. huidobrensis* (number of mines per leaf) were submitted to multiple linear regression analysis ($\alpha = 0.05$) as a function of climatic elements, natural enemy densities and plant phenological stage (1 = vegetative and 2 = reproductive) [27]. For the natural enemies that correlated significantly with the density of *L. huidobrensis*, curves of variation of their densities were constructed during the experimental period.

3. RESULTS

Five species of predators and two of parasitoids were observed attacking *L. huidobrensis* in tomato crops. The predators were spiders (Araneae), adults of the ant *Solenopsis* sp. (Hymenoptera: Formicidae), adults of Hymenoptera: Vespidae, nymphs and adults of the pirate bugs *Amphiareus* sp. and *Orius* sp. (Hemiptera: Anthocoridade) and adults of the beetle *Anthicus* sp. (Coleoptera: Anthicidae). The most abundant predators were spiders and *Solenopsis* sp. The parasitoids observed were *Diglyphus* sp. (Hymenoptera: Eulophidae) and *Opius* sp. (Hymenoptera: Braconidae) (Table 1).

The percentages of leaf mined by *L. huidobrensis* were similar between crops (Figure 1A). The number of mines per leaf varied between the tomato crops. Larger numbers of mines of *L. huidobrensis* per leaf were observed in crops 4, 5 and 6 than in crops 1, 2, 3 and 7 (Figure 1B).

In all eight tomato crops, both the percentage of mined leaves and the number of mines per leaf of *L. huidobrensis* varied according to the development of the plants. We observed that the attack intensities of *L. huidobrensis* (percentage of mined leaves and number of mines per leaf) in the eight crops showed a population peak that occurred between the middle part at the end of the crop (Figure 1).

During the experimental period, the mean air temperature was 20.98 ± 0.10 °C, the rainfall was 1153.3 mm per year and the mean photoperiod was 11.87 hours (Figure 2B).

The multiple linear regression model for the number of mines per leaf as a function of the populations of natural enemies, climatic elements and stage of the plants was significant (F = 4.91, $P = 0.046$), with coefficient of determination of 0.91. The angular coefficients associated to the densities of the parasitoids *Diglyphus* sp. and *Opius* sp. were significant ($P < 0.05$). The angular coefficient associated with the density of the parasitoid *Diglyphus* sp. was positive whereas the angular coefficient associated with the parasitoid *Opius* sp. was negative (Table 2).

The parasitoids *Diglyphus* sp. and *Opius* sp. were present in all eight tomato crops. Their densities varied between crops and along with the development of plants. We observed larger populations of *Diglyphus* sp. in crops 2, 4, 5, 7 and 8. On the other hand, the parasitoid *Opius* sp. was found at higher densities in crops 1, 2, 5, 7 and 8. The densities of *Diglyphus* sp. and *Opius* sp. showed a population peak in the final period of tomato crops (Figure 3).

4. DISCUSSION

The variation of the density of *L. huidobrensis* throughout the crop should be related to the process of colonization of the crops by this insect and the changes occurred in the plants during its development. According to Parrella (1987) [24], the leafminer insects that colonize the crops come from host plants in the areas surrounding the crop. This pattern was observed in the colonization of tomato crops by *L. huidobrensis* since the densities of this insect were low at the beginning of the crop and increased until reaching a population peak. In addition, we observed the existence of plants (weeds and other crops) close to the tomato crops that were attacked by leafminer flies.

Despite the high number of insecticide applications in tomato crops (40-50 spray during a single crop), we observed high density of *L. huidobrensis* until the final period of cultivation. According to Miranda et al. (2005) [28], the level of economic damage to leafminers in tomato crops is 20%. Thus, in the eight tomato crops of this work, *L. huidobrensis* populations reached and remained above the level of economic damage until the end of crops. This fact may have occurred due to chemical control failures, selection of resistant *L. huidobrensis* populations and reduction of the natural enemy population by the use of non-selective insecticides to natural enemies [29].

The decrease in *L. huidobrensis* attack intensity in the final part of the tomato crop probably occurred due to the control exerted by the natural enemies and the effect of the stage of plants on this pest. In relation to the effects of natural enemies, a negative correlation was observed between *L. huidobrensis* and the parasitoid *Opius* sp. In other words, the parasitoid *Opius* sp. has the potential to control the population of *L. huidobrensis*. In addition, during the development of the plants there is a change in morphological characteristics (waxes, trichomes, cuticle thickness) and chemical (secondary metabolites and nutrients) [30, 31], which may affect the biological performance of herbivorous insects. On the other hand, older leaves generally present poorer nutritional quality because they are lignified and have lower water and nutrient contents [32, 33]. In addition, leaf trichome density is higher in older tomato plants [34], which makes insect movement and oviposition difficult. In our work, we observed clearly lignified and less nutrient-rich older plants, since farmers invest less in the management of the crop at the end of the crop-cycle. Also, trichomes may contain toxins that cause mortality to pests [34, 35, 36].

Species of predators and parasitoids were observed exerting biological control of *L. huidobrensis* on tomato crops. However, both predators and parasitoids were found in low population density. This low density of natural enemies may have occurred due to the excessive and indiscriminate use of insecticides that cause high mortality to these organisms. Therefore, in programs of integrated pest management in tomato crops, practices should be adopted to allow the preservation of natural enemies populations. Among the practices that can achieve this goal are the use of selective insecticides, decision-making system and increase in plant diversity.

The selectivity of insecticides is classified as physiological and ecological [37]. The physiological selectivity consists in the application of insecticides that are more toxic to the pest than to the natural enemies [38]. In this context, there are studies reporting the control efficiency of botanical insecticides on *L. huidobrensis* and its selectivity in favor of the parasitoid *Diglyphus* sp. [39, 40]. The ecological selectivity is obtained by the application of insecticides in place and in time that reduces the contact of the natural enemies with the insecticides. To avoid the use of insecticides during the periods of higher activity of natural enemies, the sprays should be directed to the site of greater attack of the pest [41, 42, 43].

The use of the decision-making system contributes to the preservation of populations of natural enemies due to a reduction in the number of insecticide applications [44]. In this system, the insecticide applications are only carried out when the pest density reaches the level of economic damage [14, 45].

However, plant diversity contributes to the preservation of natural enemies populations because plants are a source of alternative food, shelter and nesting sites for these biological control agents [15, 46, 47]. This diversity can be obtained by the preservation of native vegetation such as forests [46], use of polyculture [46, 48] and maintenance of flowering weeds in the environs of crops [49, 50, 51].

Our results indicate that there was dependence between the attack intensity of *L. huidobrensis* and the densities of the parasitoids *Diglyphu*s sp. and *Opius* sp. since the coefficients of the multiple linear regression between these variables were significant ($P < 0.05$). These parasitoids are important in the natural biological control of *L. huidobrensis*. *Diglyphus* sp. is an ectoparasitoid of *Liriomyza* sp. larva [52] and *Opius* sp. is a larva-pupa endoparasitoid [53]. The populations of natural enemies and pests present a density-dependent relationship [15, 54]. Initially, an increase in the population of natural enemies occurs due to the increase in the pest population. This is due to the greater reproduction and migration of the natural enemies to the crops, which is caused by greater availability of food represented by the higher densities of the pest. At the second moment, there is a reduction of the populations of the pests due to the great increase of the populations of natural enemies [15, 55]. Thus, there was a positive and significant correlation ($P < 0.05$) between the populations of *Diglyphus* sp. and *L. huidobrensis* due to the effect of the greater abundance of this herbivore on this parasitoid. The negative and significant correlation ($P < 0.05$) among the populations of *Opius* sp. and *L. huidobrensis* should be due to the control effect of this parasitoid on the populations of this pest.

In conclusion, the results obtained in this work allow the understanding of the natural factors that affect the intensity of *L. huidobrensis* attack in tomato crops in tropical regions. The parasitoids *Diglyphus* sp. and *Opius* sp. are the main factors that influence the population dynamics of *L. huidobrensis* in tomato crops. Thus, in integrated pest management programs it is important to employ strategies that maintain or increase the action of these parasitoids in tomato crops.

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Table 1. Densities (mean ± standard error) of *Liriomyza huidobrensis* and natural enemies in the eight tomato crops.

Taxon	Density (individuals 10 plants^{-1})
Rate of mined leaves (%)	49.83 ± 0.33
Mines per leaf	34.24 ± 0.53
Predators	
Spiders	0.60 ± 0.05
Solenopsis sp.	0.12 ± 0.02
Vespidae	0.02 ± 0.005
Anthocoridae	0.0009 ± 0.0006
Anthicus sp.	0.004 ± 0.001
Parasitoids	
Diglyphus sp.	1.25 ± 0.05
Opius sp.	0.33 ± 0.02

