MAÍRA QUEIROZ REZENDE

EXTRAFLORAL NECTARY-BEARING TREES ENHANCE PEST CONTROL AND INCREASE FRUIT WEIGHT IN ASSOCIATED COFFEE PLANTS

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

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APROVADA: 29 de agosto de 2014.

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(Coorientador)

José Henrique Schoereder

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Madelaine Venzon
(Orientadora)

Aos meus pais, Ibraim e Elza,
por terem me ensinado que o vôo já nasce dentro dos pássaros...
e me dado a coragem para voar.

“... o que é científico?

é aquilo que caiu nas redes reconhecidas pela confraria dos cientistas. Cientistas são aqueles que pescam no grande rio ....
Mas há também os céus e as matas que se enchem de cantos de sabiás ... Lá as redes dos cientistas ficam sempre vazias.*”*

Rubem Alves

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Resumo

REZENDE, Maíra Queiroz, D.Sc., Universidade Federal de Viçosa, agosto de 2014. **Árvores com nectários extraflorais controlam pragas e aumentam o peso do fruto em plantas de café associadas.** Orientadora: Madelaine Venzon. Coorientadores: Arnoldus Rudolf Maria Janssen e Angelo Pallini Filho.

Foi avaliado o efeito da presença de uma árvore que possui nectários extraflorais (Inga sp., Fabaceae) no controle do bicho-mineiro do cafeeiro (Leucoptera coffeella) e broca-do-café (Hypothenemus hampei) em cafeeiros sob sistemas agroflorestais. A disponibilidade de néctar em árvores de Ingá aumentou o parasitismo do bicho-mineiro e diminuiu os danos em plantas de café. Para elucidar os mecanismos envolvidos no controle das pragas do café em sistemas agroflorestais foi avaliado o efeito do néctar, como uma fonte de alimento, no fitness de inimigos naturais das pragas do café. Em laboratório, foi avaliado o efeito de um alimento rico em açúcar, semelhante ao néctar, na biologia de um tripes predador que foi encontrado dentro de frutos brocados de café e se alimentando nos nectários dos Ingás nos sistemas agroflorestais. Além disso, foi avaliado o efeito da predação do tripes na população de brocas no interior de frutos brocados. A sobrevivência do tripes aumentou com a fonte de alimento rica em açúcar. No entanto, o tempo de desenvolvimento das larvas aumentou e eles não atingiram a idade adulta, a não ser quando foram alimentados com a broca-do-café. A predação do tripes não foi suficiente para diminuir a população de brocas-do-café no interior dos frutos. O efeito do néctar extrafloral do Ingá na sobrevivência de inimigos naturais do bicho-mineiro também foi avaliado. A sobrevivência de quatro espécies de parasitoides e de uma espécie de crisopídio aumentou quando se alimentaram de néctar extrafloral. Por fim, foi realizado um experimento em campo para comparar a produção do café, os danos no cafeeiro e o parasitismo e a predação das pragas entre café em monocultivo e café consorciado com árvores de Ingá. Para avaliar o efeito da proximidade das árvores na proteção do café, a produção e o controle de pragas foram avaliados ao longo de transectos de 50 m se estendendo a partir das árvores Ingá. O peso dos frutos do café aumentou quando o café foi consorciado com árvores Ingá. Os danos causados pelo bicho-mineiro e pela broca-do-café também foram menores no café consorciado e aumentou com a distância das árvores. O parasitismo e predação das pragas do café não respondeu significativamente à presença das árvores, mas mostrou a tendência esperada. Portanto, árvores de Ingá com nectários extraflorais aumentam o controle natural de pragas e a produção em cultivos cafeeiros.

Abstract

REZENDE, Maíra Queiroz, D.Sc., Universidade Federal de Viçosa, August, 2014. **Extrafloral nectary-bearing trees enhance pest control and increase fruit weight in associated coffee plants.** Adviser: Madelaine Venzon. Co-advisers: Arnoldus Rudolf Maria Janssen and Angelo Pallini Filho.

The effect of the presence of an extrafloral nectary-bearing tree (Inga sp., Fabaceae) on the control of coffee leaf miner (Leucoptera coffeella) and coffee berry borer (Hypothenemus hampei) in agroforestry coffee systems was assessed. The availability of nectar from Inga trees increased parasitism of coffee leaf miner and decreased damage on coffee plants. To uncover mechanisms behind enhanced pest control in agroforestry systems we assessed the effect of nectar feeding on fitness of natural enemies of coffee pests. Through a laboratory experiment, we assess the effect of a sugar-rich food source resembling nectar on life-history traits of a predatory thrips that was found inside bored coffee fruits and feeding on extrafloral nectar of Inga trees during surveys in coffee agroforestry systems. Also, we assessed the effect of the thrips predation on coffee berry borer population inside the berries. The predatory thrips benefited from feeding on sugar-rich food through increased survival. However, the developmental time of larvae was increased and they did not reach adulthood unless fed on coffee berry borers. Thrips did not decrease the abundance of coffee berry borer inside the berries. The effect of nectar from Inga trees on natural enemies of coffee leaf miners was also assessed. Four parasitoid species and one green lacewing species increased their survival when feeding on extrafloral nectar. Subsequently, we performed a field experiment comparing coffee yield, coffee damage and parasitism and predation of coffee pests between coffee plots with or without Inga trees. To evaluate the effect of nectar source proximity on coffee protection, we also assessed pest control and production along transects of 50 m extending from the Inga trees. Coffee fruit weight was increased when coffee was consorted with Inga trees. Damage caused by coffee leaf miners and coffee berry borers were also lower in consorted coffee plants and increased with distance from the trees. Parasitism and predation of coffee pests did not increase significantly when coffee was associated with Inga trees, but showed the proper trends. Therefore, Inga trees bearing extrafloral nectaries enhanced natural pest control of pests and production in coffee crops.

General introduction

There is an eminent challenge of managing trade-offs between food production and the capacity of the environment to provide goods and services the in long term (Tilman, Cassman et al., 2002; Foley, 2005). Intensification of agriculture has impacted atmospheric constituents and climate, polluted ground water, eutrophicated rivers and lakes, increased erosion, decreased soil fertility and reduced biodiversity (Matson, Parton et al., 1997). Among practices adopted in conventional farming that have contributed to significant environmental deterioration, the use of synthetic pesticides to control pest populations is paramount (Van Der Werf, 1996). In particular, the negative effects of pesticide use on biodiversity has limited the success of biological control of pests (Landis, Douglas A., Wratten, Stephen D. et al., 2000; Geiger, Bengtsson et al., 2010). Moreover, beyond detrimental effects on natural enemies, pesticides can ultimately lead to increased pest outbreaks due to selection of resistant pest population (Whalon, Mota-Sanchez et al., 2008).

Therefore, alternative strategies of pest regulation have been studied aiming to achieve more sustainable agriculture (Landis, Douglas A., Wratten, Stephen D. et al., 2000; Gurr, 2003; Zehnder, Gurr et al., 2007). Habitat structure, composition and plant diversity are important factors influencing pest suppression and crop yield (Langellotto and Denno, 2004; Letourneau, Armbrecht et al., 2011). Increasing plant diversification in agricultural systems usually results in decreases of herbivore population, enhancement of natural enemy efficiency and suppression of crop damage (Bianchi, Booij et al., 2006; Letourneau, Armbrecht et al., 2011). Crop yield does not always increase in more diversified crops when compared to monocultures (Poveda, Isabel Gomez et al., 2008; Letourneau, Armbrecht et al., 2011). This effect, however, can be attributed to the reduced density of the main crop due to the presence of non-crop plants (Letourneau, Armbrecht et al., 2011). Several hypotheses have been put forward to explain how vegetation diversity can enhance pest control. Increased plant diversity may repel pests, disrupt the ability of the pest to locate the host plant and increase mortality of the pest due to enhancement of natural enemies (Root, 1973; Gurr, Wratten et al., 2003; Poveda, Isabel Gomez et al., 2008). Regarding natural enemies, the ecological mechanisms proposed so far to explain their aggregation and retention in more diversified habitats are the availability of refuges, favourable microclimatic conditions, the presence of alternative prey and access to alternative resources, such as pollen and nectar (Landis, Wratten et al., 2000; Gurr, 2003; Langellotto and Denno, 2004; Bianchi, Booij et al., 2006). Many natural enemies can feed on plant-provided food (Wäckers, 2005). Nectar, for example, is a sugar-rich food that can provide energy to natural enemies and it is expected to influence various life-history traits, such as development, longevity and survival (Eubanks and Styrsky, 2005; Koptur, 2005; Lundgren, 2009).

Flowering plants are often used in agricultural systems to improve biological control because it provides pollen and nectar to natural enemies (Tylianakis, Didham et al., 2004; Heimpel and Jervis, 2005; Balmer, Pfiffner et al., 2013; Lu, Zhu et al., 2014). However, floral nectar can be restricted to the short flowering period and may not be easily accessed because most natural enemies have short mouthparts (Jervis, 1998; Wäckers, 2004; Wäckers, 2005). Extrafloral nectaries, on the other hand, are more accessible and available during all stages of plant growth, thereby extending food availability (Wäckers, 2005). Plants bearing extrafloral nectaries are known to suffer less from herbivory than plants lacking these structures (Mathews, Brown et al., 2007). Extrafloral nectaries attract and arrest natural enemies that feed on nectar and protect those plants against herbivores (Whitney, 2004; Koptur, 2005; Marazzi, Bronstein et al., 2013). Hence, extrafloral nectaries are considered as an indirect plant defence (Sabelis, Van Rijn et al., 2005; Heil, 2008; Marazzi, Bronstein et al., 2013). There is some evidence that defense provided by extrafloral nectaries extends to the plant community (Atsatt and O'Dowd, 1976; Barbosa et al., 2009; Pemberton and Lee, 1996; Rudgers and Gardener, 2004). However, the effect of extrafloral nectary-bearing plants on herbivory of neighbouring plants is poorly studied (but see Brown, Mathews et al., 2010; Jezorek, Stiling et al., 2011; Jamont, Dubois-Pot et al., 2014). Assessing whether plants bearing extrafloral nectaries can provide protection to surrounding plants may help detect important elements of diversity. This may guide the selection of functional biodiversity for diversification of agroecosystems (Heimpel and Jervis, 2005; Heil, 2008).

Coffee is one of the most valuable commodities of the world and it is cultivated under high diversified agroforestry systems in many Latin American countries (Perfecto, Rice et al., 1996; Moguel and Toledo, 1999; Perfecto, Armbrecht et al., 2007; Jha, Bacon et al., 2011; Fao, 2012). These agroforestry systems are considered a refuge for biodiversity, which preserves regional ecological processes and provides important ecosystem services such as pest control (Perfecto, Rice et al., 1996; Perfecto, Armbrecht et al., 2007; Jha, Bacon et al., 2011; Jha, Bacon et al., 2014). The center of origin of coffee is believed to lie in Ethiopia, while plantation has its roots in the Near East (Jha, Bacon et al., 2011). Coffee evolved as a forest understory plant but agronomic intensification has transformed the coffee landscape from shade to sun coffee in many countries (Jha, Bacon et al., 2011). It is estimated that more than 40% of Latin American shade coffee farms were converted to low shade or open sun systems (Jha, Bacon et al., 2011). Nowadays, countries such as Peru, Haiti, Honduras, Guatemala, Mexico and El Salvador still cultivate most of their coffee beneath shade cover, while other countries such as Colombia have converted most areas to low shade or sun coffee (Moguel and Toledo, 1999; Peeters, Soto-Pinto et al., 2003; Jha, Bacon et al., 2011). In Brazil, 95% of the coffee is cultivated in monocultures under full sun (Jha, Bacon et al., 2011). Only recently, small-scale farmers were encouraged to develop agroforestry coffee systems and adopt agroecological practices, aiming to recover environmental and social harms caused by conventional agriculture (Cardoso, Guijt et al., 2001; Sales, Méndez et al., 2012; Souza, De Goede et al., 2012). The vegetation within the coffee agroforesty systems is managed based on compatibility with coffee, biomass production, labour intensity and production diversity (Souza, Cardoso et al., 2010). Neotropical trees belonging to the genus Inga Miller (Fabaceae) are common in coffee agroforestry systems, especially because some species fix nitrogen, which is a nutrient that limits production in tropical ecosystems (Soto-Pinto, Romero-Alvarado et al., 2001; Soto-Pinto, Villalvazo-López et al., 2007; Souza, Cardoso et al., 2010; Tully, Lawrence et al., 2012). Inga trees possess foliar nectaries and may therefore play an important role within coffee agroforestry systems providing resources to natural enemies and enhancing natural regulation of coffee pests. Whether plants bearing extrafloral nectaries can provide protection to surrounding plants and therefore be used to enhance crop protection is the major question of this research. To study this relationship we used coffee crop and the nectary-bearing Inga tree as a model.

In coffee agroforestry systems, we assessed the nectary visitors on Inga trees and the effect of nectar availability from the trees on protection of surrounding coffee plants from two major coffee pests, the coffee leaf miner Leucoptera coffeella (Guérin-Mèneville) (Lepidoptera: Lyonetiidae) and the coffee berry borer Hypothenemus hampei (Ferrari) (Coleoptera: Curculionidae: Scolytinae) (Chapter I). During the surveys of the coffee agroforestry systems, a predatory thrips was found feeding on extrafloral nectar of Inga trees and inside bored coffee fruits. Therefore, a laboratory experiment was performed to assess whether this predator benefited from a sugar-rich food source such as nectar, as well as examine how this could affect predation of coffee berry borers (Chapter II). To uncover underlying mechanisms that might explain improved biological control of coffee leaf miners in coffee plants near Inga trees, we assessed the effect of extrafloral nectar of the trees on survival of natural enemies of the coffee leaf miner (Chapter III). Finally, a field experiment was performed to test whether an extrafloral nectary-bearing tree can enhance crop protection against herbivores and result in increased coffee yield. Parasitism, predation, coffee damage and coffee yield were compared between replicated coffee plots with or without Inga trees. To evaluate the effect of nectar proximity on coffee protection, pest control and production along transects extending from the Inga trees were also assessed (Chapter IV).

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Chapter I[1]

Extrafloral nectaries of associated trees can enhance natural pest control

Abstract

Plant diversity may increase natural enemy populations because plants can provide alternative food to natural enemies. Extrafloral nectar is such an alternative food and plants producing extrafloral nectar are known to suffer less from herbivory. Little is known about the effect of plants with extrafloral nectaries on herbivory of neighbouring plants. Here, we investigated whether extrafloral nectaries of an associated tree (Inga subnuda subsp. luschnathiana) can enhance natural pest control in coffee agroforestry systems. We assessed the effect of nectar availability from Inga trees on parasitism of coffee leaf miners (Leucoptera coffeella) and on damage caused by leaf miners and coffee berry borers (Hypothenemus hampei). Most of the nectary visitors were either parasitoids or predators, with most predators being natural enemies of coffee pests. Coffee plants were sampled every meter along a transect of 10-15 m extending from each Inga tree. The distance of the coffee plants from the Inga trees did not significantly correlate with coffee leaf miner parasitism, proportion of mined leaves or with the proportion of bored coffee berries. We subsequently used abundance and species richness of nectar visitors that had no known association with the leaf miners and the borers as indirect measures of nectar availability. Whereas species richness had no significant effect, leaf miner parasitism increased significantly with the abundance of nectary visitors (excluding natural enemies of the coffee pests), and the proportion of mined leaves decreased significantly with this abundance. The proportion of bored fruits decreased with increasing abundance of visitors, but this trend was not significant. Together, these results suggest that Inga trees provide alternative food to natural enemies of coffee pests, resulting in increased natural control. Thus, extrafloral nectaries of associated trees can enhance natural pest control in agroforestry systems.

Key words: Associational defence, natural enemies, agroforestry systems, Leucoptera coffeella, Hypothenemus hampei, Inga subnuda subsp. luschnathiana

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[1] Data of this chapter were collected during my master’s degree studies. During the doctorate, new analyses revealed novel results, which were published in Agriculture, Ecosystem and Environment in March 2014 (http://dx.doi.org/10.1016/j.agee.2014.02.024).