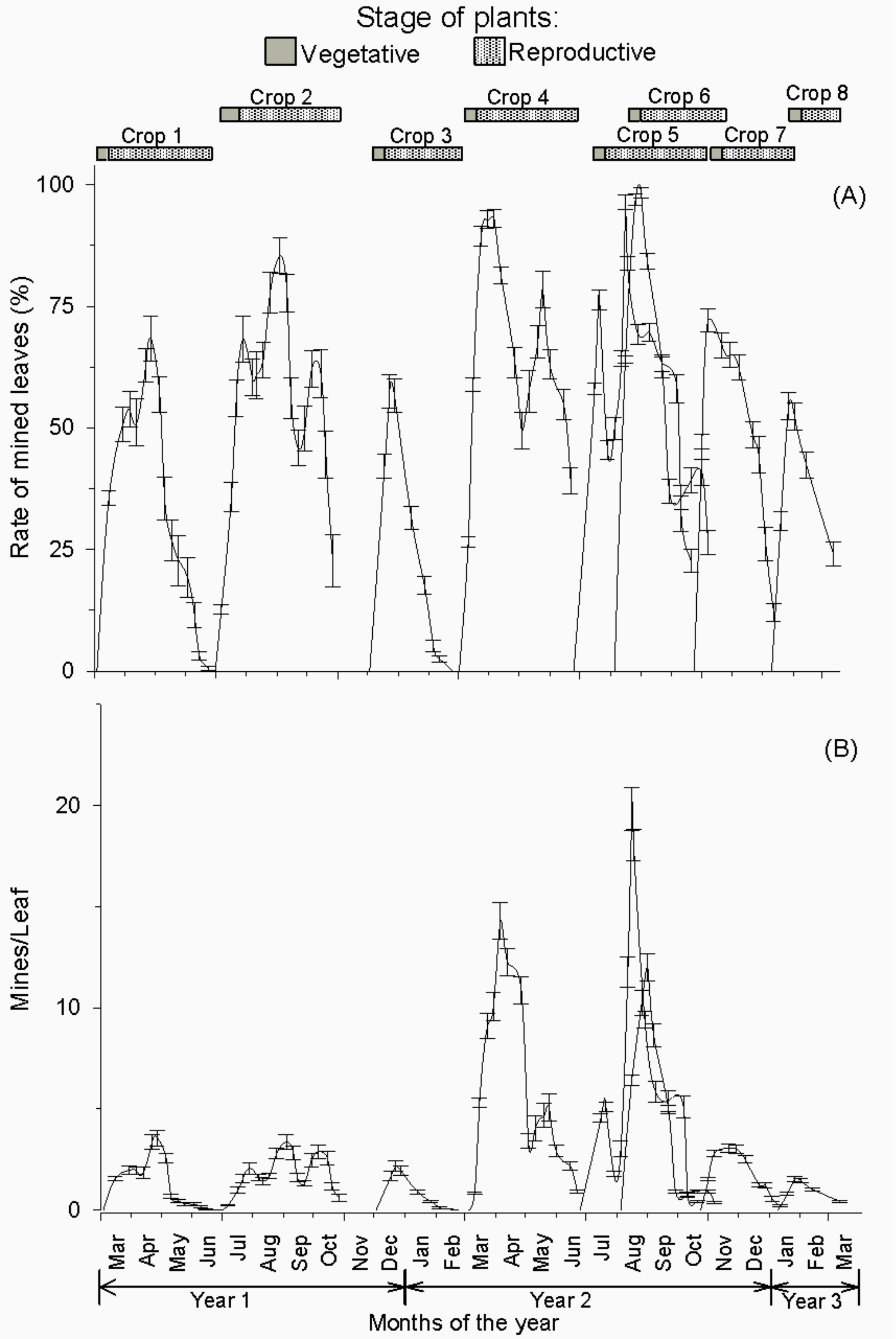

Figure 1. Densities (mean ± standard error) of *Liriomyza huidobrensis* in eight tomato crops. (A) Percentage of mined leaves and (B) number of larvae per leaf.

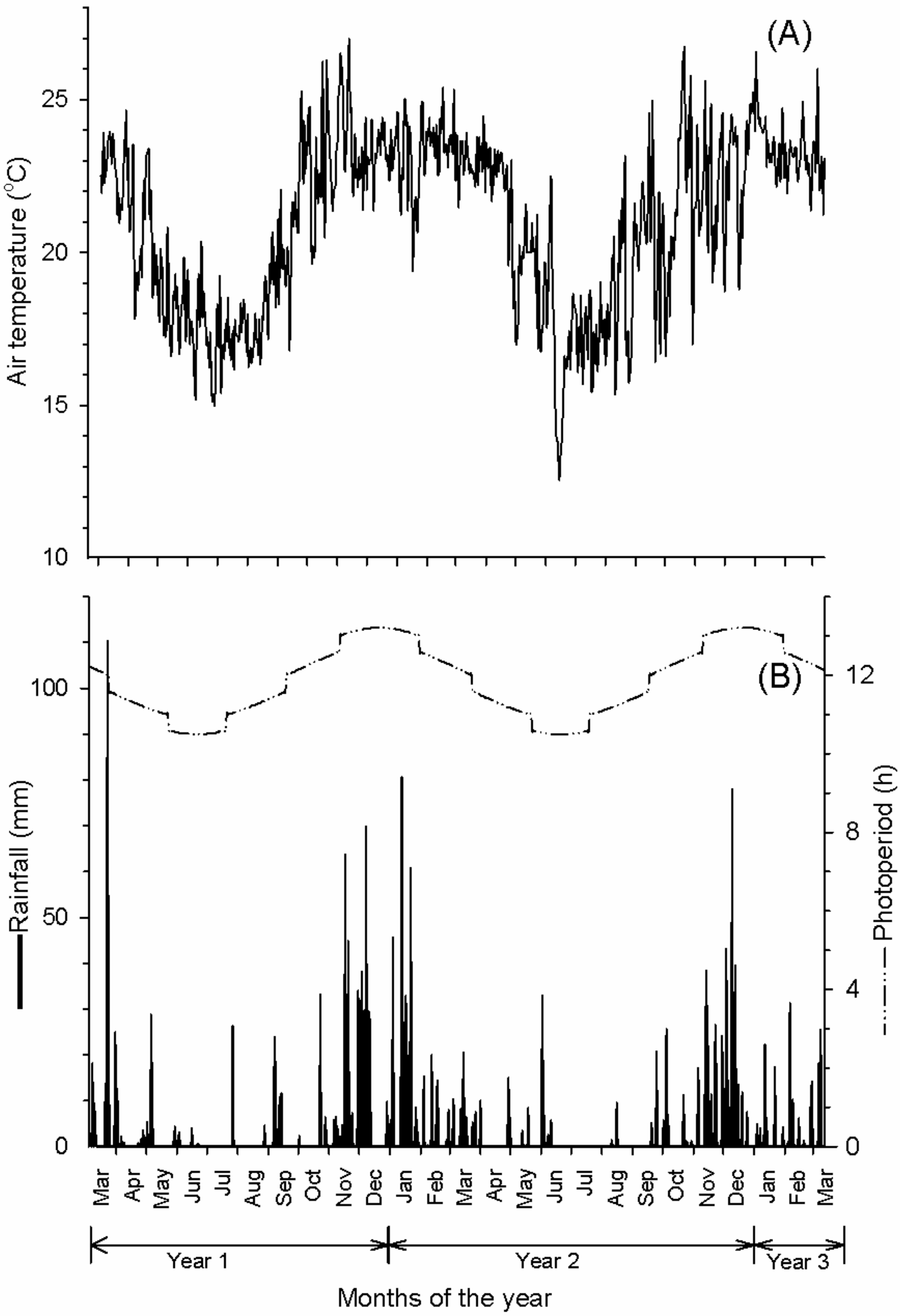

Figure 2. Variation of air temperature, photoperiod and precipitation during the period of conduction of the eight tomato crops.

Tabela 2. Angular coefficients of the multiple linear regression model of the density of *Liriomyza huidobrensis* (mines/leaf) as a function of natural enemy densities (individuals / leaf), climatic elements and plant stage (1= vegetative and 2= reproductive).

Independent variables	Angular coefficients
Spiders	4.63
Solenopsis sp.	24.10
Vespidae	47.39
Anthicidae	-1739.11
Diglyphus sp.	19.01*
Opius sp.	-36.45*
Mean temperature (ºC)	0.64
Rainfall (mm dia^{-1})	0.12
Photoperiod (h)	-2.44
Phenological growth stage	0.08

* Significant angular coefficients according to *F-test* at $P < 0.05$. $R^2 = 0.91$, $F = 4.91$ and $P = 0.046$.

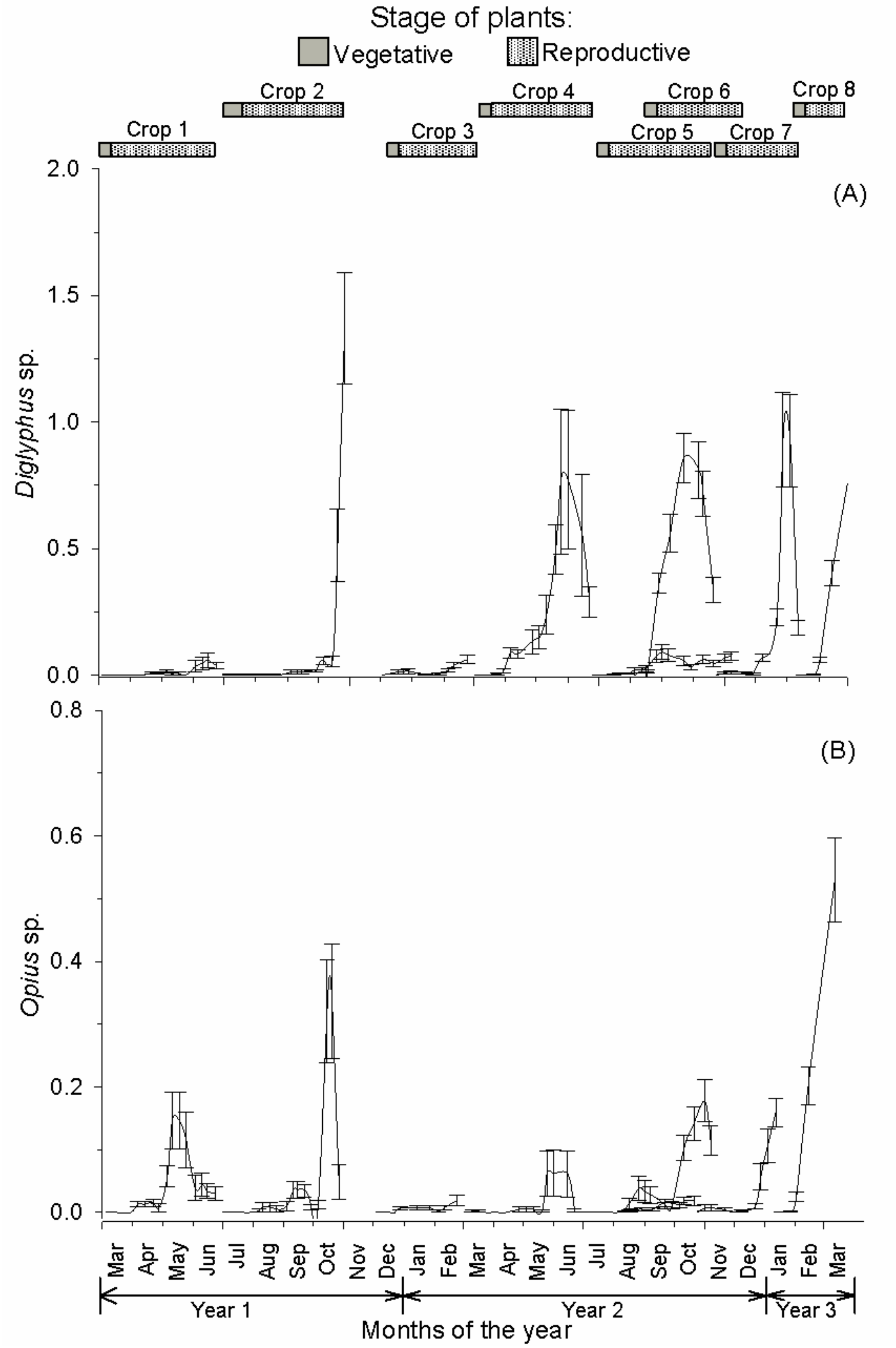

Figure 3. Densities of parasitoids (A) *Diglyphus* sp. and (B) *Opius* sp. in eight tomato crops.

CHAPTER II: PRACTICAL SAMPLING PLAN FOR *Liriomyza huidobrensis* (DIPTERA: AGROMYZIDAE) IN TOMATO CROPS

ABSTRACT- The pea leafminer *Liriomyza huidobrensis* (Blanchard) is an important pest of tomato crops worldwide. Conventional sampling plans are the starting point for the development of pest control decision-making. This work aimed to develop a conventional sampling plan for *L. huidobrensis* on the vegetative and reproductive stages of tomato. The best sampling unit for vegetative and reproductive stage tomato crops was determined. The frequency distributions of *L. huidobrensis* densities in tomato crops were assessed, and the number of samples to compose the sampling plan was determined. The basal leaf of the middle section of the plant canopy was the best plant part for sampling. Leafminer densities were fitted to the negative binomial distribution with a common aggregation parameter (Kcommon = 0.7289) that represents all tomato fields. The sampling plan consists of 73 samples per field, irrespective of field size (1, 5 or 10 ha). Evaluations using this sampling plan were performed in 47 min, 1 h 9 min and 1 h 25 min with a cost of US $1.74, 2.54 and 3.12 per sampling in fields of 1, 5 and 10 ha, respectively. The sampling plan developed in this study may be incorporated into integrated pest management programs. It leads to a more well-informed decision-making for controlling *L. huidobrensis* in tomato fields up to ten hectares, it is inexpensive (up to US $3.12 per sampling area), fast (up to 1 h 25 min per sampling area) and practicable (it can be used in tomato crops at vegetative or reproductive stages).

Key words: aggregation parameter, decision-making, leafminer, negative binomial, sampling unit

1. INTRODUCTION

The leafminer *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae) is a polyphagous insect that feeds on more than 365 host plant species (Spencer 1973, Reitz and Trumble 2002, López et al. 2010, Alves et al. 2014) and is widely distributed in the Americas, Africa, Asia and Europe (CABI and EPPO 1997, Weintraub et al. 2017). This insect is a major pest of crops worldwide such as potato (*Solanum tuberosum* L.), tomato (*Solanum lycopersicum* L.), bean (*Phaseolus vulgaris* L.), pea (*Pisum sativum* L.) (López et al. 2010, Musundire et al. 2012). *Liriomyza huidobrensis* larvae damage crops by mining, feeding, and oviposition on the leaves. Leafminers may reduce plant photosynthesis and facilitate pathogen colonization, especially bacteria (Parrella et al. 1985, Deadman et al. 2002). Additionally, high leafminer populations may cause premature leaf senescence, resulting in fruit exposure to sunlight, (Zehnder and Trumble 1984) which in turn can reduce fruit quality (Adegoroye et al. 1989).

The damage caused by *L. huidobrensis* is increasing in countries where it occurs, (Nadagouda et al. 2010, Araujo et al. 2013) and the use of chemical products for its control is also increasing (Alves et al. 2014). Additionally, there is an increasing control failure due to the development of pesticide-resistant populations and reduction of natural enemy abundance (Mason et al. 1989, Weintraub and Horowitz 1995). Furthermore, the excessive use of insecticides pollutes the environment and causes human health issues (Mason et al. 1989, Weintraub and Horowitz 1995).

Integrated pest management (IPM) decision-making systems can reduce the negative impacts of insecticide use by reducing the number of applications (Bacci et al. 2008, Lima et al. 2017). Sampling plans are an essential part of IPM programs (Lima et al. 2017, Pinto et al. 2017). Sampling plans may be conventional or sequential (Pinto et al. 2017). Conventional sampling plans consist of a fixed number of samples per field. While in sequential sampling plans the number of samples varies as a function of pest density (Pereira et al. 2016, Pinto et al. 2017).

Conventional sampling plans are the starting point for the development of pest control decision-making systems. These plans are used to determine the economic injury level and to validate sequential sampling plans (Bacci et al. 2008, Rosado et al. 2014, Pereira et al. 2016). In conventional sampling plans, the sampling unit, technique, frequency distribution of pest densities, and the number of samples per area are determined (Rosado et al. 2014, Lima et al. 2017, Pinto et al. 2017). Sampling plans must be accurate, representative, fast, low-cost and feasible (Moura et al. 2007, Rosado et al. 2014, Lima et al. 2017, Pinto et al. 2017). Besides that, they should allow for the evaluation of pest populations when a plant is either in a vegetative or reproductive stage (Pinto et al. 2017).

Despite the importance of *L. huidobrensis* as a major pest in tomato crops and of the importance of sampling plans as a tool in decision-making systems, there are no studies involving those two subjects. Thus, the objective of this work was to develop a conventional sampling plan for *L. huidobrensis* in tomato fields. In this sense, we determined the best sampling unit, frequency distribution, and sample numbers.

2. MATERIAL AND METHODS

2.1. Experimental characteristics

This study was carried out in commercial tomato fields (‘Aguamiel F1’ variety, Vilmorin®), in Coimbra, Minas Gerais State, Brazil (20º51'24"S, 42º48'10"W, 720 m above sea level and tropical weather), for 2 yr (March 2015 to March 2017).

Plants had a 0.5 × 1.0 m spacing and were established according to Silva and Vale (2007).

During this study, tomato leaves attacked by the leafminer in the field were weekly collected. The leaves were cut using a box cutter utility knife, put into a transparent plastic bag (Eco Roll, 35 × 50 cm) and immediately transported to the laboratory. In the laboratory, the leaf petioles were immersed in 30 ml containers filled with water and then placed inside an individual wooden cage (45 × 45 × 45 cm) covered with organza fabric. The adult *L. huidobrensis* samples were identified by morphological and molecular techniques (Lonsdale 2011, Parish et al. 2017).

This work was conducted in four steps. In the first step, the best sample unit to evaluate *L. huidobrensis* populations in tomato plants was selected. In the second step, the frequency distribution of *L. huidobrensis* densities was determined. In the third, the number of samples for the sampling plan was assessed. Lastly, the time and cost of the sampling plan was calculated according to the field size.

2.2. Selection of the best sampling unit to evaluate *L. huidobrensis* populations in tomato fields

This part of the study was carried out in 2 5-ha tomato fields. Plants were in the vegetative stage in one of the fields, and in the reproductive stage in the other. Fifty plants were randomly selected in each field. The canopy of the selected plants was divided into three regions: apical, middle and basal section. For this, all plant nodes were counted and numbered from the upper to lower node. The most apical node was labeled as number 1, the second most apical as number 2, and so forth. The average number of total nodes for plants at the vegetative stage was 9 and for plants at reproductive stage was 16. The total number of nodes was divided among the 3 plant sections. Thus, for plants at vegetative stage the apical section consisted of the 3 upper leaves, the middle section of the 3 middle leaves and the basal section of the 3 most basal leaves. Alternatively, for plants at reproductive stage the apical section consisted of the 6 most apical leaves, the middle section consisted of the 5 subsequent leaves and the basal section of 5 most basal leaves. In each section, the leaves were consecutively numbered from the upper to the lower part (Figure 1).

The density of *L. huidobrensis* was determined by directly counting the number of active mines on all leaves. Active mines were those that had living larvae inside them (Alves et al. 2014). The sample unit was selected using the criteria of leaf occurrence frequency in the plant, accuracy, and representativeness (Podoler and Rogers 1975, Southwood 1978, Pinto et al. 2017).

Leaf occurrence frequency in the plant was calculated using the formula (1):

(1) $Fi = (100 \times Ni)/Nt$, where Fi = leaf occurrence frequency in the plant (%), i = leaf position in the canopy section (1 to n), Ni = how many times leaf i was present in the evaluated plants, and Nt = total number of evaluated plants.

Leaves with an occurrence frequency higher than or equal to 80% were selected. This criterion was used because it is faster to search for higher frequency leaves on a plant (Pinto et al. 2017).

According to the accuracy criterion, the sampling unit was selected using the relative variance (RV) of *L. huidobrensis* densities (Bacci et al. 2006, Naranjo and Castle 2010). The RV was calculated using formula (2):

(2) $RV = ([100 \times S(\overline{x})])/\overline{x}$, where: RV = relative variance (%), $S(\overline{x})$ = standard error of *L. huidobrensis* mean densities, and $\overline{x}$ = *L. huidobrensis* mean densities.

Sample units with RVs lower than 25% were selected. This was done because samples with RVs greater than 25% generate non-feasible sampling plans (Southwood 1978).

The absolute density (mines plant^{-1}) and the relative densities for each sample (mines leaf^{-1}) were calculated according to the representativeness criterion. Samples with positive and significant correlations between absolute and relative densities by the t-test ($\alpha = 0.05$) were selected. When more than one sample had positive and significant correlations, simple linear regression analysis between those densities was performed. The angular coefficients of these regression curves were compared using their confidence intervals at $P < 0.05$, and samples with higher angular coefficients were selected (Moura et al. 2007, Bacci et al. 2008, Rosado et al. 2014).

Finally, the ideal sample to evaluate *L. huidobrensis* density in tomato fields with plants at vegetative and reproductive stages was selected among the suitable samples using the practicality criterion. This was done because feasible sampling plans are more simple and therefore more likely to be adopted by growers (Alves et al. 2014).

2.3. **Frequency distribution of *L. huidobrensis* densities**

This part of the study was carried out in 34 commercial tomato fields (11 fields at the vegetative and 23 at the reproductive stage). In each field, *L. huidobrensis* densities were evaluated on 300 plants using the sample unit previously selected. Sampled plants were distributed in a regular grid to eliminate sampling bias (Rosado et al. 2014, Pinto et al. 2017).

For each field, the mean and standard errors of *L. huidobrensis* densities were calculated, and sampling data were tested with negative binomial, Poisson and positive binominal frequency distribution models. The insect density data were considered to fit a frequency distribution when their expected and observed frequencies were not significantly different according to the Chi-square test (α = 0.05) (Bacci et al. 2006, Rosado et al. 2014). The frequency distribution at which insect densities were adjusted in most fields (≥ 70%) was used in selecting the formula to calculate the number of samples for the sampling plan (Young and Young 1998, Moura et al. 2007, Rosado et al. 2014).

2.4. Number of samples from the conventional sampling plan

The aggregation parameter values for each field were calculated according to formula (3) (Young and Young 1998) because *L. huidobrensis* densities were adjusted to the negative binomial distribution in most tomato fields:

(3) $k = \overline{x}^2/(S^2 - \overline{x})$, where: k = parameter of the negative binomial distribution, $\overline{x}$ = sample mean and S^2 = variance of the sample data.

The k value for each field was subjected to linear regression analysis using the method described by Bliss and Owen (1958) to verify the existence of a common aggregation parameter (Kcommon) that represents all tomato fields. In this analysis, the fields are considered to have a Kcommon value when the regression intercept is not significant, but its slope is significant based on a F test (α = 0.05) (Bliss and Owen 1958). Subsequently, the Kcommon parameter was used to calculate the number of samples needed for the *L. huidobrensis* sampling plan in tomato fields according to the formula (4): (Young and Young 1998, Moura et al. 2007).