Resumo

O aumento da diversidade de plantas pode aumentar a população de inimigos naturais devido ao fornecimento de recursos alternativos pelas plantas. O néctar é um desses alimentos alternativos e plantas que possuem nectários extraflorais são reconhecidas por serem mais protegidas contra herbivoria. No entanto, pouco se sabe sobre o efeito de plantas que possuem nectários extraflorais nos danos causados por herbívoros em plantas vizinhas. Neste estudo foi investigado se nectários extraflorais de uma árvore (Inga subnuda subsp. luschnathiana) associada ao café em sistemas agroflorestais pode contribuir para o controle natural de pragas do cafeeiro. Foi avaliado o efeito da disponibilidade de néctar em árvores de Ingá no parasitismo do bicho-mineiro do cafeeiro (Leucoptera coffeella) e nos danos causados pelo bicho-mineiro e pela broca-do-café (Hypothenemus hampei). A maioria dos artrópodes visitantes observados nos nectários era parasitoide ou predador, sendo que a maioria dos predadores foi reconhecida como inimigos naturais da broca-do-café. As plantas de café foram amostradas ao longo de um transecto que variou entre 10 e 15 metros. Cada transecto se iniciava a partir de uma árvore de Ingá, onde uma planta de café foi amostrada a cada metro. Não houve efeito significativo da distância do Ingá nas variáveis avaliadas. Então, foram usadas a abundância e a riqueza de visitantes nos nectários, que não tinham associação conhecida com o bicho-mineiro ou a broca-do-café, como uma medida indireta de disponibilidade de néctar. A riqueza de visitantes não apresentou qualquer relação significativa com o parasitismo ou com os danos no cafeeiro. No entanto, o parasitismo do bicho-mineiro aumentou e a proporção de folhas minadas diminui com a abundância de visitantes nos nectários (excluindo-se os inimigos naturais das pragas do café). A proporção de frutos brocados também diminuiu com a abundância de visitantes, mas essa tendência não foi significativa. Os resultados sugerem que árvores de ingá oferecem alimento alternativo para os inimigos naturais das pragas do café, resultando no aumento do controle natural. Portanto, nectários extraflorais de árvores em sistemas agroflorestais podem aumentar o controle natural de pragas.

Palavras-chave: defesa por associação, inimigos naturais, sistemas agroflorestais, Leucoptera coffeella, Hypothenemus hampei, Inga subnuda subsp. luschnathiana.

Introduction

Natural pest control is one of the most important ecosystem services and has been associated with increased habitat complexity (Bianchi, Booij et al., 2006). Diversified agroecosystems are shown to increase natural enemy abundance and enhance pest control (Langellotto and Denno, 2004; Bianchi, Booij et al., 2006). Herbivore abundance and crop damage are also lower in more diversified agroecosystems compared to lower diversity crops (Letourneau, Armbrecht, Salguero Rivera et al., 2011). The underlying ecological mechanisms that explain aggregation and improvement of natural enemies in more diversified habitats are not completely explored (Langellotto and Denno, 2004). The main explanation suggested so far is the availability of refuges, favourable microclimatic conditions, and the presence of alternative prey and food for natural enemies (Landis, D. A., Wratten, S. D. et al., 2000; Gurr, 2003; Langellotto and Denno, 2004; Bianchi, Booij et al., 2006).

Alternative food such as pollen and nectar is utilized by parasitoids and predators for survival in periods of prey scarcity (Landis, Menalled et al., 2005). Moreover, many predators can also reproduce on such alternative food (Wäckers, 2005). The availability of nectar and pollen from flowers consorted or adjacent to crops can increase natural enemy diversity and abundance, which may lead to reduction of herbivory on crop plants (Van Rijn, Van Houten et al., 2002; Tylianakis, Didham et al., 2004; Koptur, 2005). Nectar from extrafloral nectaries is also a food source for natural enemies (Bentley, 1977). Plants bearing extrafloral nectaries enhance the diversity and abundance of arthropod assemblages, which decreases herbivory and increases fitness of these plants (Cuautle and Rico-Gray, 2003; Kost and Heil, 2005; Mathews, Brown et al., 2007; Brown, Mathews et al., 2010). The nectar-feeding insects respond positively to increases in nectar flow rate and sugar content, adjusting their visitation frequency according to nectar availability (Heil, Koch et al., 2001; Ness, 2003; Schilman and Roces, 2003; Kost and Heil, 2005). In return to the food provided by the extrafloral nectaries, the natural enemies protect plants against herbivory (Whitney, 2004; Koptur, 2005). Extrafloral nectaries are therefore considered an indirect plant defence (Sabelis, Van Rijn et al., 2005). Elucidating how such indirect plant defence could be used for crop protection may help implement more sustainable practices for agroecosystem management (Heil, 2008).

Although extrafloral nectaries are thought to have community-level effects (Atsatt and O'dowd, 1976; Pemberton and Lee, 1996; Rudgers and Gardener, 2004;

Barbosa, Hines et al., 2009), little is known about the effect of nectaries on herbivory on neighbouring plants. If nectar availability increases the number of natural enemies, resulting in less herbivory on plants bearing extrafloral nectaries (Heil, Koch et al., 2001; Kost and Heil, 2005), neighbouring plants could also profit from the visitors (Jezorek, Stiling et al., 2011). Here, we investigated whether the presence of associated plants with extrafloral nectaries increases natural control of pests in an agroforestry coffee crop.

Coffee-based agroforestry systems are complex agroecosystems that usually contain a high diversity of tree species (Tscharntke, Clough et al., 2011). Trees can provide refuges for natural enemies and more diversified agroforestry systems are usually related to positive effects on coffee pest control (Armbrecht and Perfecto, 2003; Philpott, Arendt et al., 2008; Teodoro, Klein et al., 2009; Pardee and Philpott, 2011). Several plant species within coffee agroforestry systems possesses extrafloral nectaries, but one of the most common tree genera consorted with coffee and bearing foliar nectaries is Inga Miller (Fabaceae) (Soto-Pinto, Villalvazo-López et al., 2007; Souza, Cardoso et al., 2010). Coffee plants are attacked by at least 850 insect species, the major pests in the Neotropics being the coffee leaf miner Leucoptera coffeella (Guérin-Mèneville) (Lepidoptera: Lyonetiidae) and the coffee berry borer Hypothenemus hampei (Ferrari) (Coleoptera: Curculionidae: Scolytinae) (Le Pelley, 1973; Vega, Infante et al., 2009). Coffee leaf miners are small moths, whose larvae feed on the palisade parenchyma cells of coffee leaves; coffee berry borers mine galleries in coffee seeds (Souza, Reis et al., 1998; Vega, Infante et al., 2009). We investigated the effect of the presence of nectar-producing Inga trees and the visitors of extrafloral nectaries on the natural control of these two coffee pests.

Material and Methods

The study was conducted between January and May 2010 in five coffee agroforestry systems in the municipality of Araponga, state of Minas Gerais, Brazil (42°31'14" W, 20°40'01" S), located in the domain of the Atlantic Rainforest (Ab'sáber, 2003). The agroforestry systems form part of a long-term experimentation carried out by a non-governmental organization (Centre of Alternative Technologies of Zona da Mata) and local farmers since 1993 (Cardoso, Guijt et al., 2001). The diverse vegetation within the agroforesty systems is managed based on compatibility with coffee, biomass production, labour intensity and production diversity (Souza, Cardoso et al., 2010).

Several plant species consorted with coffee, such as Senna sp., Piptadenia gonoacantha (Mart.) J.F. Macbr. and Ricinus communis L., possess extrafloral nectaries. We chose Inga trees as a model because it is common in coffee agroforestry systems and farmers reported its capacity to help in coffee pest control. Hence, all systems studied here had Inga trees, but each agroforestry had a unique plant species composition (Souza, Cardoso et al., 2010). The management of these agroforesty systems was based on agroecological principles (Cardoso, Guijt et al., 2001). The region has a tropical highland climate with rainy summers and dry winters. Annual rainfall is 1200 - 1800 mm, the mean annual temperature is 18°C (Golfari, 1975; Engevix, 1995). The altitude of the studied agroforestry systems varies from 800 to 1070 m, with slopes up to 45% and the soil types are predominantly Oxisols (Golfari, 1975).

Abundance and richness of nectary visitors

Twenty-five Inga trees (Inga subnuda subsp. luschnathiana (Benth.) T.D. Penn.) were selected, five in each coffee agroforestry system. Thirty leaves per tree were checked for arthropods feeding on the nectaries during five minutes, every two hours, from 6 to 18h. All five trees in each study site were sampled during the same day. Visitors were collected in 70% ethanol for identification of morphospecies. For purposes of analysis, tree size (circumference at breast height) was scored in three classes: small: [5.0 - 31.6 cm); medium: [31.6 - 70.0 cm); large: [70.0 - 131.0 cm]. To evaluate whether abundance of natural enemies visiting the nectaries varied with the time of day (6 to 18h), we used linear mixed effects models (LMEs). Because it is known that visitation of nectaries by ants has a maximum around noon (Koptur, 1984), we entered both time of day and time of day squared as factors. The abundance of all visitors, parasitoids, ants and other predators were log(x+1)-transformed and used as response variables. The effect of tree size on the abundance and species richness of nectary visitors (excluding the natural enemies of the two coffee pests) was analyzed with a generalized linear model (GLM) with a Poisson error distribution.

Effect of Inga trees on coffee pest control

We aimed to elucidate the extent to which extrafloral nectaries result in aggregations of natural enemies and reduce damage of crop plants. The expectation was that coffee plants closer to the Inga trees would be more protected against herbivory.

We therefore assessed the proportion of herbivory and frugivory and the rate of parasitism of leaf miners on ten coffee plants on a transect extending from the Inga trees that were sampled for nectary visitors (above). Coffee plants were sampled at intervals of one meter, starting with the plant closest to the Inga tree. When a coffee plant did not have leaves or berries, we sampled an extra plant, thus extending the transect with one meter for each plant without leaves or berries. This resulted in 5 transects per field site (one per tree) extending 10 to 15 m from the Inga trees. It was not possible to assess the effect of Inga trees beyond this range because of the limited distances among the trees.

From each designated plant on the transect, the damage caused by coffee leaf miners was assessed by collecting 20 leaves distal from the fifth pair of leaves from four different branches per coffee plant (Souza, Reis et al., 1998). The four branches were located in the centre of the shrub and pointed to the four cardinal directions (Souza, Reis et al., 1998). A total of 5000 coffee leaves were sampled (200 per transect) and the proportion of mined leaves was assessed.

To estimate the damage caused by the coffee berry borer, nine fruits were randomly collected from the top, centre and lower parts of the plants per designated coffee plant on each transect (Souza and Reis, 1997). We sampled fully developed green, yellow and red fruits, which are all suitable for attacks by the coffee berry borer (Vega, Infante et al., 2009). A total of 2250 fruits were sampled (90 per transect) and the proportion of bored fruits per coffee plant was assessed.

To assess the parasitism rate of leaf miners, one mined leaf was collected from each coffee plant along the transects (10 leaves per transect, summing to a total of 250 mined leaves). We chose leaves with intact mines, thus assuring that the leaf miners had not been attacked by predatory wasps and that parasitoids had not emerged yet. Each mined leaf was incubated in a separate Petri dish in the laboratory until the emergence of leaf miners or parasitoids. The petioles of the leaves were inserted in water to maintain turgidity (Pereira, Picanço et al., 2007). The parasitism rate was calculated by dividing the total number of emerged parasitoids by the total number of insects that emerged from the mines (parasitoids and coffee leaf miners) per coffee plant.

Visitation by nectar-feeding insects is positively correlated to nectar production (Ness, 2003; Schilman and Roces, 2003; Kost and Heil, 2005). Therefore, we used abundance and species richness of visitors as a measure of nectar availability. This can potentially lead to spurious correlations, i.e. when more natural enemies of coffee berry borers and coffee leaf miners are present in the field for whatever reason, they are also more likely to be found visiting the nectaries. To circumvent this problem, we specifically excluded those visitors known to be natural enemies of the coffee borers and coffee leaf miners. Hence, the assessment of the stand-in measure of nectar availability was independent of the abundance and species richness of the natural enemies of the coffee pests studied here.

The effect of Inga trees on the parasitism rate of coffee leaf miners, the proportion of mined leaves and bored fruits was analyzed using linear mixed effects models (LME of the package nlme) (Pinheiro, Bates et al., 2010). These response variables were square-root transformed to stabilize variance. We used the following tree characteristics and their interactions as factors: distance of the coffee plant to the tree, tree size, and the abundance and species richness of the nectary visitors (but excluding the natural enemies of the two coffee pests). The data were analyzed for each of the response variables separately. Because coffee plants were sampled at various distances from the Inga trees, tree identity was treated as a random factor. We first formulated a full model and then removed non-significant interactions and factors using the ANOVA function of R (2010). Factor levels were compared through a post-hoc analysis by grouping factor levels (Crawley, 2007). All analyses were performed using R software (R Development Core Team, 2011) and residuals were analysed to check for the suitability of the models and distributions used (Crawley, 2007).

Results

Abundance and species richness of nectary visitors

We collected 287 visitors of 79 morphospecies feeding on extrafloral nectaries of Inga trees (Appendix A and Appendix B). They belonged to the classes Arachnida and Insecta. Within the Insecta, we identified seven orders, which included natural enemies such as parasitoids, ants and other generalist predators. Parasitoids represented 16.7% of the visitors, predators accounted for 59.9% of the visitors, with most of them being ants (53.3%). The other 23.4% consisted of pollinators such as bees (0.7%), herbivores (4.5%) and other unidentified species. Sixteen of the recorded predators were reported before as predators of either coffee leaf miners or coffee berry borers (Appendix 2). The thrips Trybomia sp. (Thysanoptera: Phlaeothripidae) that was found visiting extrafloral nectaries of Inga trees was observed inside bored coffee fruits and feeding on coffee berry borers, which was never reported before.

The abundance of nectary visitors varied significantly with the time of day and was highest at noon (LME, time of day (h): Likelihood ratio = 8.38, d.f. = 1, P = 0.0038; time of day squared (h^2): L-ratio = 6.62, d.f. = 1, P = 0.01). This was mainly caused by the high abundance of ants during midday (Figure 1: LME, L-ratios = 6.96 and 7.01, d.f.s = 1, Ps = 0.0083 and 0.0081 for h and h^2, respectively). Parasitoids were more abundant at the end of the day (Figure 1: LME, L-ratios = 15.29 and 0.32, d.f.s = 1, Ps = 0.0001 and 0.574 for h and h^2, respectively). The abundance of other generalist predators did not vary with the time of day.

The abundance and species richness of nectary visitors varied significantly with tree size (Figure 2: GLM, Abundance: deviance = 22.95, d.f. = 2.22, P < 0.001; Richness: deviance = 11.79, d.f. = 2.22, P = 0.0028). Trees of intermediate size harboured the highest abundance and species richness of nectary visitors.

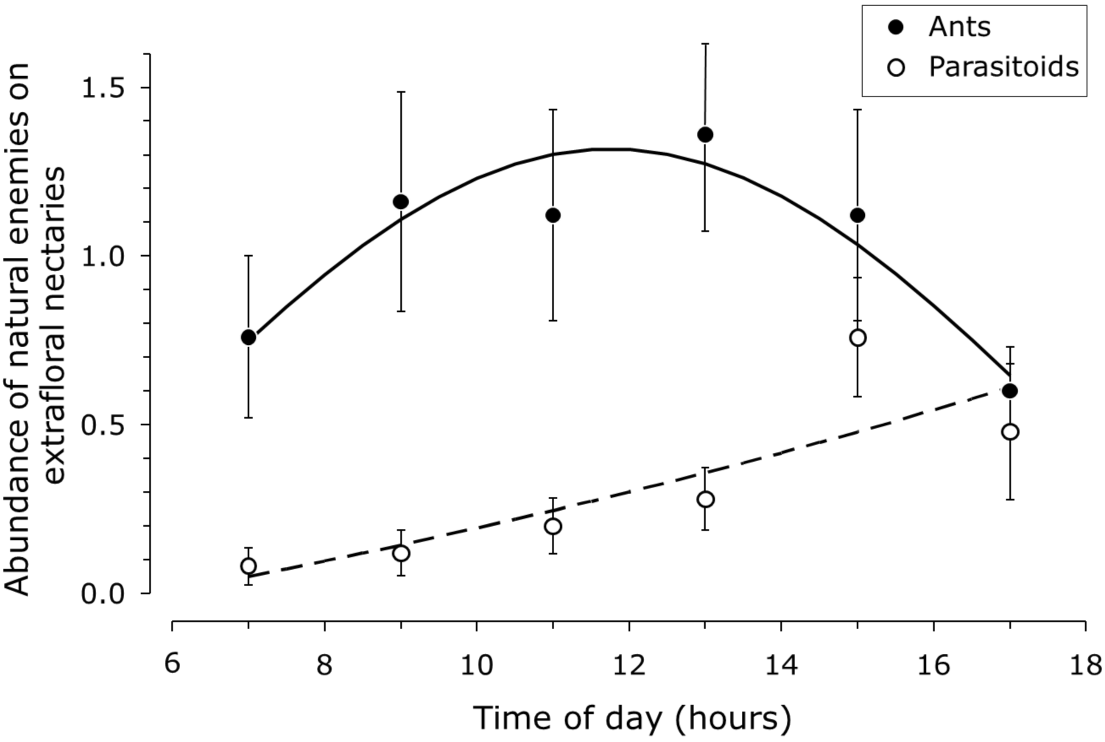

Figure 1. Average number of ants (closed circles) and parasitoids (open circles) visiting extrafloral nectaries of Inga trees throughout the day. The curves were fit to the averages shown here using a linear model with time of day and time of day squared as factors. Ant and parasitoid abundance varied significantly with time of day (ants: $y = \exp(a + bx + cx^2) - 1$, with a = -0.878, b = 0.292 and c = -0.0124, $R^2 = 0.894$, $F_{2,3} = 12.61$, P = 0.035; parasitoids: $y = \exp(a + bx) - 1$, with a = 0.251 and b = 0.043, $R^2 = 0.746$; $F_{1,4} = 11.76$; P = 0.027).