(4) $NA = \frac{1}{C^2} \times (\frac{1}{\overline{x}} + \frac{1c}{kc})$, where *NA* = number of samples, *C* = maximum allowed error; $\overline{x}$ = population mean, and *kc* = common aggregation parameter of the negative binomial frequency distribution (kc = 0.7289).

The error values used initially were 0.05, 0.10, 0.15, 0.20, and 0.25 (five, ten, 15, 20 and 25%) (Moura et al. 2007, Pinto et al. 2017). These errors were used to determine the number of samples because they are considered acceptable for decision-making in on-farm integrated pest management programs (Southwood 1978).

2.5. Time and cost of the sampling plan as a function of field size

Samples were collected in three tomato fields of one, five and ten hectares each. These fields were used because they are representative of tomato field sizes in Brazil. The sample unit (last leaf of the plant middle section) and the sample numbers (73 samples per field) were chosen based on the previous experiments. The time spent on sample evaluation and movement between samples in each field were measured. Finally, costs for *L. huidobrensis* sampling in each of the three plot sizes were calculated using the formula:

(5) $SC = CM + ((TS + TW) \times LC)$

where *SC* is the sampling cost, *CM* is the cost of materials (rubber, pencil, paper and drawing board: US $0. 042), *TS* is the time spent sampling (hours), *TW* is the time spent walking between samples (hours), and *LC* is the labor costs (rural worker wage and nonwage labor costs: US $2.17/h) (Moura et al. 2007, Rosado et al. 2014). For cost of materials, a durability period of 1 yr was considered.

3. RESULTS

3.1. Selection of the best sample unit to evaluate *L. huidobrensis* populations in tomato fields

In plants at the vegetative stage, only the leaves of the apical and middle canopy section were adequate for *L. huidobrensis* sampling by the criterion of leaf occurrence frequency in the plant (≥ 80%) (Figure 1 and Table 1). Among these, the most basal leaf of the apical section and all leaves of the middle section were suitable for sampling by the accuracy criterion. This occurred because pest densities in these samples had relative variances lower than 25%. According to the representativeness criterion, *L. huidobrensis* relative densities (mines leaf^{-1}) showed positive significant correlations ($P < 0.05$) with their absolute densities (mines plant^{-1}). Among the four leaves selected, the most basal leaf of the middle section presented a regression curve (insect relative density of as a function of absolute density) with the highest angular coefficient (Table 1).

In plants at the reproductive stage, the first leaf of the basal section and all leaves of the apical and middle sections were adequate for *L. huidobrensis* sampling by the criterion of leaf occurrence frequency in the plant (≥ 80%). Among these, the first leaf of the basal section and all leaves of the middle section were adequate for sampling by the accuracy criterion. Pest densities in these samples had relative variances lower than 25%. According to the representativeness criterion, *L. huidobrensis* relative densities (mines leaf^{-1}) showed positive and significant correlations ($P < 0.05$) with their absolute densities (mines plant^{-1}) only for leaves of the middle section of the plant. Among the leaves of the middle section, the most apical and basal leaves presented regression curves (insect relative density of as a function of absolute density) with the highest angular coefficient (Figure 1 and Table 1).

Considering the criterion of practicality, the basal leaf of the plant middle section was the sampling unit selected for evaluating *L. huidobrensis* in tomato fields at both vegetative and reproductive stages.

3.2. Frequency distribution of *L. huidobrensis* densities

Liriomyza huidobrensis densities fitted a negative binomial distribution in 28 of the 34 (82.35%) fields evaluated since the chi-square values were non-significant in those 28 fields ($P > 0.05$). On the other hand, none of the *L. huidobrensis* density data fit the Poisson or positive binomial distributions (Table 2). Thus, the number of samples from *L. huidobrensis* conventional sampling plan in tomato fields was calculated using the negative binomial distribution formula.

3.3. Number of samples from the conventional sampling plan

Simple linear regression for the common aggregation parameter (Kcommon) of *L. huidobrensis* in 34 tomato fields as a function of the individual k parameters of each field showed a significant slope ($P < 0.05$) and a non-significant intercept ($P > 0.05$) (Table 3). Therefore, there was a common aggregation parameter (Kcommon) between *L. huidobrensis* densities in all tomato fields.

The number of samples needed for sampling *L. huidobrensis* stabilized at the maximum allowed error of 15% (Figure 2). Thus, this was the level of error used to determine the number of samples from the conventional sampling plan. Using this error (15%), the number of samples from the sampling plan was 73 per field.

3.4. Time and cost of the sampling plan as a function of field size

The total distance walked between samples was 1727.85, 3863.56 and 5463.95 m taking 17, 39 and 55 min for fields of one, five and ten hectares respectively. The time required to evaluate the 73 samples was 30 minutes. Therefore, the total time required for sampling *L. huidobrensis* in fields of one, five and ten hectares was 47 min, 1 h and 9 min, and 1 h and 25 min respectively. Based in field size, the cost for *L. huidobrensis* sampling was US $1.74, 2.54 and 3.12 for fields of 1, 5 and 10 ha respectively.

4. DISCUSSION

The basal leaf of the plant middle section of the canopy was the best plant part to evaluate *L. huidobrensis* populations in tomato fields with plants both at the vegetative and reproductive stage based on the criteria of leaf occurrence frequency in the plant, accuracy, and representativeness. The fact that the same sampling unit can be used to assess *L. huidobrensis* density in tomato fields with plants at different phenological stages makes the sampling plan easier, practicable and could increase its adoption by growers (Lima et al. 2014). Besides that, the selection of a leaf easily located (present in every plant) that allowed an accurate sampling (RV < 25%) resulted in the development of a faster sampling plan with fewer samples (Rosado et al. 2014, Lima et al. 2017). In addition, this sampling plan determines relative densities (mines leaf^{-1}) that represent the absolute density of the insect (mines plant^{-1}). Thus, the density determined in this leaf-sample represents the whole-plant insect density (Rosado et al. 2014, Lima et al. 2017).

Tomato plant sites with high *L. huidobrensis* densities are generally ideal for sampling because it generates accurate and representative sampling plans (Southwood 1978, Moura et al. 2007, Bacci et al. 2008, Pinto et al. 2017). This fact was observed in this work, since the selected sample had high *L. huidobrensis* densities. This can be related to the fact that *L. huidobrensis* adults prefer the middle section of tomato plants to oviposit (Minkenberg 1988). This behavior may occur because this plant region has larger and thicker leaves, which ensures more space and food for the larvae (Issa and Marcano 2002).

Liriomyza huidobrensis densities adjusted to the negative binomial frequency distribution in most fields (82.35%). This occurred because sample variances were greater than the means. This was due to many samples with high insect densities (Taylor 1961, Rosado et al. 2014).

Populations of the same species may have different values for the aggregation parameter (k) of the negative binomial distribution in different fields. When this occurs, it is necessary to calculate the number of samples for each field individually, (Young and Young 1998) which makes it difficult to determine a sampling plan that can be used across all fields (Alves et al. 2014). However, *L. huidobrensis* densities presented a common aggregation parameter (Kcommon) between the different tomato fields in this study. This fact indicates that the sampling plan proposed here can be used to evaluate *L. huidobrensis* populations across different tomato fields. In addition, this sampling plan can be used in tomato fields with plants at the vegetative or reproductive stages since both plant stages were used to determine a common aggregation parameter (Kcommon).

Feasibility is a concern when developing a sampling plan for a pest in order to enable a rapid and inexpensive sampling process (Rosado et al. 2014, Lima et al. 2017, Pinto et al. 2017). Sampling plans are feasible when it is possible to collect data in the field, process the information, and come to a decision within a day period (i.e., during the morning or the afternoon) (Moura et al. 2007). Here, the use of a 15% error resulted in a feasible sampling plan since the time it takes to enact in fields up to 10 ha would be less than a day period.

Sample evaluation and the time required to walk between samples must be considered in a sampling plan. In this study, we developed a sampling plan that consisted of 73 samples and an average time of 30 minutes to evaluate *L. huidobrensis* in a tomato field. We observed that the distance between samples and therefore the time walking between samples increased in larger fields. Thus, the sampling cost was higher in larger fields because labor is the main component of the total cost.

5. CONCLUSION

The sampling plan developed in this study can be incorporated into integrated pest management programs to evaluate *L. huidobrensis* densities in tomato fields up to ten hectares with plants in vegetative or reproductive stages. This sampling plan consists of 73 samples. The basal leaf of the middle section of the canopy is the best plant part to evaluate the number of mines with *L. huidobrensis* larvae. This sampling plan is inexpensive (up to US $3.12 per sampling) and feasible because it is accurate, representative and fast (up to 1 h 25 min).

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Table 1. Selection of leaf to sample *Liriomyza huidobrensis* in tomato plants at the vegetative and reproductive stages

Canopy section	Leaf position†	Density (mines leaf^{-1} ± SEM)	Characteristics used in sample selection‡			
			Freq	RV (%)	r	b ± SEM
			Vegetative stage plants			
Apical	1	0.04 ± 0.03	100 §	69.99	0.056^{ns}	-
	2	0.24 ± 0.11	100 §	46.96	0.072^{ns}	-
	3	1.30 ± 0.20	100 §	15.73 §	0.306* §	0.38 ^{ns} ± 0.17
Middle	1	6.44 ± 0.74	100 §	11.56 §	0.606* §	0.65* ± 0.19
	2	10.48 ± 0.86	100 §	8.19 §	0.714* §	0.93* ± 0.15
	3	7.78 ± 0.89	100 §	11.48 §	0.862* §	1.82* ± 0.15 §
Basal	1	6.30 ± 0.89	66	14.11 §	0.668*	-
	2	4.00 ± 0.50	14	12.41 §	0.366*	-
	3	4.00 ± 0.08	2	-	0.183^{ns}	-
			Reproductive stage plants			
Apical	1	0.00 ± 0.00	100 §	-	0.000^{ns}	-
	2	0.00 ± 0.00	100 §	-	0.000^{ns}	-
	3	0.00 ± 0.00	100 §	-	0.000^{ns}	-
	4	0.02 ± 0.02	100 §	100	0.063^{ns}	-
	5	0.10 ± 0.05	100 §	51.51	0.399*	-
	6	0.40 ± 0.13	100 §	31.94	0.451*	-
Middle	1	0.96 ± 0.19	100 §	20.18 §	0.544* §	1.18* ±0.12 §
	2	1.88 ± 0.26	100 §	13.81 §	0.446* §	0.90* ± 0.09
	3	2.92 ± 0.31	100 §	10.67 §	0.612* §	0.92* ± 0.07
	4	2.94 ± 0.31	100 §	10.67 §	0.417* §	0.91* ± 0.06
	5	1.94 ± 0.27	100 §	13.93 §	0.423* §	1.13* ± 0.07 §
Basal	1	0.81 ± 0.16	96 §	19.88 §	0.057^{ns}	-
	2	0.30 ± 0.12	80 §	38.78	0.006^{ns}	-
	3	0.13 ± 0.05	46	37.33	0.153^{ns}	-
	4	0.00 ± 0.00	18	-	0.000^{ns}	-
	5	0.00 ± 0.00	8	-	0.000^{ns}	-

† Leaves were consecutively numbered from the upper to the lower part in each section.

‡ Freq = leaf occurrence frequency in the evaluated plants (%); RV = relative variance; r = correlation coefficient between leaf relative density (mines leaf^{-1}) and the absolute density (mines plant^{-1}) of the insect, and b = curve slope of the relative density as a function of the absolute density of the insect.

§ Indicates that the leaf was selected based on the column characteristic.
* r or b significant ($\alpha = 0.05$). ^{ns} r or b non-significant ($\alpha = 0.05$).

Table 2. Chi-square test (χ^2) between observed and expected frequencies according to the negative binomial, Poisson, and positive binomial distributions of *Liriomyza huidobrensis* densities in 34 tomato fields

Field	Density (mines leaf^{-1} ± SEM)	Negative binomial		Poisson		Positive binomial	
		χ^2	df	χ^2	df	χ^2	df
		Vegetative stage plants					
1	0.54 ± 0.070	8.13^{ns}	6	4.26x10^{4*}	7	3.42x10^{3*}	7
2	0.68 ± 0.064	2.59^{ns}	5	5.93x10^{2*}	6	5.14x10^{3*}	6
3	1.76 ± 0.250	28.42^{ns}	9	4.85x10^{4*}	10	1.74x10^{8*}	10
4	2.56 ± 0.132	20.21^{ns}	10	1.13x10^{3*}	11	2.64x10^{11*}	11
5	9.98 ± 0.409	50.47^{ns}	26	7.88x10^{4*}	27	3.08x10^{52*}	27
6	7.33 ± 0.283	33.24^{ns}	20	9.18x10^{3*}	21	2.56x10^{38*}	21
7	3.43 ± 0.154	7.10^{ns}	11	3.61x10^{2*}	12	4.93x10^{15*}	12
8	0.73 ± 0.081	12.30^{ns}	7	1.64x10^{5*}	8	3.88x10^{4*}	8
		Reproductive stage plants					
9	4.13 ± 0.26	7.13^{ns}	11	3.28x10^{3*}	12	4.01x10^{24*}	12
10	0.54 ± 0.06	6.90^{ns}	5	2.09x10^{3*}	6	1.56x10^{4*}	6
11	6.26 ± 0.44	37.21*	10	2.74x10^{3*}	11	2.19x10^{6*}	11
12	0.83 ± 0.07	5.91^{ns}	3	82.50*	4	3.35x10^{3*}	4
13	4.09 ± 0.47	4.55^{ns}	7	94.82*	8	8.24x10^{16*}	8
14	2.66 ± 0.22	12.84^{ns}	13	4.33x10^{5*}	14	6.87x10^{11*}	14
15	1.19 ± 0.24	2.06^{ns}	4	59.31*	5	4.37x10^{4*}	5
16	3.29 ± 0.40	7.70^{ns}	7	78.80*	8	2.05x10^{11*}	8
17	1.20 ± 0.14	7.67^{ns}	8	6.01x10^{4*}	9	1.17x10^{7*}	9
18	5.46 ± 0.34	40.70*	21	6.91x10^{6*}	22	1.58x10^{42*}	22
19	26.04 ± 1.23	39.92*	12	3.83x10^{10*}	13	4.17x10^{167*}	13
20	5.29 ± 0.45	27.04*	16	2.32x10^{5*}	17	2.81x10^{29*}	17
21	1.38 ± 0.33	7.05^{ns}	4	186.23*	5	2.95x10^{3*}	5
22	3.14 ± 0.25	9.96^{ns}	13	2.09x10^{5*}	14	2.15x10^{19*}	14
23	1.05 ± 0.10	4.65^{ns}	6	2.41x10^{3}	7	1.23x10^{6*}	7
24	1.63 ± 0.13	13.24^{ns}	10	1.32x10^{5*}	11	2.19x10^{6*}	11
25	1.96 ± 0.16	24.21*	11	1.70x10^{5*}	12	8.56x10^{7*}	12
26	1.15 ± 0.26	7.20^{ns}	7	5.91x10^{3*}	8	3.14x10^{3*}	8
27	11.58 ± 1.11	12.76^{ns}	21	2.89x10^{3*}	22	1.44x10^{89*}	22
28	0.55 ± 0.06	9.15^{ns}	4	462.61*	5	2.20x10^{3*}	5
29	0.34 ± 0.04	3.30^{ns}	2	108.22*	3	3.76x10^{2*}	3
30	1.20 ± 0.10	7.98^{ns}	7	3.16x10^{3*}	8	6.12x10^{5*}	8
31	4.21 ± 0.29	50.73*	17	1.08x10^{6*}	18	3.90x10^{22*}	18
32	2.25 ± 0.14	13.94^{ns}	10	3.47x10^{3*}	11	4.81x10^{10*}	11
33	2.20 ± 0.16	7.71^{ns}	11	2.54x10^{4*}	12	1.72x10^{13*}	12
34	0.44 ± 0.06	4.80^{ns}	4	668.94*	5	936.45*	5

^{ns}Non-significant. *Significant at 5% probability. df = degrees of freedom.

Table 3. Analysis of variance of *Liriomyza huidobrensis* density (mines leaf^{-1}) data from 34 tomato fields to verify the existence of a common aggregation parameter (Kcommon) using a negative binomial distribution

Variance source	df	Sum of squares	Mean squares	F
Slope 1/kc	1	977.30	977.30	55.97*
Intercept	1	36.96	36.96	2.12^{ns}
Error	31	541.27	17.46	
Kcommon = 0.7289				

*Significant at 5% probability. ^{ns}Non-significant. df = degrees of freedom.

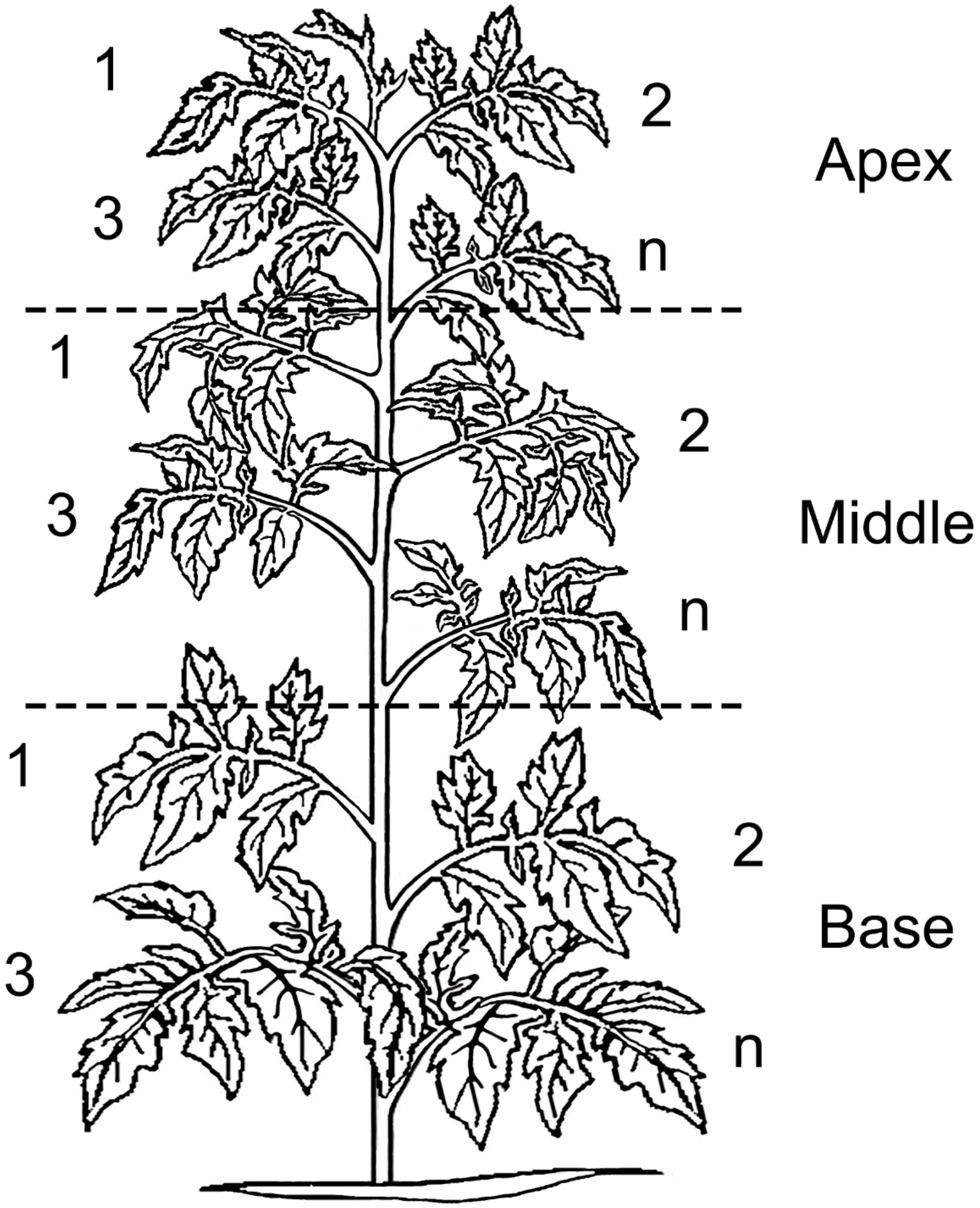

Figure 1. Canopy division and leaf enumeration used to evaluate tomato plants in both vegetative and reproductive stages.