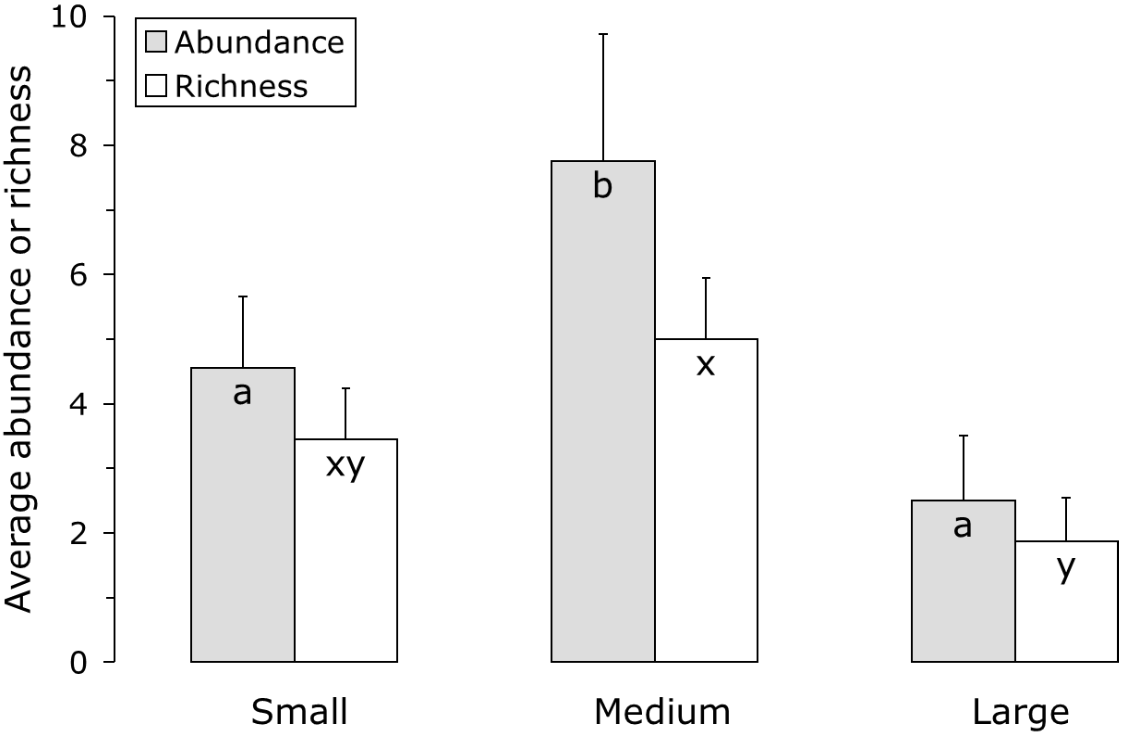

Figure 2. The average (+ s.e.) abundance (grey bars) and species richness (white bars) of visitors of Inga nectaries as a function of tree size. Tree size is based on circumference at breast height and was scored in three classes: small: [5.0 - 31.6 cm); medium: [31.6 - 70.0 cm); large: [70.0 - 131.0 cm]. Within abundance or richness, bars with different letters differ significantly ($P < 0.05$).

Effect of Inga trees on coffee pests control

The proportion of leaves with mines significantly decreased with increasing abundance of nectary visitors (Figure 3, LME, Likelihood ratio = 4.98, d.f. = 1, P = 0.026) and was affected by the size of the tree (Figure 4, LME, L-ratio = 7.74, d.f. = 2, P = 0.021), but there was no significant interaction between these two factors. The proportion of mined leaves was smallest near small trees (Figure 4). Species richness of the nectary visitors and distance from the Inga tree had no significant effects (LME, L-ratios = 0.16, 0.007, d.f.s = 1,1, P = 0.69, 0.93, respectively).

The proportion of bored fruits was not significantly affected by tree size, distance from the tree, or the abundance and species richness of nectary visitors or their interactions (LME, L-ratios = 0.19, 0.63, 1.10, 0.07 d.f.s = 1, 2, 1, 1, P = 0.91, 0.43, 0.29, 0.79, respectively), but there was a trend of the proportion of damaged fruits decreasing with increasing abundance of nectar visitors.

We recorded 104 parasitoids of seven different species from the mined coffee leaves (Table 1). The parasitism rate of coffee leaf miners increased significantly with abundance of nectary visitors (Figure 5: LME, L-ratio = 6.46, d.f. = 1, P = 0.011), and there was no significant effect of species richness, tree size, distance or their interactions (LME, L-ratios = 0.70, 2.50, 0.051, d.f. = 1, 2, 1, P = 0.40, 0.29, 0.82, respectively).

Table 1. Identity and number of parasitoids emerged from mined leaves of coffee plants collected in agroforestry systems (n=5).

Parasitoids of coffee leaf-miner	Species distribution[1]	Abundance
Stiropius reticulatus Penteado-Dias, 1999	4	52
Centistidea striata Penteado-Dias, 1999	2	3
Cirrospilus sp. indet.	1	1
Closterocerus coffeellae Ihering, 1914	4	7
Entedoninae	1	1
Horismenus sp. aff. inflatus	4	14
Proacrias coffeae Ihering, 1914	4	26
Total abundance		104

[1] The number of agroforestry systems (n=5) in which the species occurred.

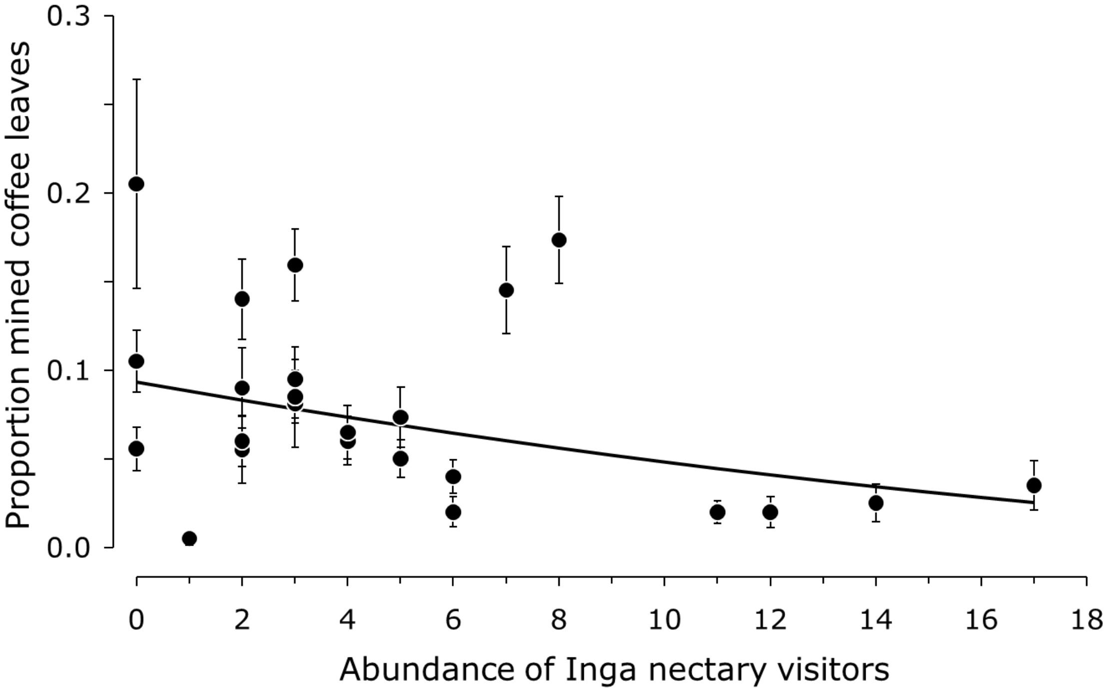

Figure 3. The proportion of coffee leaves mined as a function of the number of nectary visitors on a nearby Inga tree. Shown are averages (± s.e.) per tree, the curve was fitted to these averages for illustrative purposes ($y = a^2 + 2abx + b^2x^2$, with a = 0.305 and b = -0.0085, R^2 = 0.160, $F_{1,23}$ = 4.36, P = 0.048).

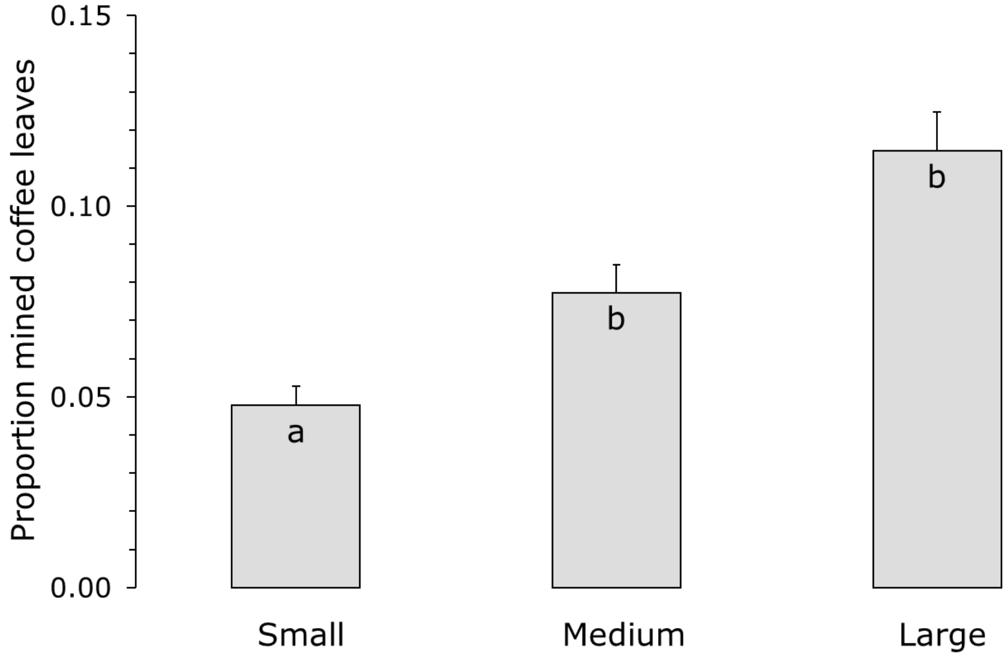

Figure 4. The average proportion (+ s.e.) of mined coffee leaves as a function of the size of the nearby Inga tree. Bars with different letters differ significantly ($P < 0.05$). See the legend to Figure 2 for tree size classes.

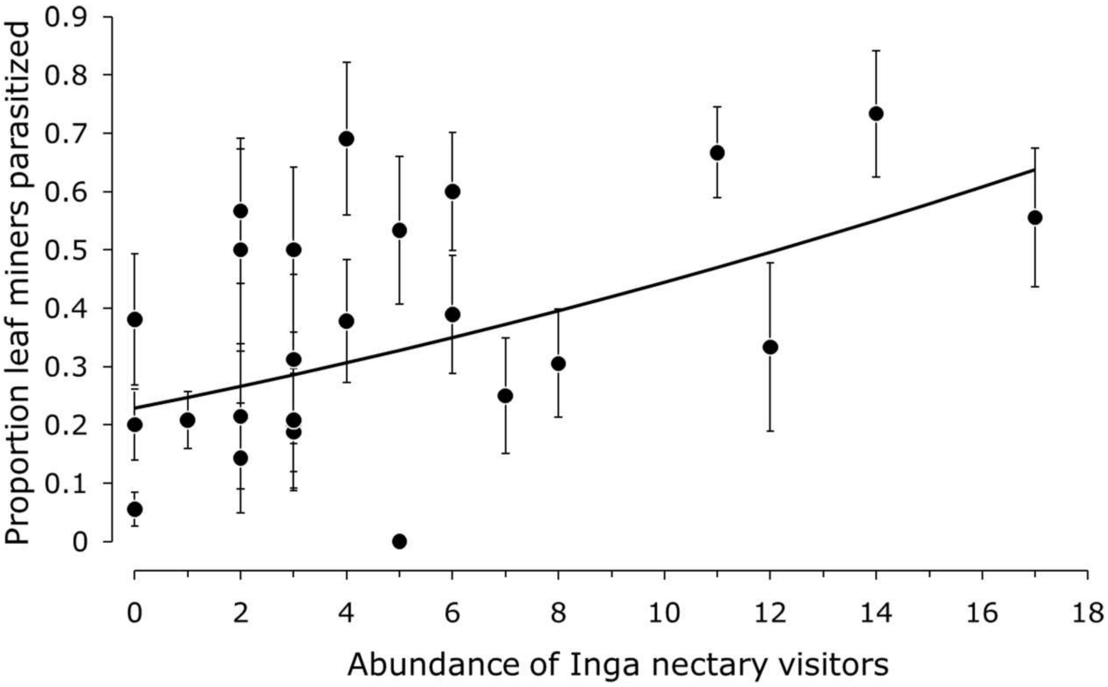

Figure 5. The proportion of coffee leaf miners parasitized on coffee plants as a function of the number of nectary visitors on nearby Inga trees. Shown are averages (± s.e.) per tree, the curve was fitted to these averages for illustrative purposes ($y = a^2 + 2abx + b^2x^2$, with $a = 0.479$ and $b = 0.019$, $R^2 = 0.176$, $F_{1,23} = 4.91$, $P = 0.037$).

Discussion

Alternative food such as nectar and pollen can increase longevity, fecundity, dispersal capacity and host-finding efficacy of natural enemies (Lewis, Stapel et al., 1998; Tylianakis, Didham et al., 2004; Jamont, Crepelliere et al., 2013). Natural enemies may aggregate around higher quality patches and the increased availability of food may also result in an increased numerical response of the natural enemy populations (Langellotto and Denno, 2004). As a result, herbivore populations on plants offering non-prey resources to natural enemies may suffer increased attacks from natural enemies compared to herbivores on plants that do not supply such resources (Sabelis, Van Rijn et al., 2005). For example, plants that produce extrafloral nectar suffer less from herbivory (Kost and Heil, 2005; Mathews, Brown et al., 2007; Brown, Mathews et al., 2010). The foraging of the nectar visitors, however, is not necessarily restricted to the plants possessing nectaries, but they could also attack herbivores on neighbouring plants (Jezorek, Stiling et al., 2011). We found here that coffee plants, which do not bear extrafloral nectaries, indeed seem to profit from the vicinity of nectary-possessing Inga trees.

Because proximity to floral resources was found to increase abundance of natural enemies, parasitism and predation rates and to decrease pest populations (Tylianakis, Didham et al., 2004; Lavandero, Wratten et al., 2005; Letourneau, Armbrecht, Salguero Rivera et al., 2011), and similar effects were observed with extrafloral nectaries (Jazorek et al. 2011), we expected comparable effects for extrafloral nectaries of Inga trees. However, the distance of the coffee plants from the Inga trees did not affect the parasitism rate of coffee leaf miners or the damage of coffee leaves and fruits, in agreement with studies on other agroecosystems with nectary-bearing plants (Brown, Mathews et al., 2010). Perhaps the Inga trees in our study system were too close to each other, limiting the possibilities to detect an effect of distance from the trees.

It is known that nectaries that produce more nectar attract and arrest more nectar-feeding insects (Schilman and Roces, 2003). Hence, the density of nectary visitors is expected to correlate with nectar production. We therefore used the number and species richness of visitors to nectaries as a stand-in measure for nectar production. By excluding those visitors that are known natural enemies of the two pests of coffee plants that we studied here, we avoided spurious correlations. Our results show that the abundance of nectary visitors was positively correlated with parasitism rate of coffee leaf miners (Figure 5) and negatively correlated with damage caused by coffee leaf miners (Figure 3), suggesting that nectar availability affected natural control of this coffee pest. Associational defence mediated by extrafloral nectaries of neighbouring plants has been found in natural systems (Jezorek, Stiling et al., 2011) and tested in agroecosystems, although most studies did not demonstrate increased crop protection (Spellman, Brown et al., 2006; Brown and Mathews, 2008; Brown, Mathews et al., 2010).