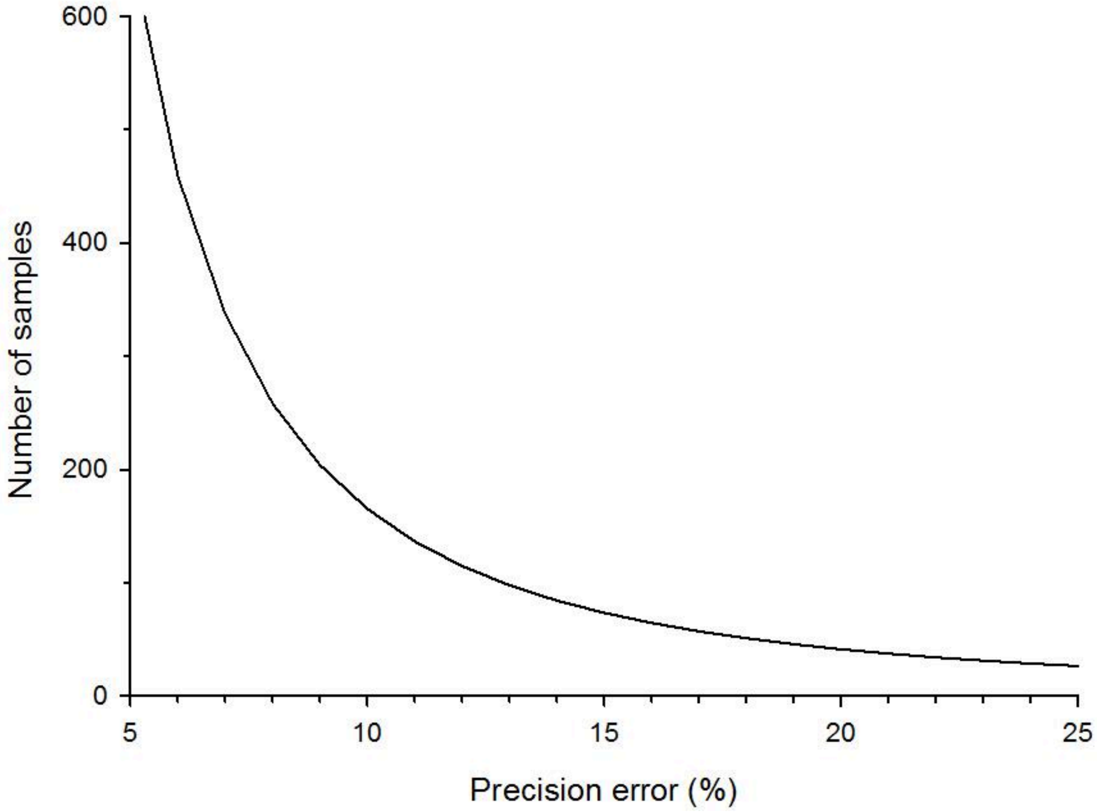

Figure 2. Number of samples of *L. huidobrensis* (mines leaf^{-1}) as a function of different precision error levels.

CHAPTER III: ECONOMIC INJURY LEVEL AND SEQUENTIAL SAMPLING PLAN FOR *Liriomyza huidobrensis* (Diptera: Agromyzidae) MANAGEMENT IN TOMATO CROPS

ABSTRACT- Decision-making systems, comprised of sampling plans and decision indices, are essential components of integrated pest management programs. Sampling plans allow verifying if the pest reached the economic injury level (EIL). Although conventional sampling plans are reliable, they are composed of more samples and the related cost can discourage their use. Sequential plans usually require fewer samples to reach a decision, reducing the time and cost of sampling. *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae) is one of the major pests of tomato crops. Despite the importance of *L. huidobrensis*, to date no decision-making system has been established for this pest in tomato. Thus, this study aimed to determine the EIL and a sequential sampling plan for *L. huidobrensis* in tomato. The cost of *L. huidobrensis* control in tomato crops was US $ 335.38 per hectare. *Liriomyza huidobrensis* reduced the productivity of tomato crops by up to 15%. The EIL was 3.24 larvae per leaf. The sequential sampling plans were validated using field-collected data (providing correct decisions in 100% of the crops and an average time saving of 86.75%), and operational characteristic curves and average sample number. This study provides relevant insights into the decision-making process of *L. huidobrensis* control in tomato crops by providing a formal criterion to reduce economic losses (EIL) and an accurate and highly cost-effective sampling plan.

Keywords: Agromyzidae, control cost, EIL, sequential sampling plan, tomato yield

1. INTRODUCTION

Decision-making systems, comprised of sampling plans and decision indices, are essential components of integrated pest management programs (IPM).[1–4]

Sampling plans can be conventional or sequential.[2] In conventional plans, decision making (control or not control) is made after the evaluation of a fixed number of samples, whereas in sequential plans this number is variable.[2,5] Sequential plans usually lead to fewer samples, which reduces the time and cost of sampling.[4,6,7]

The economic injury level (EIL) is the most widely adopted decision-making index. This threshold corresponds to the lowest pest population density causing economic damage.[1,8,9] In the decision-making process, the pest density obtained by sampling is compared to the EIL. When the pest density equals this level, control must be applied.[10,11] The EIL is influenced by several factors, including management cost, crop market value, intensity of injury, and plant response to injury.[4,12,13]

Tomato (*Solanum lycopersicum* L.) has great economic importance, ranking as the second most consumed vegetable in the world.[14,15] The global area planted is 4,800,000 hectares and the world tomato production is over 182 million tons.[16] In addition to the economic importance, tomatoes have great nutritional value due to their high content of nutrients, such as vitamins C and E, flavonoids and lycopene.[17]

The leafminer *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae) is one important pests of tomato crops.[18–20] This pest is distributed in the Americas, Africa, Asia and Europe.[20,21] Larvae of *L. huidobrensis* consume the leaf mesophyll, which reduces plant photosynthesis and facilitates the entry of phytopathogens.[22] This pest, in high infestations, can also cause premature leaf senescence, resulting in the fruits exposure to the sunlight.[23]

Despite the importance of *L. huidobrensis*, to date no decision-making system has been established for this pest in tomato. Thus, this study aimed to determine the EIL and a sequential sampling plan for *L. huidobrensis* in tomato crops.

2. MATERIAL AND METHODS

2.1. General procedures

This study was carried out in commercial tomato crops (cultivar Aguamiel), in Coimbra (20º51'24"S, 42º48'10"W, altitude 720 m and tropical climate), Minas Gerais State, Brazil, from March 2015 to March 2017. Plants, spaced 0.5 x 1.0 m, were grown as recommended.[24]

Tomato leaves attacked by leafminer flies were collected and transported to the laboratory. The leaf petioles were immersed in 30 mL containers filled with water. These containers were kept in wooden cages (45 x 45 x 45 cm) until adult emergence. Molecular and morphological analyses (using the genitalia of dissected males) were applied for the identification of *L. huidobrensis*.[25,26]

Initially, the EIL for *L. huidobrensis* in tomato crops was determined. Subsequently, a sequential sampling plan for this pest was developed and validated.

2.2. Economic injury level (EIL)

In order to determine the EIL, the control cost and the curves of tomato yield and production value (as a function of *L. huidobrensis* density) were estimated.

2.2.1. Control cost

A survey was carried out with producers and technicians involved in tomato production in order to determine the products (insecticides and adjuvants), equipment, and frequency of insecticide application to control the leafminer. The selected products were the most used by the growers, adopting the principles of insecticide rotation.[4,27] Then, the chemicals and equipment were budgeted in the major producing regions of tomato in Brazil.[1,11,12]

2.2.2. Yield of the tomato crops as a function of *L. huidobrensis* densities

In this part of the study, six commercial tomato crops were evaluated weekly. In each crop 300 plants, evenly spaced to avoid directional tendencies, were sampled.[2,28] Active mines of *L. huidobrensis* were counted in the most basal leaf of the median section of the plant canopy [29]. Fruits from the sampled plants were harvested and weighed to determine the yield (ton ha^{-1}).

Subsequently, the yield data as a function of the mean *L. huidobrensis* density were submitted to regression analysis ($\alpha = 0.05$). The regression models tested were linear simple, negative exponential and hyperbolic decreasing. These models were used due to their suitability to describe the relationship of plant yield against pest densities.[5,12] Significance (*p*-value) and coefficient of determination (R^2) were used to select the best model.

2.2.3. Production value of the tomato crops as a function of *L. huidobrensis* density

During the experimental period, the retail price of tomato (average price US$ 0.512/kg) was monitored.[30] The yield data obtained in the previous section were used to calculate the production value using formula 1.

(1) $PV = Yd \times Pu$, where:

PV is the production value of tomato (US$), Yd is the yield of each one of the six crops evaluated (t ha^{-1}), and PU is the tomato selling price (US$ t^{-1}).

The data of the production value of the tomato crops as a function of the *L. huidobrensis* density were submitted to regression analysis, as described in section 2.2.2.

2.2.4. Economic injury level (EIL)

We calculated the percentual yield loss of the tomato crop (PP, %) corresponding to EIL using formula 2.[3,10,12]

(2) $PP = (C \times 100) \div (V_o \times K)$, where:

C is the pest control cost (US$ ha^{-1}), V_o is the tomato production value without the pest attack (i.e., when *L. huidobrensis* density equals zero) (US$ ha^{-1}), K is the pest control coefficient. The value of $K = 0.8$ was adopted because this is the minimum efficiency required for registering an insecticide in Brazil.[31–33]

Then, the production value when the *L. huidobrensis* attack equals the EIL (V_{DL}, US$ ha^{-1}) was calculated using formula 3.[3]

(3) $V_{DL} = V_o \times (100 - PP) \div 100$

EIL was determined by applying the value of V_{DL} in the equation of the crop production value as a function of the leafminer density. In this equation, V_{DL}

was the dependent variable and *L. huidobrensis* density was the explanatory variable.

2.3. Sequential sampling plan

2.3.1. Decision-making boundaries

The sequential sampling plan was developed based on the binomial sequential probability ratio test.[7,34,35] The minimum and maximum number of samples, in addition to the lower and upper boundaries (not control and control limits, respectively), composed the sampling plan.

The lower (LB_n) and upper boundaries (UB_n) were calculated using formulas 4 and 5.[7,36]

(4) $LB_n = h_0 + S \times n$

(5) $UB_n = h_1 + S \times n$, where:

h_0 the *y*-intercept of the lower boundary curve, h_1 is the *y*-intercept of the upper boundary curve, S is the slope of the lower and upper boundaries curves, and n is the number of sampling units used (up to 73, the sample number of the conventional plan.

The values of h_0, h_1, and S were calculated using formulas 6, 7, and 8.[36,37]

(6) $h_0 = \frac{ln\left(\frac{\beta}{1-\alpha}\right)}{ln\left[\frac{m_1\,(m_0+k)}{m_0\,(m_1+k)}\right]}$

(7) $h_1 = \frac{ln\left(\frac{1-\beta}{\alpha}\right)}{ln\left[\frac{m_1\,(m_0+k)}{m_0\,(m_1+k)}\right]}$

(8) $= k\,\frac{ln\left[\frac{m_1+k}{m_0+k}\right]}{ln\left[\frac{m_1\,(m_0+k)}{m_0\,(m_1+k)}\right]}$, where:

Ln is the natural logarithm, α is the type I error, β is the type II error, m_0 is the critical pest density of the lower limit, m_1 is the critical pest density of the upper limit, and k is the common aggregation parameter of the negative binomial distribution of frequency.

For the m_0 and m_1 parameters, the value of 50% and 100% of the EIL, respectively, were adopted. For α and β, the value of 0.10 was applied.[4,5,38] The value of 0.7289 was used for the common aggregation parameter (*k*), as previously determined. [29]

2.3.2. Sampling plan validation

The sequential plan was validated using operating characteristic (OC) and average sample number (ASN) curves, and field-collected data.

2.3.2.1. Operating characteristic (OC) and average sample number (ASN) curves

The OC curve indicates the probability of the decision making (control or not control), at different pest densities, to measure the accuracy of the sampling plan. ASN curve shows the expected number of samples to reach a decision, aiming to determine the cost-effectiveness of the sequential plan.[36,38]

Formulas 9 and 10 were used to determine OC and ASN curves.[36,38]

$$(9)\ OC = \frac{\frac{(1-\beta)^{h}-1}{\alpha}}{\frac{(1-\beta)}{\alpha}-\frac{(\beta)^{h}}{1-\alpha}}$$

$(10)\ ASN = \frac{OC_n(h_0-h_1)+h_1}{m-S}$, where:

m is the mean number of larvae leaf^{-1} and *h* is an auxiliary dependent variable. The other variables (α, β, h_0, h_1, and *S*) were presented in previous sections of this work.

2.3.2.2. Validation using field-collected data

The density of *L. huidobrensis* was evaluated in 46 tomato crops at the vegetative and reproductive stages. The conventional sampling plan [29] and the developed sequential plan were used in the assessments. Active mines of *L. huidobrensis* were counted in the most basal leaf of the median section of the plants (sample unit for both plans). [29]

It was determined for each crop the decision provided by both plans and, for the sequential plan, the number of samples taken. Then, the percentage of correct decisions and the time saved by the sequential plan were calculated using formulas 11 and 12, respectively.[4,5,11]

(11) $Ac = 100\ (DSq/\ TLv)$, where:

Ac is the percentage of correct in the decision making when adopting the sequential plan, *DSq* is the number of sequential plan decisions similar to those of the conventional plan, and *TLv* is the total number of crops evaluated.

(12) $Ec = 100\ (NCv - NSq)/\ NCv$, where:

Ec is the time saving when adopting the sequential sampling plan (%), *NCv* is the number of samples of the conventional plan, and *NSq* is the number of samples of the sequential plan to reach the decision.

3. RESULTS

3.1. Economic injury level

The cost of *L. huidobrensis* control in tomato crops was US$ 335.38 per hectare. Insecticides, equipment and services, and adjuvant accounted for 80.88%, 14.67%, and 4.45% of this value, respectively (Table 1).

The curves fitted with decreasing hyperbolic function provided the best models for the relationship between yield and production value with the *L. huidobrensis* density ($R^2 = 0.91$, $P = 0.011$). For low *L. huidobrensis* densities (<2 larvae leave^{-1}), little change occurred in yield and production value (up to 0.26%). Conversely, for higher leafminer densities, yield and production value reduced steadily. In this density range (2 to 7.40 larvae leave^{-1}), there was a reduction of about 15% in both variables (Figures 1A and 1B). The EIL for *L. huidobrensis* was 3.24 larvae per leaf (Figure 1B).

3.2. Sequential sampling plan

3.2.1. Decision-making boundaries

The lower (m_o) and upper (m_1) boundaries of the sequential sampling plan were 1.62 and 3.24 larvae per leaf, respectively. The slope (S), and lower intercept (h) and upper intercept (h_1) values were 2.27, -13.03 and 13.03, respectively. The generated plan requires a minimum of six samples to reach a decision (control or not control) (Figure 2).

3.2.2. Validation

In the OC curve, the probability of not controlling *L. huidobrensis* is 90% when its density is below m_0. Conversely, the probability of not controlling the pest is 10% when its density equals EIL (m_1). The mean number of samples to provide the decisions to not control (ASN intercept with m_o) and to control *L.*

huidobrensis (ASN intercept with m_1) were 16 and 11, respectively. The maximum number of samples required to reach a decision is 19 (Figure 3).

In the validation using field-collected data, the sequential and conventional plans provided the same decisions in 100% of crops. The sequential plan provided an average time saving of 86.75% compared to the conventional plan. This saving was 89.25% and 85.76% at the vegetative and reproductive stages, respectively (Table 2).

4. DISCUSSION

The control cost of *L. huidobrensis* represents about 1% of the production value while the damage caused by this pest can reach 15% of the tomato grower revenue. Due to this relatively advantageous cost-benefit ratio, most tomato growers adopt the calendar spray of insecticides. This practice leads to several environmental (side-effects on natural enemies and pollinators, and environmental pollution), social (human health impacts) and technical problems (development of resistance of *L. huidobrensis* populations to applied products, pest resurgences and secondary pest outbreaks).[33,39–43] On the other hand, the non-control of *L. huidobrensis* occurring at high densities results in significant economic losses. Thus, the EIL established in this study (3.24 larvae per leaf) is a guideline for decision-making in *L. huidobrensis* management, minimizing the use of insecticides while providing a formal criterion to reduce economic losses.

The determination of models describing the crop yield as a function of the density of indirect pests (i.e., feeders on non-commercial plant parts) is generally a hard task, especially under field conditions.[5,11] This is due to the difficulty of isolating the effect of the pest attack from those caused by other factors (climate conditions, crop management, phytopathogens, and weeds) at open field scale.[1,5] Here, robust models were generated for *L. huidobrensis*. The tomato plants were tolerant to the attack of *L. huidobrensis* since the yield and production value curves as function of the pest attack were hyperbolic concave downward.[12] The increase of *L. huidobrensis* density up to 2 larvae per leaf did not decrease yield of tomato crops. However, when the pest density was higher than 2 larvae per leaf, yield decreased steadily. Although less frequent than negative exponential models, the hyperbolic decay in crop yield under pest attack has already been reported.[5,11,44,45]

Sampling plans are important in IPM programs since they allow to monitor pest populations (i.e., verify if the pest reached the EIL), besides to assess the treatment efficacy.[46] Although conventional plans are reliable, they are composed of more samples and the related cost can discourage their use by growers. In addition, *L. huidobrensis* presents high growth potential in tropical conditions, which requires faster decision making.[47] Here, the generated conventional plan was assertive in the decision making of a wide range of *L. huidobrensis* densities (by the field collected data and OC function), and presented a time saving of 87% compared to the conventional plan. As presented in the ASN curve, the generated plan requires a maximum of 19 samples to reach a decision.

In conclusion, this study provides relevant insights into the decision-making process of *L. huidobrensis* control in tomato crops. The EIL for *L. huidobrensis* is 3.24 larvae per leaf. The developed sequential sampling plan is accurate and highly cost-effective and can be incorporated into IPM programs of this important pest.

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Table 1. Cost (US$/ha) of equipment, insecticides, and adjuvant used for *Liriomyza huidobrensis* control in tomato crops. U.C. = Unity cost (US$), S.C. = Spray cost ha^{-1} (US$).

Inputs	Unit	U.C.	Quantity	S.C.
Equipment:				
PPE [†]	ud	36.93	0.10^{a}	3.69
Tractor [‡]	h	33.33	0.10^{a}	3.33
(1) Subtotal				7.03
Insecticides:				
Cartap hydrochloride 500 SP	kg	41.27	1.25^{b}	51.58
Cyantraniliprole 100 OD	L	87.47	0.45^{b}	39.36
Cyromazine 750 WP	kg	817.78	0.08^{b}	65.42
Spinosad 480 SC	L	264.00	0.17^{b}	44.88
Spinetoram 250 WG	kg	536.27	0.08^{b}	42.91
Acephate 750 SP	kg	32.00	0.62^{b}	19.84
Abamectin 18 EC	L	10.69	0.68^{b}	7.27
(2) Average cost of insecticide per application				38.75
Adjuvant				
(3) Mineral oil 856 g/L	L	4.27	0.5	2.13
(4) Cost of one spray: (1) + (2) + (3)				47.91
(5) Total control cost = (4) x 7 applications per crop				335.38

[†] The personal protective equipment (PPE) consisted of a respirator, protective eyewear, long pants, rubber boots, and chemical-resistant suit, gloves, and apron.

[‡] Operational cost of tractor +bar sprayer + labor + social charges.

[a] Value obtained according to the durability of the PPE and tractor.

[b] Value obtained according to the recommended dose of the insecticide.

Table 2. *Liriomyza huidobrensis* (larvae leaf^{-1}) density (D) in tomato crops (Lv) at the vegetative (V) and reproductive (R) stages. The decision making (Ct = control, Nc = Not control) for both sampling plans (Cv = conventional, Sq = sequential) is presented. For the sequential plan, the number of samples to reach a decision (NSq) and the time saving compared to the conventional plan (Ec) is also given.

Lv	St	D	NSq	Decision		Ec
				Cv	Sq	(%)
1	V	0.86	10	Nc	Nc	86.30
2	V	0.40	6	Nc	Nc	91.78
3	V	0.67	7	Nc	Nc	90.41
4	V	1.01	10	Nc	Nc	86.30
5	V	2.52	10	Nc	Nc	86.30
6	V	0.99	12	Nc	Nc	83.56
7	V	1.14	7	Nc	Nc	90.41
8	V	0.03	6	Nc	Nc	91.78
9	V	12.97	6	Ct	Ct	91.78
10	V	9.11	6	Ct	Ct	91.78
11	V	1.15	7	Nc	Nc	90.41
12	V	0.34	7	Nc	Nc	90.41
13	V	0.59	8	Nc	Nc	89.04
14	R	4.78	6	Ct	Ct	91.78
15	R	1.10	10	Nc	Nc	86.30
16	R	0.99	8	Nc	Nc	89.04
17	R	0.27	6	Nc	Nc	91.78
18	R	31.85	6	Ct	Ct	91.78
19	R	37.32	6	Ct	Ct	91.78
20	R	33.03	6	Ct	Ct	91.78
21	R	20.10	6	Ct	Ct	91.78
22	R	1.40	8	Nc	Nc	89.04
23	R	1.97	42	Nc	Nc	42.47
24	R	0.52	7	Nc	Nc	90.41
25	R	0.21	6	Nc	Nc	91.78
26	R	11.16	6	Ct	Ct	91.78
27	R	14.37	6	Ct	Ct	91.78
28	R	8.05	6	Ct	C	91.78
29	R	9.97	6	Ct	Ct	91.78
30	R	11.78	6	Ct	Ct	91.78
31	R	13.34	6	Ct	Ct	91.78
32	R	8.93	6	Ct	Ct	91.78
33	R	14.77	6	Ct	Ct	91.78
34	R	0.49	7	Nc	Nc	90.41
35	R	1.34	12	Nc	Nc	83.56
36	R	1.74	44	Nc	Nc	39.73
37	R	0.32	9	Nc	Nc	87.67
38	R	3.90	9	Ct	Ct	87.67
39	R	4.67	6	Ct	Ct	91.78
40	R	6.23	6	Ct	Ct	91.78
41	R	4.64	6	Ct	Ct	91.78
42	R	2.19	43	Nc	Nc	41.10
43	R	0.37	6	Nc	Nc	91.78
44	R	2.23	12	Nc	Nc	83.56
45	R	0.36	11	Nc	Nc	84.93
46	R	0.19	7	Nc	Nc	90.41