The relationship between nectary visitors and damage caused by coffee leaf miners and coffee berry borers showed the same trend, but the negative correlation between number of nectary visitors and damaged fruits was not significant. One possible explanation is that some of the most important natural enemies of the coffee berry borer are ants (Gonthier, Ennis et al., 2013), which have a stronger mutualistic relationship with extrafloral nectaries. Thus, in the presence of extrafloral nectaries, they may spend less time foraging for coffee berry borers. However, they must forage for protein and the nectaries do not provide this. Another explanation is that ants are known to tend nectaries and deter other natural enemies (Koptur, 1984; Rudgers and Gardener, 2004; Rosumek, Silveira et al., 2009), which could impair pest control by the latter. However, we actually found a positive correlation between the numbers of ants visiting a leaf with nectaries and the number of other visitors of that leaf on the same day (LME, L-ratio = 6.49, d.f. = 1, $P = 0.011$). Hence, ants do not exclude other visitors, but maybe the latter avoid competition and other interactions with ants by visiting the nectaries at other periods of the day than ants do (Figure 1). Besides the competition among visitors, the variation in abundance of ants and parasitoids on the nectaries observed during the day could also be related to variation in nectar flow rate, sugar concentration or nectar viscosity (Koptur, 1985; Schilman and Roces, 2003; Rudgers and Gardener, 2004; Nicolson, 2007). Koptur (1984) observed that the nectar secretion of Inga species did not vary with time of day, but ants visited the nectaries more frequently around noon, when the sugar concentrations in the nectar were higher due to water evaporation. Ants probably responded to the sugar concentration because they are more adapted to feed on nectar with higher viscosity (Nicolson, 2007). In contrast, the mouthparts of parasitoids allow consumption of more liquid nectar (Jervis, 1998). Hence, nectaries with a higher production are more visited by ants and other visitors, but competition among visitors and nectar properties may affect diurnal patterns of visitation. Clearly, the effects of Inga extrafloral nectaries on the interactions among natural enemies and its effects on coffee pest control needs further study. Nevertheless, we found a positive effect of increased nectary visitation on natural control of coffee pests.

Besides the availability of extrafloral nectaries, it is likely that other factors affected pest control (Bianchi, Booij et al., 2006; Vandermeer, Perfecto et al., 2010).

For example, coffee berry borer density has been reported to decrease with relative humidity, canopy cover and tree diversity and increase with temperature (Teodoro, Mein et al., 2008). The incidence of coffee leaf miners was also found to decrease with increased relative humidity, canopy cover and agroforestry complexity and increase with temperature (Teodoro, Mein et al., 2008; Lomeli-Flores, Barrera et al., 2010). Agroforestry systems may affect all these parameters, for example, the shading by trees may reduce average temperature, but increase humidity and canopy cover. Higher mortality rates of leaf miners are also related to rainfall (Pereira, Picanço et al., 2007), although another study showed mined leaves to be more abundant during the rainy season (Lomeli-Flores, Barrera et al., 2010). All in all, Inga trees will affect coffee pest control not only due to nectar provision, but also because trees provide shelter from rainfall and modify local temperature and humidity (Lomeli-Flores, Barrera et al., 2010). Although all the study systems here were agroforests, there are undoubtedly variations in these abiotic factors. We nevertheless found effects of extrafloral nectaries on coffee pests, suggesting that the nectaries are an important factor of the agroforestry systems. As a next step, manipulative field experiments are needed to confirm that the increased natural control is indeed caused by the presence of plants bearing nectaries.

We found the highest abundance and species diversity on nectaries of trees of intermediate size. This may have been caused by small trees possessing low numbers of leaves, and large trees possessing many old leaves that no longer produce nectar (Koptur, 1984). The proportion of mined leaves was lowest in coffee plants near small Inga trees (Figure 4), but it has to be realized that part of these mines contained parasitized leaf miners (Figure 5). Because leaf miner parasitism was not assessed for all leaves examined, we cannot directly assess whether there are significant differences in the proportions of coffee leaves containing unparasitized leaf miners. This is a subject of further studies.

Interplanting crops and plants that supply alternative food stimulates diversity and effectiveness of natural enemies (Landis, Menalled et al., 2005). Whereas effects of floral resources on pest control defence are well studied (Koptur, 2005), the effects of extrafloral nectaries on natural control are not. However, plants with extrafloral nectaries may be even more suitable for crop protection than flowering plants because they excrete higher rates of nectar and produce nectar over more extended periods than floral nectaries (Wäckers, 2005; Pacini and Nepi, 2007). The availability of extrafloral nectar might be an important cause of the aggregation and efficiency of natural enemies in complex habitats. We suggest that plants bearing extrafloral nectaries can provide associational defence to neighbouring crop plants without such nectaries and crop protection strategies using intercropping of plants with extrafloral nectaries therefore deserve more attention.

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Chapter II

Sugar-rich food affects predatory thrips and coffee berry borer interactions

Abstract

Plant-provided food may improve the fitness of omnivorous predators and thus increase natural pest control. Trees that bear extrafloral nectaries can provide alternative food to natural enemies, and this can help decrease damage caused by herbivores on these trees and on surrounding plants. We have found a predatory thrips (Trybomia sp.) feeding on extrafloral nectaries of Inga trees (Inga sp.) in coffee agroforestry systems and inside bored coffee fruits. We investigated whether the predatory thrips was able to feed on coffee berry borers (Hypothenemus hampei) and how a sugar-rich food source affected its survival, development and predation rate. To assess life-history parameters and predation efficiency, we conducted an experiment where immature thrips were placed into arenas made of Inga sp. leaf discs containing one of the four diets: water, honey solution, coffee berry borers plus water and coffee berry borers plus honey solution. Survival, development and number of coffee berry borer predated were assessed daily. To evaluate the ability of thrips to feed on coffee berry borer inside coffee fruits we compared number of coffee berry borers within fruits exposed and unexposed to the thrips. The predatory thrips benefited from feeding on sugar-rich food by increasing survival. However, the developmental time of larvae also increased and they did not reach adulthood unless fed on coffee berry borers. Predatory thrips that fed on coffee berry borer oviposited, and their survival rate was higher than thrips that fed only on honey solution. In addition, provision of alternative food reduced the number of coffee berry borers consumed by the thrips. Moreover, the presence or absence of thrips had no measurable effect on the infestation by coffee berry borers. The omnivorous thrips may benefit from feeding on extrafloral nectar of Inga trees and on prey inhabiting the surrounding plants (i.e., coffee). Therefore, Inga trees may favor the recruitment of natural enemies into coffee plantations and enhance their fitness by providing them with an important source of energy (nectar). Despite its low predation rate, the thrips might provide additional level of coffee berry borer regulation in concert with other natural enemies.

Keywords: Nectar, extrafloral nectaries, agroforestry systems, natural enemies, predator, Thysanoptera.

Resumo

Alimentos alternativos podem melhorar o fitness de predadores onívoros e contribuir para o controle natural de pragas. Plantas que possuem nectários extraflorais podem oferecer alimento alternativo para inimigos naturais e contribuir para a diminuição de danos causados por herbívoros em plantas adjacentes. Em cafeeiros cultivados em sistemas agroflorestais foram observados tripes predadores (Trybomia sp.) dentro de frutos de café brocados e também se alimentando em nectários extraflorais de Ingá (Inga sp.). Por isso, investigamos se esse tripes predador é capaz de predar a broca-do-café (Hypothenemus hampei) e se uma fonte de alimento rica em açúcar pode afetar a sua sobrevivência, seu desenvolvimento e a taxa de predação. Foram realizados experimentos com larvas de tripes em arenas de folhas de Inga sp. contendo uma das seguintes dietas: água, solução de mel, broca-do-café e água, broca-do-café e solução de mel. A sobrevivência e o desenvolvimento dos tripes e o número de brocas predadas foram registrados diariamente em cada tratamento. Para avaliar a capacidade do tripes de se alimentar da broca-do-café dentro do fruto brocado, foi comparado o número de brocas dentro de frutos brocados expostos ou não ao tripes. Os resultados mostraram que os tripes se beneficiam do alimento rico em açúcar, pois tripes que se alimentaram de solução de mel tiveram maior sobrevivência comparado ao controle. No entanto, o tempo de desenvolvimento das larvas foi maior e somente os tripes que se alimentaram de brocas-do-café alcançaram a fase adulta. Tripes que se alimentaram de brocas chegaram a ovipositar e sua sobrevivência foi maior quando comparada aos tripes que se alimentaram somente da solução de mel. Além disso, tripes que se alimentaram de mel predaram um menor número de brocas-do-café. O número de brocas-do-café dentro dos frutos brocados não foi influenciado pela presença ou ausência do tripes. É possível que o tripes onívoro associado às árvores de Ingá se beneficie do néctar e de presas presentes em plantas vizinhas (ex. café). Dessa forma, as árvores de Ingá podem contribuir para o estabelecimento de inimigos naturais em cafezais e fornecer a eles um alimento rico em energia (néctar) que pode influenciar positivamente seu fitness. Apesar da baixa taxa de predação, é possível que os tripes contribuam para a regulação natural da broca-do-café juntamente com outros inimigos naturais.

Palavras-chave: Néctar, nectários extraflorais, sistemas agroflorestais, inimigos naturais, predador, Thysanoptera.

Introduction

The regulation of herbivore populations by natural enemies is one of the most important ecosystem services provided to humans, and generalist predators play an important role in natural pest control (Symondson, Sunderland et al., 2002; Letourneau, Jedlicka et al., 2009). This is in part because many of them can benefit not only from prey but also from alternative food sources provided by plants (Coll and Guershon, 2002; Symondson, Sunderland et al., 2002). Their omnivorous feeding habits allows them to exploit a wider range of food, thus reducing the likelihood of starvation or emigration from crops when herbivorous arthropods are at low densities (Coll and Guershon, 2002).

Alternative food sources such as nectar are thought to have coevolved in a mutualistic relationship with natural enemies, including omnivorous predators (Heil, 2008; Agren, Stenberg et al., 2012). For example, extrafloral nectaries, which are specialized tissues that secrete nectar, can help protect plants against herbivory (Koptur, 2005). Nectar is an aqueous solution of sugar, but also contains amino acids and other organic compounds (Nicolson and Thornburg, 2007). This source of energy is expected to influence various life-history traits of omnivorous predators, such as development, longevity and survival (Eubanks and Styrsky, 2005; Van Rijn and Sabelis, 2005). The expected improved fitness of natural enemies leads to higher predator densities, which could help prevent or reduce herbivory on neighboring plants (Harmon, Ives et al., 2000; Eubanks and Styrsky, 2005). Thus, plants that offer an alternative food source may provide associational defense to surrounding plants and therefore can be used to increase natural pest control in agroecosystems (Harmon, Ives et al., 2000; Heil, 2008; Barbosa, Hines et al., 2009; Rezende, Venzon et al., 2014). Here we investigated whether an omnivorous predator benefits from a sugar-rich food source, and examine how this could affect natural pest control in coffee crops.

Coffee is one of the most important crops worldwide, and it is attacked by several arthropod pests (Le Pelley, 1973). The coffee berry borer Hypothenemus hampei (Ferrari) (Coleoptera: Curculionidae: Scolytinae) is a serious pest that bores galleries into the endosperm of coffee berries causing significant yield losses (Damon, 2000; Jaramillo, Borgemeister et al., 2006; Vega, Infante et al., 2009). Management of coffee berry borers is rather difficult because they spend most of their life cycle inside coffee fruits, where few natural enemies have access. Therefore, little success has been achieved in biological control of this coffee pest (Vega, Infante et al., 2009). Most known predators of coffee berry borers are ants, which provide natural control by preventing the borer from damaging coffee fruits, removing adults from inside the berries and feeding on immatures (Armbrecht and Gallego, 2007; Vega, Infante et al., 2009; Larsen and Philpott, 2010; Gonthier, Ennis et al., 2013). Recently, adults of a predatory thrips Karnyothrips flavipes Jones (Thysanoptera: Phlaeothripidae) were reported feeding on immature stages of coffee berry borer (Jaramillo, Chapman et al., 2010). Thrips have a broad diet and opportunistic predation is widespread among many species of Thysanoptera (Mound, 2005). Predatory thrips have been recorded feeding on mites, whiteflies, lepidopteran eggs, scales and other thrips, and some species have been recognized as potential biological control agents (Hoddle, Robinson et al., 2000; Kakimoto, Inoue et al., 2006; Jaramillo, Chapman et al., 2010).

In coffee agroforestry systems in southeast Brazil, thrips were documented feeding on extrafloral nectaries of Inga trees (Inga spp., Fabaceae), a common tree among these systems, and observed inside coffee fruits infested by the berry borer (Rezende, Venzon et al., 2014). The species of thrips observed belongs to the genus Trybomia (Thysanoptera: Phlaeothripidae) and a preliminary taxonomic evaluation (Mound, unpublished) suggests that it could be a new species. In preliminary trials in the laboratory, we found that this Trybomia sp. is able to feed on coffee berry borer eggs, larvae, pupae and adults outside berries. However, its potential to control coffee berry borers under more natural conditions (i.e. inside coffee berries) is not known. In this study we investigated the suitability of a sugar-rich food (resembling extrafloral nectar) and coffee berry borer as food sources for Trybomia sp.. We quantified thrips survival and development on these foods and the predation rate on coffee berry borers. We also investigated whether predation by Trybomia sp. would reduce coffee berry borer populations inside berries.

Material and Methods

Study organisms

Predatory thrips (Trybomia sp.) were collected from Inga trees in coffee agroforestry systems in the municipality of Araponga, state of Minas Gerais, Brazil (42°31'14" W, 20°40'01" S), one day prior to the experiment. Coffee berry borer eggs and larvae used in the experiments originated from a laboratory colony maintained at IAPAR (Agronomic Institute of Paraná, Brazil) and were reared on artificial diet

(Portilla and Streett, 2006) under controlled conditions (27± 1 °C, 70 ± 10% R.H., 0L:24D).

Predator survival, development and predation rate

We investigated the effect of coffee berry borers as a prey and of a sugar-rich source of food on survival, development and predation by the Trybomia sp. Because it was not possible to assess thrips development under field or greenhouse conditions, the experiment was carried out on arenas made from Inga leaves placed inside plastic Petri dishes (6 cm of diameter) in a laboratory (25± 2 °C, 70± 10% R.H., 12L:12D). The leaves were collected from Inga trees in the field and cut in 5 cm diameter discs. The discs were sterilized by dipping them in a 1% sodium hypochlorite solution for 10 min and then rinsing with distilled water. After drying, the leaf discs were laid in Petri dishes filled with an agar solution (0.02g/ml), before solidification, to maintain and extend leaf disc turgidity. The arenas made from Inga leaves were replaced weekly.

Leaves detached from Inga trees ceased nectar production. Therefore, it was necessary replace nectar with other sugar-rich source of food. Natural enemies may survive equally well feeding on nectar and honey solution and previous studies used an aqueous solution of honey to simulate a nectar source (Lavandero, Wratten et al., 2006; Rose, Lewis et al., 2006; Wu, Yun et al., 2011). Thus, we used a honey and water solution (1:1) to simulate a sugar-rich source of food. A small ball of cotton was soaked with this solution and was placed on each leaf disc. Treatments without honey solution had the same cotton ball dipped into only water. The honey solution and water source were replaced every two days.

Four diets were offered to the thrips on Inga leaves: (I) a honey solution, (II) coffee berry borers (eggs and larvae) plus water, (III) coffee berry borers plus honey solution and (IV) water (control). Each replicate consisted of one leaf disc containing one first instar thrips larva with one of the four diets. Each treatment was replicated 42 - 44 times, in three different blocks over time. For the diets with coffee berry borers, five eggs and five larvae were offered daily on each leaf disc. First instar thrips obtained from the field were randomly and individually assigned to each arena. Their survival and developmental stage were observed daily. Thrips were checked daily for molting, and mortality in each instar was recorded. The number of days required to reach adulthood and the number of thrips that turned into adults on each diet were also recorded.

To assess the predation rate and the role of the alternative food on prey consumption, we recorded the number of coffee berry borers consumed in the treatments with and without honey solution every day. Coffee berry borers killed by thrips were recognized as empty or withered eggs and dead or wounded larvae, where hemolymph was exuding (Figure 1). Every day, dead coffee berry borers were replaced with new eggs and larvae.

Figure 1. a. Thrips feeding on coffee berry borer eggs; b. Coffee berry borer eggs (red arrow shows an empty egg).

Predation of coffee berry borer inside berries

We determined the efficiency of the thrips in preying on coffee berry borers inside coffee berries by comparing the number of coffee berry borers within fruits that were exposed or unexposed to the thrips. Red non-bored coffee berries were collected from the coffee agroforestry systems and their surface was sterilized by washing with detergent for 15min, rinsing with water, then dipping in a 2% sodium hypochlorite solution for 10 min and rinsing again with sterile distilled water to avoid fungal contamination. After drying, the coffee berries were exposed to adult female berry borers for 24 hours. The bored berries selected for the experiment were placed in a separate plastic vial (50ml) with a hole at the top covered with a mesh to allow ventilation. Ten days after infestation by coffee berry borers, 26 vials received one first instar thrips collected from the field. Another 26 vials remained without predators. The thrips were observed daily for survival. After all the thrips died, the bored berries were dissected under a stereomicroscope and the number of coffee berry borers (eggs, larvae, pupae and adult) was assessed. The vials were kept under controlled conditions in a climate room (25± 2 °C, 70± 10% R.H., 12L:12D).