- Correct decisions in the decision making when adopting the sequential plan = 100.00%
- Average time saving = 86.75%
- Time saving in crops at the vegetative stage = 89.25%
- Time saving in crops at the reproductive stage = 85.76%

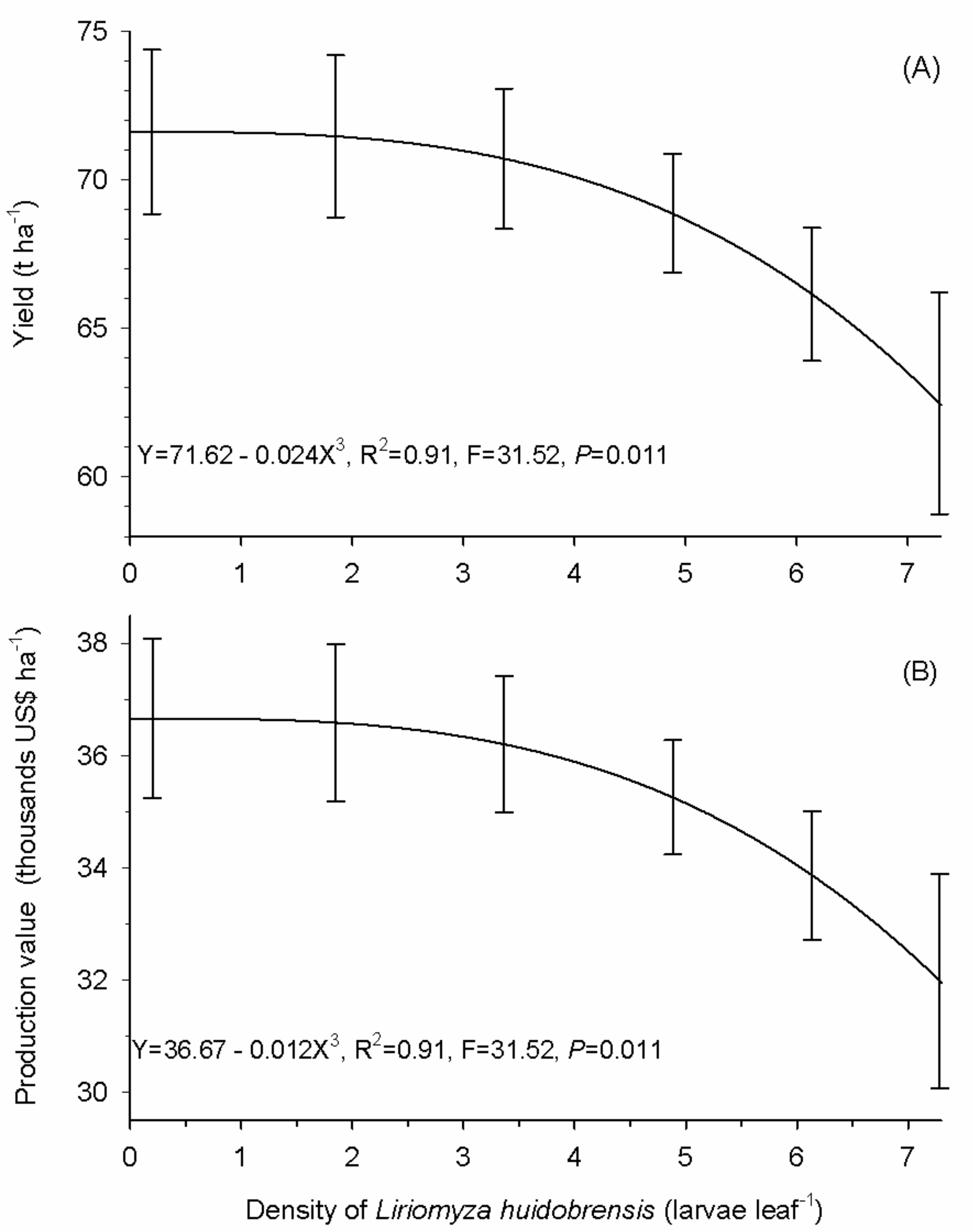

Figure 1. Curves (A) yield and (B) production value of tomato crops as a function density of *Liriomyza huidobrensis* larvae. The vertical line segments represent the confidence interval at 95% probability.

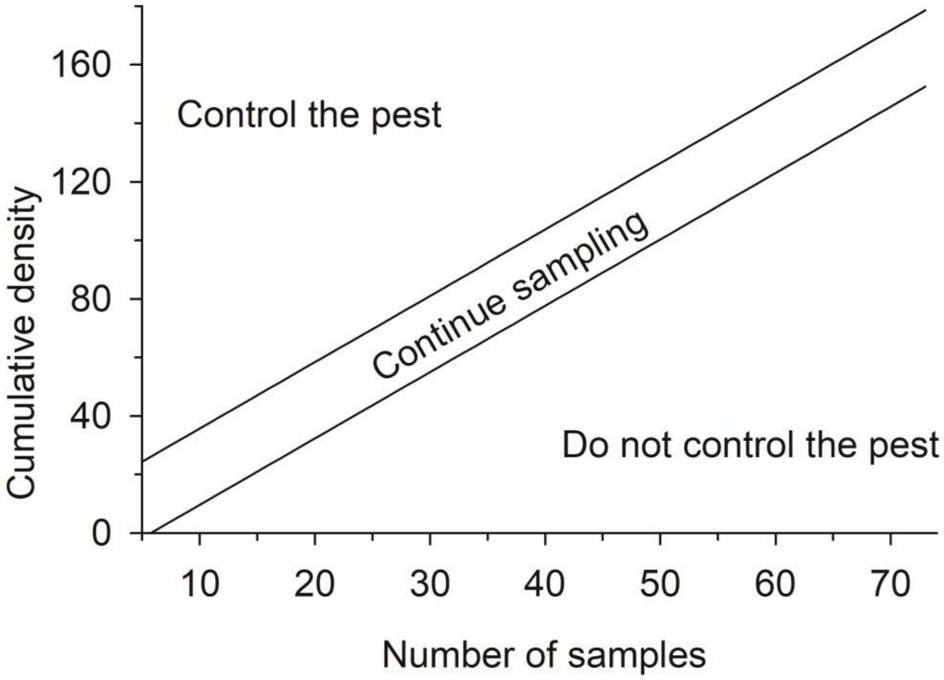

Figure 2. Decision-making limits of the sequential sampling plan for *Liriomyza huidobrensis* in tomato crops.

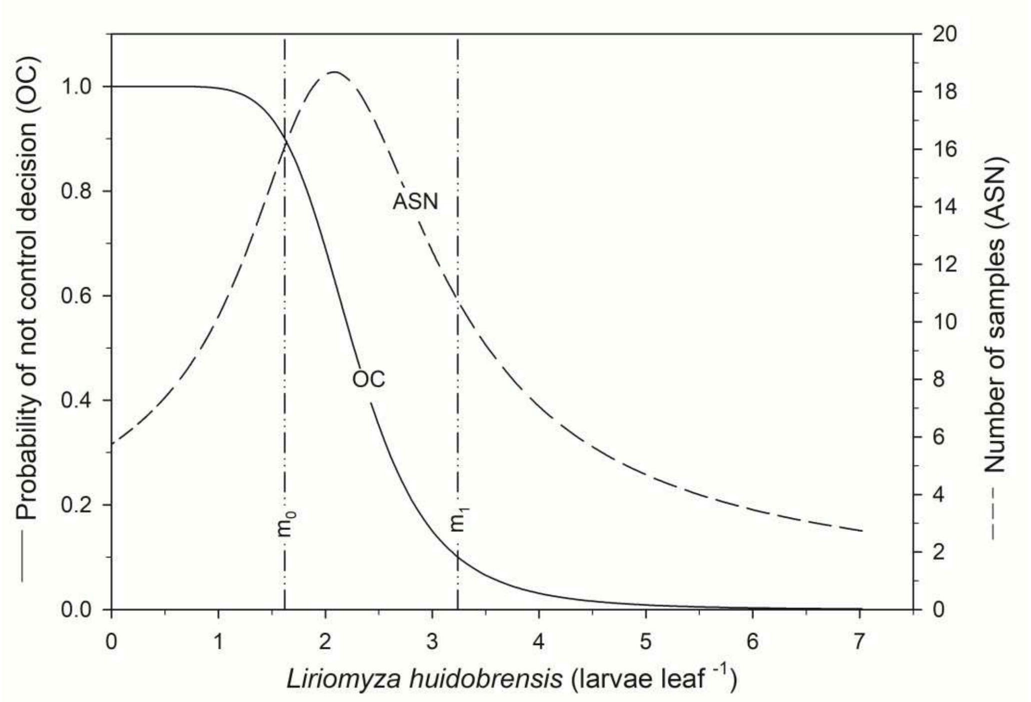

Figure 3. Validation of the sequential sampling plan for *Liriomyza huidobrensis* in tomato crops using the operational (OC) and average sample number (ASN) curves.

CONCLUSÕES GERAIS

Este estudo elucida os fatores de regulação populacional de *L. huidobrensis* e demonstra a importância da preservação de seus inimigos naturais. Nele também foram determinados sistemas de tomada de decisão para *L. huidobrensis.*

Os parasitóides *Diglyphus* sp. e *Opius* sp. são os principais fatores de regulação das populações de *L. huidobrensis* em cultivos de tomateiro.

O plano de amostragem convencional para *L. huidobrensis* em lavouras de tomate, é composto por 73 amostras. A amostra ideal para avaliação das populações de *L. huidobrensis* é a folha mais basal do terço mediano das plantas de tomate e nela avalia-se o número de minas com larvas. Este plano convencional é praticável por ser preciso, representativo, rápido (até 1 h: 25 min.) e de baixo custo (US$ 3.12 por amostragem).

O nível de dano econômico para *L. huidobrensis* em cultivos de tomate é de 3.24 larvas por folha. O plano de amostragem sequencial determinado neste trabalho toma decisões corretas com menor número de amostras que o plano de amostragem convencional.