Statistical analyses

The longevity of thrips and survivorship of larva instars were assessed using Kaplan–Meier survival analysis (Kaplan and Meier, 1958). We compared survival rates among diets using a log-rank test. The time required for thrips to complete development on each diet were compared through analysis of variance (ANOVA), using generalized linear models (GLMs) with Poisson errors distribution. The number of days required for thrips to reach adulthood was used as a response variable and diets used as an explanatory variable.

To analyze the coffee berry borer predation rate with and without honey solution, we performed a two way ANOVA, using generalized linear mixed models (GLMMs) with Poisson errors distribution. The number of coffee berry borers eaten per day was used as a response variable, whereas diet and thrips instars were used as explanatory variables. Because we made repeated measures on the same individuals, the number of days was used as a random effect to avoid temporal pseudoreplication (Crawley 2007). A similar analysis was performed within thrips instars to determinate predation differences between diets.

To evaluate differences in number of coffee berry borers inside the berries with or without thrips, we conducted an analysis of variance (ANOVA), using generalized linear models (GLMs) with quasiPoisson errors distribution to correct for overdispersion. The numbers of coffee berry borers from each stage remaining inside the berries was used as a response variable, whereas the presence of thrips was used as an explanatory variable. All analyses were performed using R 2.13 software (R Development Core Team, 2011) and were followed by residual analyses to check for the suitability of the models (Crawley, 2007).

Results

Predator survival, development and predation rate

Survival of thrips differed among diets ($\chi^2 = 89.20$, df = 3, $p < 0.001$). Thrips survived longer (mean days ± SE) when fed on a honey solution (17.38 ± 1.68 days), on coffee berry borers plus water (18.21 ± 1.61 days), or on coffee berry borers plus a honey solution (20.48 ± 1.89 days) than when fed only with water (7.07 ± 0.70 days) ($\chi^2 = 40.20$, df = 1, $p<0.0001$, Figure 2).

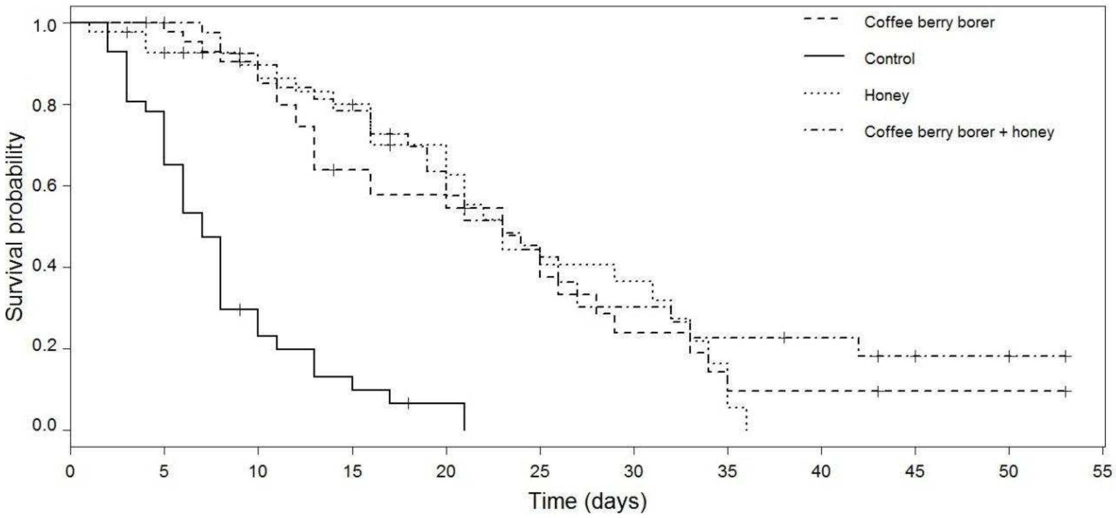

Figure 2. Kaplan-Meier estimates of survivorship functions of thrips (Trybomia sp.) fed on water (control), honey solution, coffee berry borer and water and coffee berry borer and honey solution.

The post-embryonic development of the thrips (Trybomia sp.) involved two larval instars, three pupal instars and the adult stage (Figure 3). Survival among the different developmental stages of thrips differed between diets ($\chi^2 = 45.90$, df = 3, $p<0.0001$). Thrips that fed only on water and only on honey solution had higher mortality in the first stages compared to thrips that fed on coffee berry borers and coffee berry borers plus a honey solution ($\chi^2 = 19.7$, df = 1, $p < 0.0001$, Figure 4). Thrips larvae that fed only on water and on honey solution did not reach adulthood (Figure 4). Thrips completed development only when they were fed with coffee berry borers (23.26%) or with coffee berry borers plus a honey solution (34.09%) (Figure 4). Also, the number of days (mean days ± SE) required to complete development was not different for thrips fed with coffee berry borer plus water (20.5 ± 1.75) and thrips fed with coffee berry borer and honey (20.6 ± 1.19) ($\chi^2_{1,23} = 28.01$, $p = 0.96$).

Figure 3. Developmental stages of Trybomia sp. a. Egg; b. First instar; c. Second instar; d. Third instar (pupa); e. Fourth instar (pupa); f. Adult. Fifth instar is not exemplified in the pictures.

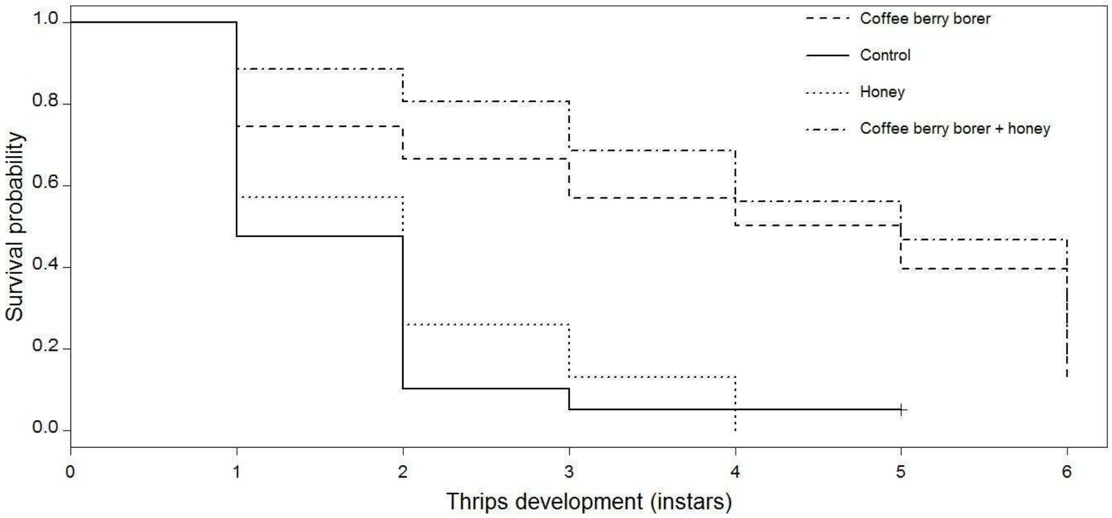

Figure 4. Kaplan-Meier estimates of survivorship functions of thrips (Trybomia sp.) along its developmental stages when fed with: (a) water (control); (b) honey solution; (c) coffee berry

borer and water; and d) coffee berry borer plus honey solution. Stages are represented by numbers at the explanatory axis: first instar (1), second instar (2), third instar (3), forth instar (4), fifth instar (5), adult (6).

The number of coffee berry borers preyed (eggs and larvae) differed between diets ($\chi^2_{1,7}$ = 15.59, p < 0.0001) and among instars of the thrips ($\chi^2_{2,7}$ = 51.68, p < 0.0001). Predation rate of first and second instar thrips decreased when honey solution was offered ($\chi^2_{1,5}$ = 12.28, p = 0.0005; $\chi^2_{1,5}$ = 14.12, p = 0.0002, respectively; Figure 5). For adults, the predation rate of coffee berry borer did not differ between treatments ($\chi^2_{1,5}$ = 0.16, p = 0.69; Figure 5).

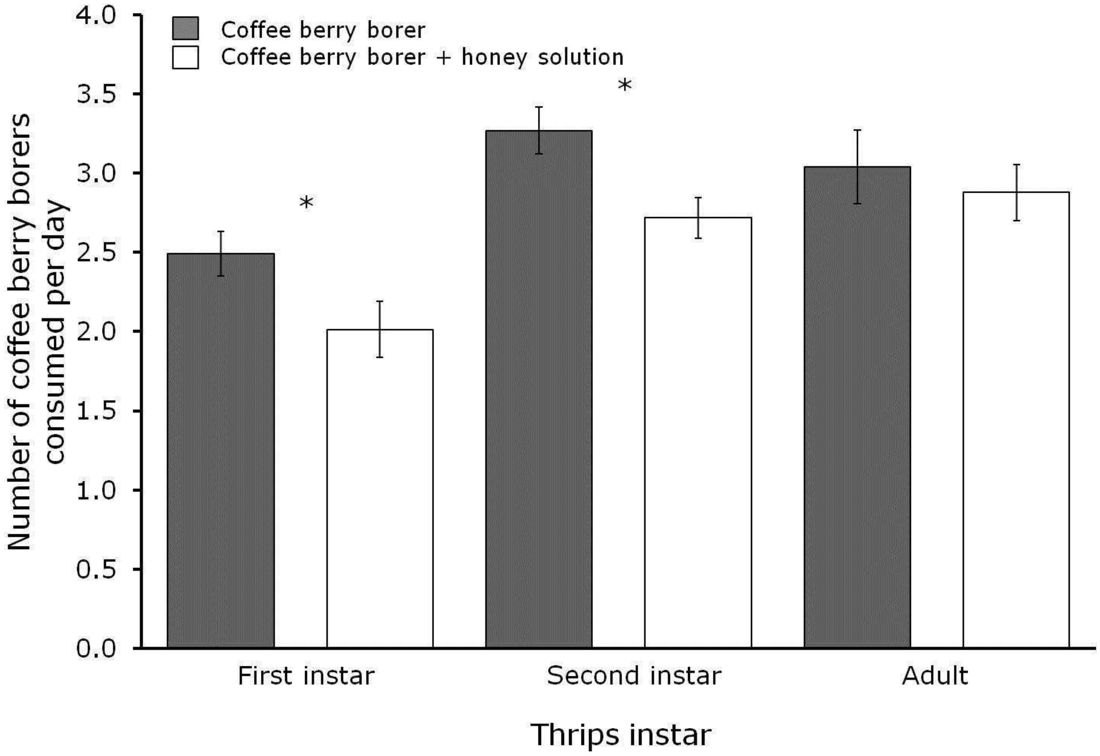

Figure 5. Average number of coffee berry borers (eggs and larvae) predated per day (+SE) by different instars of thrips (Trybomia sp.) in the presence (white bars) or absence (grey bars) of honey solution. Bars result from fitting data to generalized mixed models with Poisson errors distributions. Asterisk represents significant difference between bars.

Predation inside the coffee berries

The number of coffee berry borer eggs, larvae, pupae and adults per fruit did not differ between bored berries without and with thrips predators ($\chi^2_{1,50}$ = 323.79, p = 0.19; $\chi^2_{1,50}$ = 345.46, p = 0.64; $\chi^2_{1,50}$ = 161.06, p = 0.15; $\chi^2_{1,50}$ = 162.53, p = 0.94, respectively; Figure 6).

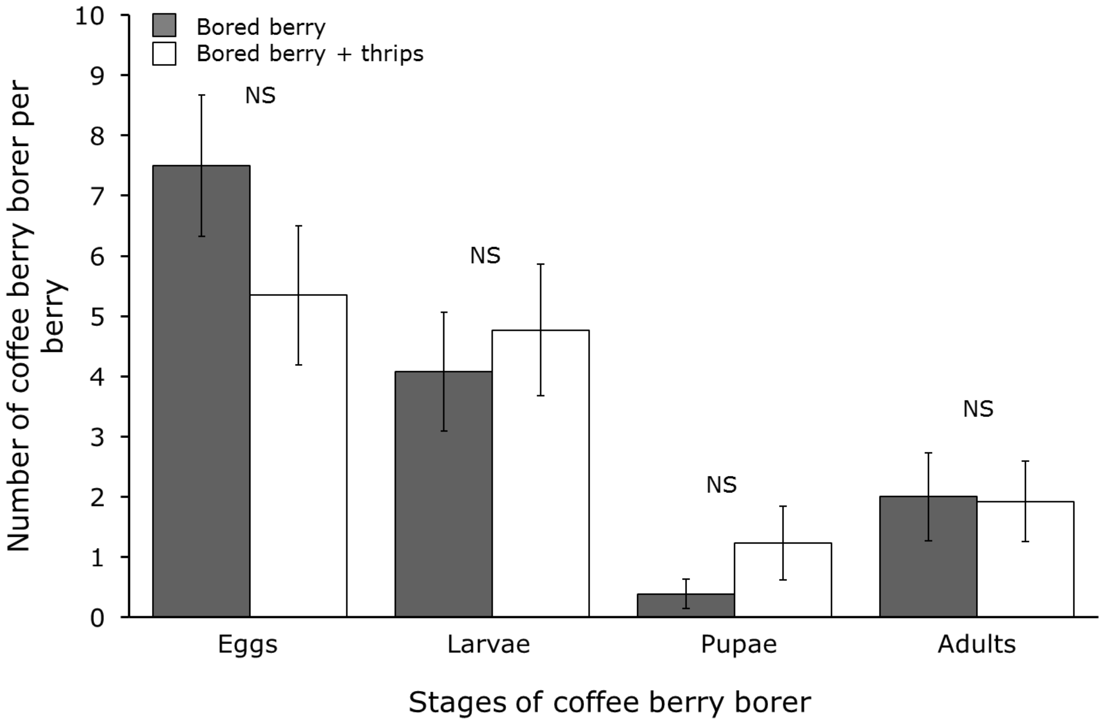

Figure 6. Average number of coffee berry borers inside bored coffee fruits in the presence (white bars) or absence (grey bars) of the thrips (Trybomia sp.). Bars result from fitting data to generalized mixed models with Poisson errors distributions. NS represents non-significant difference between bars.

Discussion

We report here a new predator-prey relationship between coffee berry borers and the thrips Trybomia sp., an omnivorous predator hosted by an extrafloral nectary-possessing tree (Inga subnuda). This tree is planted along with coffee in agroforestry systems in many countries of Latin America (Soto-Pinto, Romero-Alvarado et al., 2001; Souza, Cardoso et al., 2010). Feeding experiments revealed that a honey solution, used as a food source to resemble nectar, prolonged thrips survival as much as coffee berry borers alone and the mixture of these two food sources. However, feeding on a honey solution only did not enable the thrips to complete their development. Thrips fed with a honey solution and thrips fed only with water had equally high larval mortality. Complete development and oviposition was observed only for thrips fed diets with coffee berry borers, but mortality at larval stages was also high. Moreover, alternative food played an important role for thrips predation, especially for immatures, since coffee berry borer predation rate was lower in the presence of sugar-rich food. Regarding predation of coffee berry borers inside the berries, the thrips was not an effective predator.

Sugar feeding is reported to extend the developmental time of phytophagous thrips larvae but does not enable development to the next stage (Teulon and Penman, 1991; Varikou, Tsitsipis et al., 2010). Little is known about the effects of sugar feeding on predatory thrips. Other predators benefit from sugar-rich food such as nectar to increase survival and delay starvation when prey is scarce (Lundgren, J.G., 2009). Nectar may also enhance long-term survival or fecundity of predators until high quality food becomes available (Symondson, Sunderland et al., 2002; Lundgren and Seagraves, 2011). It is possible that nectar from Inga trees enhances survival of thrips enabling them to persist in the field between coffee fructification seasons, when coffee berry borer and other herbivorous are at low densities.

The low survivorship observed among thrips fed with coffee berry borers (23.26%) and coffee berry borer plus a sugar-rich food (34.09%) indicates that coffee berry borers may not be a high quality food to the thrips. Nevertheless, females that developed into adults were able to oviposit. Although predators are known to forage for high nutritional prey, prey consumption may be related to many other factors such as prey size and abundance, effects on predator fitness, such as oviposition rate or offspring survival, and availability of alternative food (Eubanks and Denno, 1999; Symondson, Sunderland et al., 2002; Venzon, Janssen et al., 2002; Schmidt, Sebastian et al., 2012). Some predators may also respond to prey densities, which result in consumption of a large number of nutritionally poor but aggregated prey (Symondson, Sunderland et al., 2002). Therefore, foraging behavior of the thrips needs to be assessed to elucidate whether it can affect coffee berry borer population despite their apparently low nutritional value.

Thrips predation was affected by alternative food consumption because thrips consumed fewer berry borers in presence of nectar solution. Other omnivorous thrips are known to shift between plant food and prey depending on plant quality and prey availability (Agrawal, Kobayashi et al., 1999). Many studies show that predators may forage less for prey in the presence of alternative food, and that prey consumption may depend on alternative food abundance, quality and content (Cottrell and Yeargan, 1998; Spellman, Brown et al., 2006; Wilder and Eubanks, 2010; Stenberg, Lehrman et al., 2011). Nevertheless, from a long term perspective, alternative food might increase an omnivorous population and result in suppression of herbivore populations, even when the predation rate by individual predators is low (Eubanks and Denno, 2000). Therefore, positive effects from plant-provided food, such as increased survival by an omnivorous population, might outweigh decreased predation rates by individual predators (Eubanks and Denno, 1999; Eubanks and Denno, 2000).

The number of coffee berry borers inside the coffee berries did not decrease due to thrips presence. Many factors could have affected thrips predation on coffee berry borers. For example, thrips may not have entered the berries or the predation rate may have been too low to be distinguished from the control. The first option does not seem probable since we observed thrips inside bored berries in field. However, we used ripe berries in the experiment, and thrips might have preference or capability for other fruit stages. Previous studies found that over ripe coffee berries, which fell on the ground, are the main reservoir of parasitoids (Jaramillo, Chabi-Olaye et al., 2009). Also, the other predatory thrips of coffee berry borers (K. flavipes) was found in berries that had fallen to the ground (Jaramillo, Chapman et al., 2010). Over ripe fruits are less humid, which might facilitate the passage of natural enemies through the galleries made by coffee berry borer.

Nevertheless, natural pest control in coffee agroforestry systems are driven by complex ecological interactions (Vandermeer, Perfecto et al., 2010). Pest populations can be controlled by natural enemy communities when their feeding niches are complementary (Cardinale, Harvey et al., 2003; Cardinale, Srivastava et al., 2006; Snyder, Snyder et al., 2006). Therefore, thrips might provide an additional level of regulation in concert with other natural enemies. We can thus infer that this omnivorous predator associated to a nectary-possessing tree in coffee agroforestry systems benefits from sugar feeding by increasing its survival, but depends on a protein food source to complete its development. The thrips feed on coffee berry borer and, despite its low efficiency as a predator, it might be part of the food web responsible for natural control of coffee berry borers in the field.

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Chapter III

Natural enemies of coffee leaf miners increase survival feeding on extrafloral nectar

Abstract

Nectar is an important source of food for many natural enemies. Plants bearing nectaries may attract natural enemies that protect those plants against herbivory. Therefore, nectary-bearing plants can be used as a strategy to enhance crop protection against pests. In coffee agroforestry systems, the availability of extrafloral nectar from Inga (Inga sp.) trees increased parasitism of coffee leaf miners and decreased damage on coffee plants. To assess possible mechanisms of enhanced pest control in coffee agroforestry systems, we evaluated the effect of extrafloral nectar of Inga trees on survival of parasitoids and of a predator of coffee leaf miners. We selected four parasitoids of coffee leaf miners (Horismenus sp., Orgilus niger, Proacrias coffeae and Stiropius reticulates) and one predator (Chrysoperla externa) to perform a greenhouse experiment. Newly emerged parasitoid females were placed inside clipcages that were fixed either to Inga leaves exuding extrafloral nectar or to Inga leaves lacking extrafloral nectaries (control). The same procedure was followed to second instar larvae of C. externa. All coffee leaf miner parasitoids and the predator survived longer when feeding on extrafloral nectar. Our results suggest that extrafloral nectar of Inga trees can increase fitness of natural enemies of coffee leaf miner through survival enhancement. This can be one of the mechanisms that explain increased parasitism and decreased coffee damaged when extrafloral nectar is available in agroforestry coffee systems.

Key words: Parasitoids, Inga edulis, Leucoptera coffeella, extrafloral nectaries.

Resumo

Nectar é uma fonte importante de alimento para muitos inimigos naturais. Plantas que possuem nectários podem atrair inimigos naturais que irão protegê-las contra herbivoria. Por isso, plantas que possuem nectários podem ser utilizadas como uma estratégia de proteção contra pragas em cultivos agrícolas. Em cafeeiros sob sistemas agroflorestais, a disponibilidade de néctar extrafloral em árvores de Ingá (Inga sp.) aumentou o parasitismo do bicho-mineiro do cafeeiro e diminuiu danos em plantas de café. Para acessar os possíveis mecanismos relacionados ao controle de pragas observado nos sistemas agroflorestais foi avaliado o efeito do néctar extrafloral de árvores de Ingá na sobrevivência de parasitoides e de um predador do bicho-mineiro. Foram selecionadas quatro espécies de parasitoides (Horismenus sp., Orgilus niger, Proacrias coffeae and Stiropius reticulates) e um predador (Chrysoperla externa) para os experimentos realizados em casa de vegetação. Fêmeas de parasitoides recém-emergidas foram acondicionadas em clipcages que foram fixados em folhas de Ingá com nectários extraflorais ou em partes das folhas que não possuiam nectários (controle). O mesmo procedimento foi realizado para larvas de segundo instar de C. externa. Todos os parasitoides e o predador avaliados sobreviveram por mais tempo quando se alimentaram com o néctar das árvores de Ingá. Os resultados sugerem que o néctar das ávores de Ingá pode aumentar o fitness dos inimigos naturais do bicho-mineiro do cafeeiro, pois seu consumo aumenta suas longevidades. Isso pode ser um dos mecanismos que explicam o aumento do parasitismo do bicho-mineiro e a diminuição nos danos em plantas de café quando néctar está disponível em sistemas agroflorestais.

Palavras-chave: Parasitoides, Inga edulis, Leucoptera coffeella, nectários extraflorais.

Introduction

Nectar is an aqueous solution of sugar and amino acids produced by plants (Pacini and Nicolson, 2007). To many insects nectar is an important source of food that provides energy for activities such as flying, mating and foraging (Jervis, Kidd et al., 1996; Koptur, 2005; Wäckers, 2005). Natural enemies often require plant-provided food, at least during part of their life cycle. For example, predators are usually facultative consumers of nectar, using it to complement their prey diet (Coll and Guershon, 2002; Eubanks and Styrsky, 2005). Parasitoids, however, can be obligatory nectar consumers during their adult stage (Jervis, Kidd et al., 1996). Nectar mediates important interactions between insects and plants. Floral nectar is one of the primary rewards for animals visiting flowers and is involved in pollination processes (Heil, 2011). Extrafloral nectaries, on the other hand, are located outside the flowers and animals attracted to it are likely to encounter herbivorous preys. Therefore, insects that feed on extrafloral nectar usually act as plant protectors (Koptur, 2005; Nicolson, 2007).

Plants bearing floral nectaries are often used in agricultural systems to improve biological control (Landis, Wratten et al., 2000; Heimpel and Jervis, 2005; Lu, Zhu et al., 2014). Although less studied, extrafloral nectaries can be especially suitable for this purpose because they produce nectar for longer periods and are usually more easily accessible than floral nectaries (Wäckers, 2005). Nectar can attract and arrest predators and parasitoids, thus increasing their density in crop areas (Jamont, Dubois-Pot et al., 2014). Moreover, nectar can enhance fitness of natural enemies by increasing, for example, fecundity or longevity (Tylianakis, Didham et al., 2004; Lee and Heimpel, 2008; Lundgren, Jonathan G., 2009; Lundgren and Seagraves, 2011). The combination of higher densities and enhanced fitness of natural enemies may lead to higher parasitism and predation levels of herbivores in field (Harmon, Ives et al., 2000; Tylianakis, Didham et al., 2004; Jamont, Dubois-Pot et al., 2014). Therefore, the presence of plants bearing nectaries in agricultural systems can ultimately lead to decreased pest densities (Heimpel and Jervis, 2005).

In agroforestry coffee systems, associated trees bearing extrafloral nectaries were found to enhance natural pest control (Rezende, Venzon et al., 2014). The trees belong to the genus Inga Miller (Fabaceae), which is commonly found in coffee agroforestry systems (Soto-Pinto, Romero-Alvarado et al., 2001; Romero-Alvarado, Soto-Pinto et al., 2002; Siles, Harmand et al., 2010; Souza, Cardoso et al., 2010). The availability of extrafloral nectar of Inga trees increased parasitism of coffee leaf miners, a coffee pest, and decreased damage on coffee plants (Rezende, Venzon et al., 2014). Coffee leaf miner Leucoptera coffeella (Guérin-Mèneville) (Lepidoptera: Lyonetiidae) is a small moth and its larvae feed on the parenchyma between surfaces of coffee leaves, reducing photosynthesizing foliar area and causing early senescence of infested leaves. This can reduce coffee yield as well as bean weight and quality (Souza, Reis et al., 1998; Pereira, Eliseu José G., Picanço, Marcelo C. et al., 2007). Several studies suggest that coffee leaf miner population dynamics is strongly affected by natural enemies (Pereira, Eliseu José G., Picanço, Marcelo C. et al., 2007; Lomeli-Flores, Barrera et al., 2009). There are at least 30 species of coffee leaf miner parasitoids that can cause around 10% of larval mortality (Pereira, Eliseu José G., Picanço, Marcelo C. et al., 2007; Lomeli-Flores, Barrera et al., 2009). Important predators of coffee leaf miners are wasps, ants and green lacewings and mortality due to predation can be as high as 69% (Souza, 1979; Ecole, Silva et al., 2002; Lomeli-Flores, Barrera et al., 2009). The mechanisms underlying coffee pest control by natural enemies near trees bearing extrafloral nectaries remain to be studied (Rezende, Venzon et al., 2014). Here we assessed the effect of extrafloral nectar of Inga trees on survival of parasitoids and of a predator of coffee leaf miners.

Material and methods

Parasitoids of coffee leaf miner

Parasitoids of coffee leaf miners were obtained from mined coffee leaves sampled on a farm at the municipality of Paula Cândido, Minas Gerais, Brazil (20°48’24.47”S 42°59’01.85”W). One mined coffee leaf was sampled from each of 104 different coffee plants every fifteen days during 22 weeks between September 2013 and January 2014. A total of 1248 mined leaves were collected. We sampled leaves with intact mines to assure that larvae of coffee leaf miners had not been attacked by predatory wasps and that parasitoids had not emerged. Each mined leaf was incubated in a separate plastic vial with the petioles inserted in water to maintain turgidity (Reis Jr., Lima et al., 2000; Pereira, E.J.G., Picanço, M.C. et al., 2007). The vials were checked daily for emergence of parasitoids and coffee leaf miners. When parasitoids emerged from mined leaves they were identified and sexed. Females of the most abundant parasitoid species, Horismenus sp. (Hymenoptera: Eulophidae), Orgilus niger Penteado-Dias, 1999 (Hymenoptera: Braconidae), Proacrias coffeae Ihering, 1913 (Hymenoptera:

Eulophidae) and Stiropius reticulatus Penteado-Dias, 1999 (Hymenoptera: Braconidae), were used in the experiment. Other parasitoid species were too rare to allow for sufficient replicates.

Predator of coffee leaf miner

Most adult lacewings feed on pollen, nectar and honeydew, whereas immatures have omnivorous habits, feeding on soft-bodied arthropod prey, honeydew and plant-provided food (Limburg and Rosenheim, 2001; Venzon, Rosado et al., 2006; Hogervorst, Wackers et al., 2008). The green lacewing Chrysoperla externa (Hagen, 1861) (Neuroptera: Chrysopidae) has been reported as a predator of coffee leaf miners and it is often found in coffee crops (Ecole, Silva et al., 2002). Larvae of C. externa were reared at the Laboratory of Entomology at Agriculture and Livestock Research Enterprise of Minas Gerais (EPAMIG). Adults were kept in plastic cages (8 x 11 cm) wrapped with paper and covered with plastic film. They were fed with a diet of yeast and honey (1:1) offered on a parafilm strip suspended inside the cage. Water was provided on a soaked cotton ball inside a 10 ml vial. Food and water were replaced twice a week. Eggs of C. externa were transferred to separate plastic vials (50 ml) covered with plastic film and newly emerged larvae were fed with eggs of the flour moth Anagasta kuehniella (Zeller) (Lepidoptera: Pyralidae) until pupation. The insects were kept in a climate controlled room (25± 2 °C, 70± 10% R.H., 12: 12 L:D).

Survival experiment

To assess the effect of extrafloral nectar of Inga trees on longevity of natural enemies of the coffee leaf miner, we performed a greenhouse experiment. Fifty Inga (Inga edulis) seedlings were transplanted to 3 L pots containing a mixture of soil and substrate (1:1). Plants were fertilized with N-P-K (25:5:20), and watered daily. No pesticides were used. The experimental unit consisted of a clipcage (3 cm diameter) with one parasitoid or one predator inside (Figure 1). Whenever a female parasitoid emerged, it was placed inside a clipcage and fixed to an Inga leaf with a nectary secreting nectar, thus, allowing the parasitoid to feed on nectar. When the extrafloral nectary ceased nectar secretion the clipcages were moved to a new nectary. The next emerging parasitoid of the same species would serve as a control and was therefore placed in a clipcage on an Inga leaf region lacking extrafloral nectaries. Sixty eight

Horismenus sp. parasitoids fed on extrafloral nectar and 64 served as a control (deprived from food); 24 S. reticulatus fed on nectar and 22 were control; 11 O. niger fed on nectar and 10 were control; 9 P. coffeae fed on nectar and 11 were control.

The same methodology was applied to C. externa. Sixty tree second instar larvae were selected for the experiment. Thirty one were placed in clipcages with access to extrafloral nectar and 32 were placed in clipcages on leaf areas lacking extrafloral nectaries (control). Parasitoids and predators were observed daily and survival of individuals that fed with extrafloral nectar and deprived from food was compared for each species. Longevity of parasitoids and predator were estimated by Kaplan–Meier survival analysis (Kaplan and Meier, 1958), with equations adjusted to a non-linear model identity. We determined the significance of survival curves by Cox's test.

Figure 1. Clipcage used to condition natural enemies of coffee leaf miner on Inga leaves.

Results

Parasitoids of coffee leaf miner

All parasitoids recorded from mined coffee leaves survived longer when feeding on extrafloral nectar of Inga trees. Horismenus sp. survived 9.88 ± 1.24 days (mean ± SE) when they had access to extrafloral nectaries and 2.43 ± 0.16 days on leaf areas lacking extrafloral nectaries (Cox's test: $\chi_{1,68}$= 55.6; p<0.0001; Figure 2). Stiropius reticulatus survived 9.58 ± 2.07 days when fed on extrafloral nectar and 1.86 ± 0.16 days when received no food (Cox's test: $\chi_{1,24}$= 25.1; p<0.0001; Figure 3). Proacrias coffeae survived 4.22 ± 1.42 days when fed on nectar and 1.73 ± 0.19 days when they did not feed (Cox's test: $\chi_{1,11}$= 6.3; p<0.01; Figure 4). Finally, O. niger survived 4.18 ± 1.39 days when fed on extrafloral nectar and 1.60 ± 0.22 when they did not feed (Cox's test: $\chi_{1,11}$= 5.4; p<0.02; Figure 5).

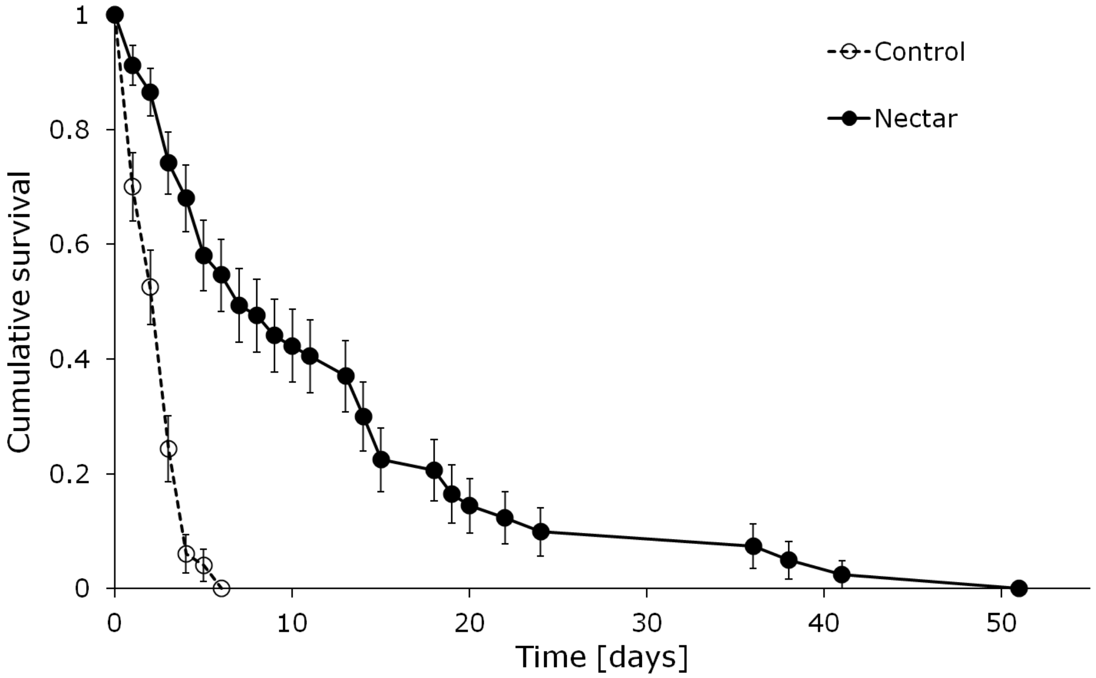

Figure 2. Kaplan-Meier estimates of survivorship functions of a coffee leaf miner parasitoid (Horismenus sp.) fed with extrafloral nectar and without food (control) (Cox's test: $\chi_{1,68}$= 55.6; p<0.0001).

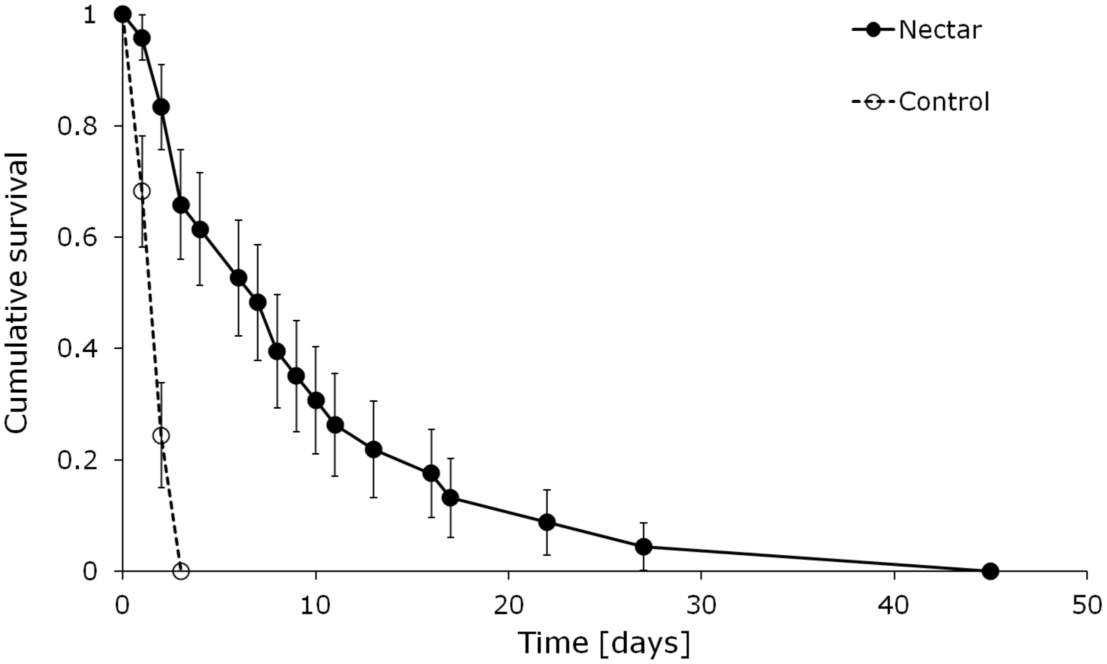

Figure 3. Kaplan-Meier estimates of survivorship functions of a coffee leaf miner parasitoid (Stiropius reticulatus) fed with extrafloral nectar and without food (control) (Cox's test: $\chi_{1,24}$= 25.1; p<0.0001).

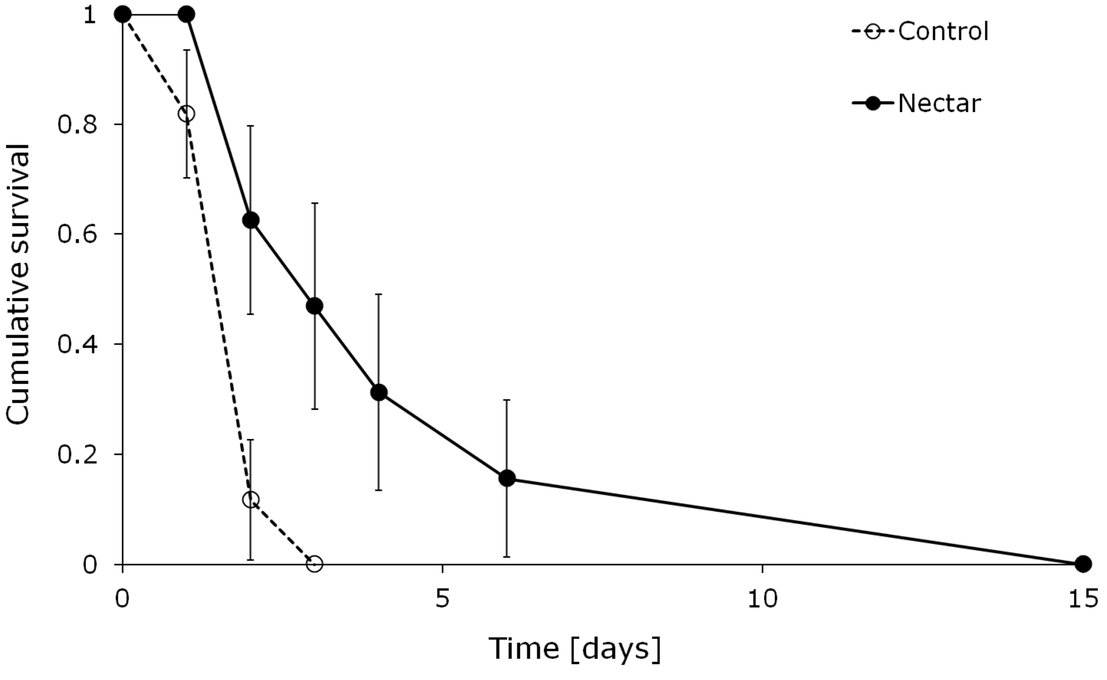

Figure 4. Kaplan-Meier estimates of survivorship functions of a coffee leaf miner parasitoid (Proacrias coffeae) fed with extrafloral nectar and without food (control) (Cox's test: $\chi_{1,11}$= 6.3; p<0.01).

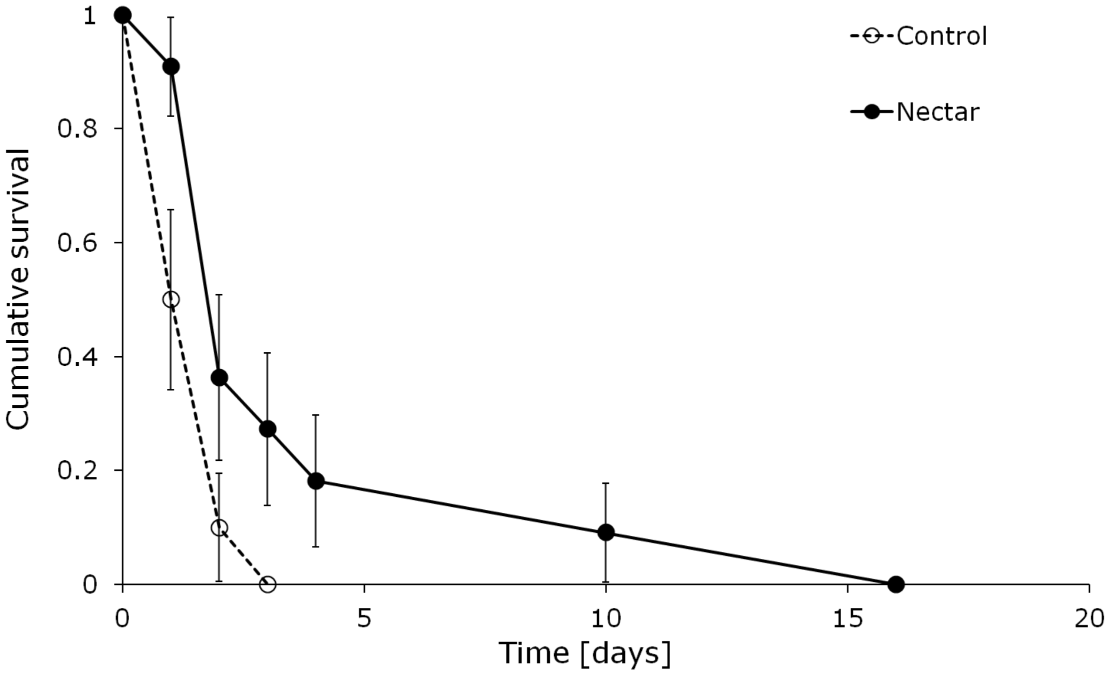

Figure 5. Kaplan-Meier estimates of survivorship functions of a coffee leaf miner parasitoid (Orgilus niger) fed with extrafloral nectar and without food (control) (Cox's test: $\chi_{1,11}$= 5.4; p<0.02).

Predator of coffee leaf miner

Larvae of C. externa also survived longer with access to extrafloral nectaries (Cox's test: $\chi_{1,32}$= 12.5; p<0.0004; Figure 6). It survived 9.62 ± 1.70 days (mean ± SE) when fed on extrafloral nectar and 4.13 ± 0.74 days when they were deprived from food. The second instar larvae molted into third instar in both treatments, but none of them turned into pupae.

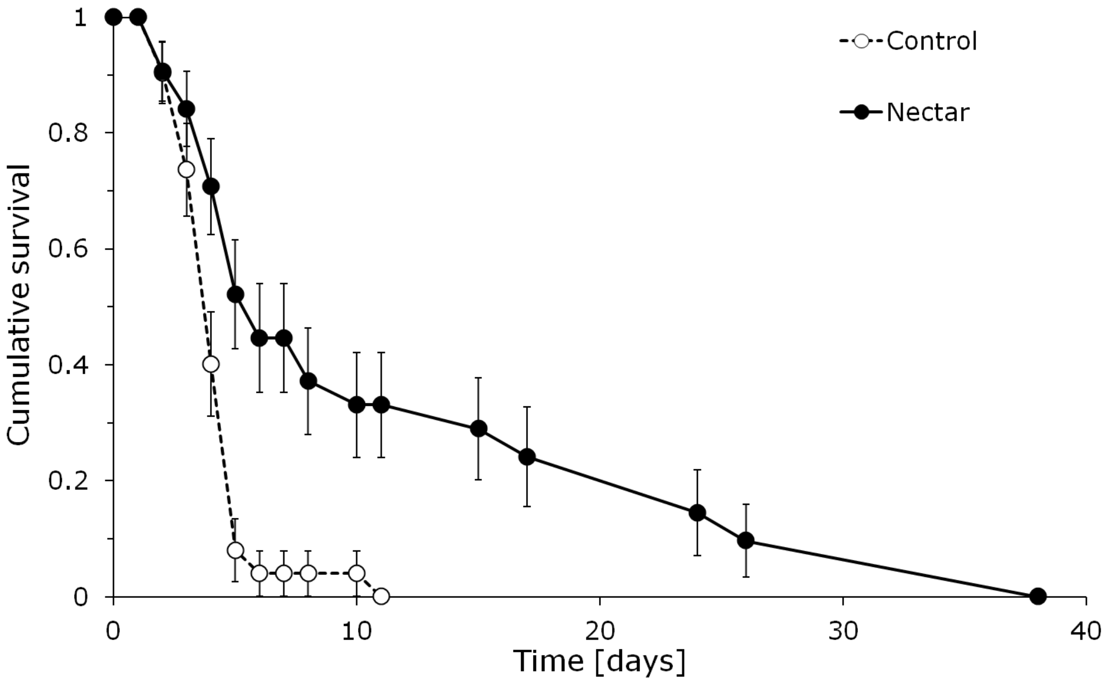

Figure 6. Kaplan-Meier estimates of survivorship functions of a coffee leaf miner predator (Chrysoperla externa) fed with extrafloral nectar and without food (control) (Cox's test: $\chi_{1,32}$= 12.5; p<0.0004).

Discussion

The parasitoids and the green lacewing were able to feed on extrafloral nectar of Inga trees and this increased their longevity compared to starved individuals. Our results show the importance of extrafloral nectar as a food source for natural enemies of coffee pests. Several studies show that survival of parasitoids and predators can be increased by nectar feeding (Koptur, 2005; Wäckers, 2005; Lundgren, Jonathan G., 2009). However, few studies investigated the effect of plant-provided food on fitness of natural enemies of coffee leaf miners (but see Rosado, 2007). Little information is available on the biology and life history of parasitoids of coffee leaf miners (Penteado-Dias, 1999; Miranda, 2009). Overall, adult parasitoids needs to balance the trade-off between time spent feeding and searching for hosts (Lewis, Stapel et al., 1998). Therefore, time can be limiting if a parasitoid dies before exhausting its supply of mature eggs (Heimpel, Mangel et al., 1998; Rosenheim, 1999). Increased longevity due to nectar feeding can have major impact in realized fecundity of parasitoids (i.e. number of parasitized hosts) because more time is available for host location and egg maturation (Tylianakis, Didham et al., 2004; Heimpel and Jervis, 2005). Moreover, prolonged longevity and attraction of parasitoids from surrounding areas can lead to aggregation of parasitoids in areas providing food (Bianchi and Wäckers, 2008). Therefore, it is expected that nectar provision in agricultural systems can lead to increased parasitism rates (Lewis, Stapel et al., 1998; Tylianakis, Didham et al., 2004; Balmer, Pfiffner et al., 2013; Jamont, Crepelliere et al., 2013; Jamont, Dubois-Pot et al., 2014; Rezende, Venzon et al., 2014). Considering that all parasitoids evaluated here had increased survival when feeding on extrafloral nectaries, it is plausible that it is one of the mechanisms driving increased coffee leaf miner parasitism observed in coffee agroforestry systems (Rezende, Venzon et al., 2014).

Nectar feeding can affect the extent of time that predators can remain in field when pest densities are low (Eubanks and Styrsky, 2005; Lundgren, Jonathan G., 2009; Lundgren and Seagraves, 2011). In concert with other studies, we found that green lacewings survived longer when feeding on extrafloral nectar (Limburg and Rosenheim, 2001; Rosado, 2007). Therefore, extrafloral nectar can be important in delaying starvation of green lacewings when prey are scarce (Limburg and Rosenheim, 2001). Moreover, if predators are able to persist in the field prior to the build-up of prey populations, they can prevent pest increases of population (Lundgren, 2009). Therefore, green lacewings and parasitoids in concert with other natural enemies that also benefit from nectar feeding could lead to decreased population of coffee leaf miner and damage on coffee plants near Inga trees.

Fitness enhancement of several predators and parasitoids due to feeding on floral nectar has been reported repeatedly (Tylianakis, Didham et al., 2004; Koptur, 2005; Lee and Heimpel, 2008). However, studies regarding the effect of extrafloral nectar on fitness of natural enemies are less common (Taylor and Bradley, 2009; Jamont, Crepelliere et al., 2013). Extrafloral nectar is distinct in composition from floral nectar (Nicolson and Thornburg, 2007; Heil, 2011). Extrafloral nectar of Inga trees is hexose-dominant and have higher sugar concentration compared to Inga floral nectar, which is sucrose-dominant (Koptur, 1994). Nectar composition may determine the spectrum of nectar consumers because insects differ in their nutritive preferences (Heil, 2011). For example, coccinelids are known to feed more frequently on extrafloral nectaries than on floral nectaries (Lundgren, 2009). Extrafloral nectar can be more easily accessible and available over longer periods (Wäckers, 2005; Pacini and Nepi, 2007). Thus, depending on feeding preference of natural enemies, plants bearing extrafloral nectaries can be more suitable for enhancing natural pest control in crop fields than flowering plants. Our results suggest that extrafloral nectar of Inga trees can increase fitness of natural enemies of coffee leaf miners through increases in survival. This can be one of the mechanisms that explain increased parasitism and decreased coffee damaged when extrafloral nectar is available in agroforestry coffee systems (Rezende, Venzon et al., 2014). Further investigations should consider the effect of extrafloral nectar on other parameters of life histories of coffee leaf miner natural enemies.

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Chapter IV

Extrafloral nectary-bearing trees enhance pest control and increase fruit weight in associated coffee plants

Abstract

Plants bearing extrafloral nectaries might provide protection to neighboring plants against herbivory because nectar can increase fitness of natural enemies. Therefore, extrafloral nectary-bearing plants could be use to enhance crop protection. Here we took an experimental approach to test whether an extrafloral nectary-bearing tree can increase natural control of coffee leaf miners (Leucoptera coffeella) and coffee berry borers (Hypothenemus hampei) in associated coffee crops. We compared parasitism, predation, coffee damage and coffee yield between replicated coffee plots with or without Inga (Inga densiflora) trees. To evaluate the effect of proximity of the nectar source on coffee protection, we also assessed pest control and production along transects of 50 m extending from the Inga trees. Damage caused by coffee leaf miners and coffee berry borers were lower in coffee with Inga trees and increased with distance from the trees. Coffee fruits were heavier in coffee consorted with Inga and production per coffee plant was equal in both systems. Parasitism of coffee leaf miners did not increase in coffee consorted with Inga, neither decreased with distance from the trees. Predation by wasps, on the other hand, did not differ between consorted coffee and monoculture but tented to decrease as distance from Inga trees increased. Natural enemies of coffee berry borers were found more often inside berries near Inga trees, but there was no difference in the proportion of berries with natural enemies between coffee with trees and monoculture. Predation of coffee berry borer inside the coffee berries did not differ between monoculture and consorted coffee, neither responded to distance from the trees. However, the method used to assess predation was not completely successful. Therefore, Inga trees increased natural control of coffee pests and enhanced coffee fruit weight. Thus, indirect plant defense provided by extrafloral nectaries can indeed decrease herbivory on neighboring plants. Hence, plants bearing extrafloral nectaries can be used to enhance natural pest control in crop fields.

Key words: Agroforestry systems, associational defence, insectary plants, Inga densiflora, coffee leaf miner, coffee berry borer.

Resumo

Plantas que possuem nectários extraflorais podem possivelmente proteger plantas vizinhas contra herbivoria, pois o néctar pode aumentar o fitness dos inimigos naturais. Por isso, é provável que plantas com nectários extraflorais possam ser usadas como uma estratégia de proteção em cultivos agrícolas. Através de um experimento em campo foi testado se árvores que possuem nectários extraflorais podem aumentar o controle natural do bicho-mineiro do cafeeiro (Leucoptera coffeella) e da broca-do-café (Hypothenemus hampei) em cafezais. Foram comparados o parasitismo, a predação, os danos e a produção do café entre parcelas replicadas de café em monocultivo e café consorciado com árvores de Ingá (Inga densiflora). Para avaliar o efeito da proximidade dos nectários na proteção do café, também foram avaliados o controle de pragas e a produção ao longo de transectos de 50 m a partir das ávores de Ingá. O parasitismo no bicho-mineiro não aumentou no café consorciado ao Ingá e também não diminuiu com a distância das árvores. Por outro lado, a predação por vespas não diferiu entre o café consorciado e o monocultivo, mas tendeu a diminuir com o aumento da distância das árvores. Inimigos naturais da broca-do-café foram encontrados mais frequentemente dentro de frutos de café próximos às árvores de Ingá, mas não houve diferença na proporção de frutos com inimigos naturais entre monocultivo e café consorciado com Inga. A predação da broca-do-café dentro dos frutos brocados não diferiu entre monocultivo e café consorciado, tampouco respondeu à distância das árvores. No entanto, o método utilizado para avaliar a predação não foi completamente bem sucedido. Os danos causados pelo bicho-mineiro e pela broca-do-café foram menores no café consorciado pelo ingá e aumentaram com a da distância das árvores. O peso do fruto do café foi maior nas plantas de café consorciadas, e a produção por planta de café foi igual nos dois sistemas. Por tanto, as árvores de Ingá aumentaram o controle natural das pragas e o peso do fruto do café. De fato, a defesa indireta das plantas promovida pelos nectários extraflorais pode diminuir a herbivoria em plantas vizinhas. Por isso, plantas que possuem nectários extraflorais podem ser usadas para aumentar o controle natural de pragas em cultivos agrícolas.

Palavras-chave: Sistemas agroflorestais, defesa por associação, plantas companheiras, Inga densiflora, bicho-mineiro do cafeeiro, broca-do-café.

Introduction

Diversified agroecosystems can increase populations of natural enemies and decrease herbivory and crop damage (Bianchi, Booij, et al., 2006, Letourneau, Armbrecht, et al., 2011). Understanding the mechanisms that enhance natural pest control in diversified systems is important for the management of functional biodiversity to generate such a critical ecosystem service (Letourneau, Armbrecht, et al., 2011). So far, it is believed that natural pest control is favored by plant diversification mainly because it can regulate host-plant selection by disrupting host plant finding and may improve conditions and provide resources to natural enemies (Finch and Collier, 2000, Gurr, Wratten, et al., 2003, Landis, Wratten, et al., 2000). For example, many natural enemies can feed on food sources provided by plants (Lundgren, 2009). Plant-provided nectar is an important source of carbohydrates that provides energy to insects (Koptur, 2005). Nectar may increase survival, fecundity, longevity, flight activity and also enhances foraging behavior of natural enemies, thus leading to increased control of herbivores (Jamont, Dubois-Pot, et al., 2014, Koptur, 2005, Lavandero, Wratten, et al., 2005, Tylianakis, Didham, et al., 2004, Wäckers, 2005).

Nectar may be related to plant reproduction or protection, depending on which plant structure it is produced (Pacini and Nicolson, 2007). Nectar released by flowers usually rewards pollinators, whereas nectar secreted by extrafloral nectaries rewards more often natural enemies that protect those plants against herbivory (Koptur, 2005, Pacini and Nicolson, 2007). Therefore, extrafloral nectaries are considered as an indirect plant defense, because it attracts and arrests natural enemies, leading to decreased damage on the nectary-bearing plants (Cuautle and Rico-Gray, 2003, Heil, 2008, Mathews, Brown, et al., 2007, Sabelis, van Rijn, et al., 2005). Despite some evidence that defense provided by extrafloral nectaries extends to the plant community (Atsatt and O'Dowd, 1976, Barbosa, Hines, et al., 2009, Pemberton and Lee, 1996, Rudgers and Gardener, 2004), only recent studies suggested that plants bearing extrafloral nectaries could also provide protection to neighboring plants (Jamont, Dubois-Pot, et al., 2014, Jezorek, Stiling, et al., 2011, Rezende, Venzon, et al., 2014). Flowering plants have been used as insectary plants to improve natural pest control in agroecosystems for a long time (Balmer, Pfiffner, et al., 2013, Fiedler, Landis, et al., 2008, Harmon, Ives, et al., 2000, Heimpel and Jervis, 2005, Landis, Wratten, et al., 2000, Lu, Zhu, et al., 2014), but there are few field experiments regarding the use of extrafloral nectary-bearing plants for this purpose (but see Brown and Mathews, 2008, Brown, Mathews, et al., 2010, Jamont, Dubois-Pot, et al., 2014). We therefore took an experimental approach to test whether an extrafloral nectary-bearing tree can enhance coffee crop protection against herbivores and result in increased crop yield.

Coffee is a cash crop covering more than 10 million ha, mainly in tropical developing countries (FAO, 2012). In Latin America, coffee was traditionally cultivated in agroforestry systems, but agronomic intensification resulted in a transformation of many understory coffee to full-sun cropping (Jha, Bacon, et al., 2011, Moguel and Toledo, 1999, Peeters, Soto-Pinto, et al., 2003, Perfecto, Rice, et al., 1996). In Brazil, coffee has predominantly been cultivated in monocultures under full sun (Jha 2011). Recently, environmental and social harms caused by conventional agricultural practices encouraged small-scale farmers to develop agroforestry coffee systems and to adopt agroecological practices, aiming to recover the soil, reduce erosion, improve nutrient recycling and increase food security and sovereignty (Cardoso, Guijt, et al., 2001, Sales, Méndez, et al., 2012, Souza, de Goede, et al., 2012). Coffee agroforestry systems are considered a refuge of biodiversity that preserve regional ecological processes and provides important ecosystem services, such as pest control (Bedimo, Dufour, et al., 2012, Jha, Bacon, et al., 2014, Jha, Bacon, et al., 2011, Perfecto, Armbrecht, et al., 2007, Vandermeer, Perfecto, et al., 2010).

Trees play an important role within coffee agroforestry systems, providing resources to natural enemies that enhance natural regulation of coffee pests (Railsback and Johnson, 2014; Rezende et al., 2014; Staver et al., 2001). However, little information on the effect of individual tree species on pest suppression is available (Righi et al., 2013; Staver et al., 2001). Many trees selected for intercropping with coffee possess extrafloral nectaries (Soto-Pinto et al., 2001; Souza et al., 2010); some of the most common species belong to the genus Inga Miller (Fabaceae) (Siles et al., 2010; Soto-Pinto et al., 2001). A previous study showed that the production of extrafloral nectar by associated Inga trees was correlated to increased natural control of coffee pests (Rezende, Venzon, et al., 2014). Because correlation does not always prove causation, manipulative field experiments were performed here to confirm that the presence of nectar-producing Inga trees indeed result in increased natural control of coffee pests. Pests causing major damage in coffee are the coffee leaf miner Leucoptera coffeella (Guérin-Mèneville) (Lepidoptera: Lyonetiidae), which disrupts coffee photosynthesis due to premature drop of mined leaves, and the coffee berry borer Hypothenemus hampei (Ferrari) (Coleoptera: Curculionidae: Scolytinae), which depreciates coffee fruits due to the galleries bored into coffee seeds (Souza et al., 1998;

Vega et al., 2009). Thus, to assess the effect of extrafloral nectary-bearing trees on natural pest control, we compared coffee damage, coffee yield, parasitism and predation between replicated coffee plots with or without Inga trees. To evaluate the effect of nectar proximity on coffee protection, we also assessed pest control and production along transects extending from Inga trees.

Material and Methods

Study area

Experiments were conducted on a farm at the municipality of Paula Cândido, Minas Gerais, Brazil (20°48’24.47”S 42°59’01.85”W), within the Atlantic Rainforest domain (Ab'Sáber, 2003). The altitude at the farm is 777 m and the predominant soil type is Oxisols. The region is characterized by a tropical highland climate, with rainy summers and dry winters. The mean annual temperature is 18.5 °C and mean rainfall is 1403 mm (Valverde, 1958). The farm is conventionally managed, implying that coffee is grown under full sun and fertilizers and pesticides are used. However, insecticides were not used during the study period.

To assess the effect of Inga trees on natural control of coffee pests, we conducted a field experiment, comparing coffee in monoculture with coffee consorted with Inga trees. To elucidate the extent to which these trees enhance natural control of coffee pests, the effect of distance from the trees was assessed. The experimental design consisted of four blocks of two plots (with and without Inga trees), each plot measuring $400m^2$ (20 x 20 m) within a coffee field (Coffea arabica L., variety "Oeiras") and four transects of 50 m extending from the trees (Figure 1). In November of 2012, 196 seedlings of Inga edulis Mart. (Fabaceae) were planted in one plot of each block (49 trees per plot). The trees had an average height of 60 cm. Trees were distributed in seven rows in each plot and spaced 2.5 m. The other plot in the block, with only coffee plants, served as control. Each block had one treatment and one control plot. They were interspersed and 22 m apart. Natural enemies of coffee pests, coffee damage and coffee yield were assessed between February 2013 and February 2014. Sampling was performed every fifteen days, during 51 weeks. On every sample date, ten coffee plants in each plot were randomly chosen and sampled (excluding plants from the edge), and one coffee plant was sampled every 10 meters in the transects (0 - 50m).

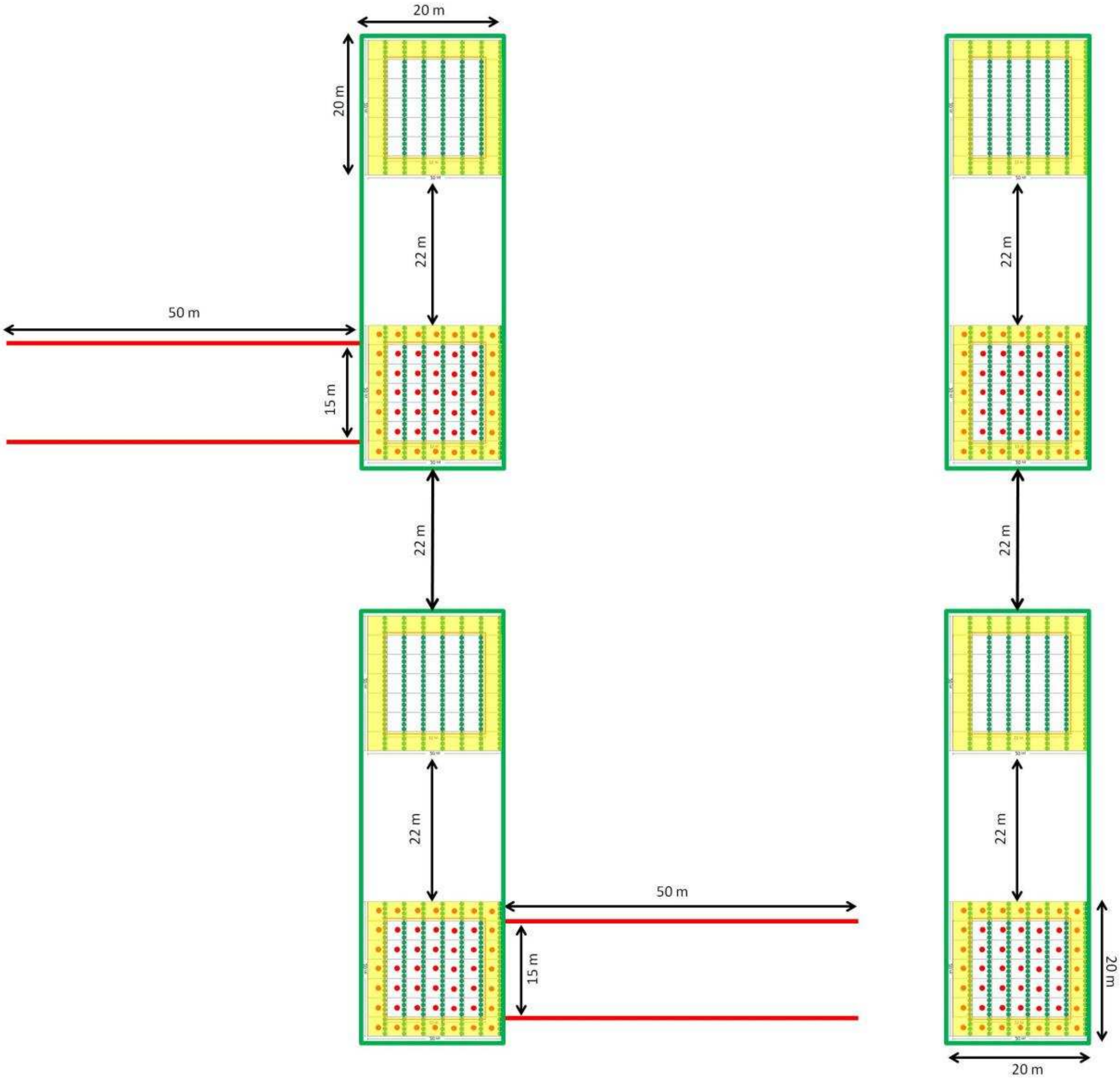

Figure 1. Sketch of the experimental design. Green rectangles delimit the blocks where the experiments were performed. Green dots represent coffee plants and red dots represent Inga trees, which were planted in one plot of each block; the other plot within the block served as control. Each plot within blocks measured 400m^2. The red lines are the transects (50 m) used to assess the distance effect from the Inga trees. Sampling was performed inside plots excluding the edge (yellow strips) and every 10 m at the transects.

Coffee yield

The coffee yield was estimated by collecting the total number of fruits from 10 coffee plants on each plot and from one coffee plant every 10 meters on each transect in May 2013 and May 2014. A total of 104 coffee plants was sampled to assess coffee yield in each year. Coffee productivity was expressed as the total weight of fruits per coffee plant (kg/plant) and as the mean weight per fruit (g/100 fruits per coffee plant).

Coffee damage

To assess the damage caused by coffee leafminers, we measured the proportion of mined leaves. Ten coffee plants were fortnightly sampled in each plot and one coffee plant was sampled every 10 meters on each transect. Eight leaves were collected from primary plagiotropic branches at the center of the canopy of each coffee plant. We sampled the fourth pair of leaves from each side of the plant (north, south, east and west) (Oliveira, 2003, Souza, Reis, et al., 1998). The proportion of mined leaves per coffee plant was assessed based on a total sample size of 21632 leaves.

Damage caused by coffee berry borers was calculated as the proportion of bored coffee berries. We conducted a non-destructive sampling method on the same coffee plants as sampled for mined leaves. Fifty fruits were observed on each coffee plant from one branch at the center of the plant (Souza and Reis, 1997). The proportion of bored fruits per coffee plant was assessed based on a total sample size of 135200 berries. After coffee harvesting, a new survey was conducted to evaluate damage caused by coffee berry borers. The proportion of bored fruits per coffee plant was assessed based on a total of 10400 coffee fruits per year for two consecutive years.

Natural enemies

To access the parasitism rate of coffee leaf miners we collected one mined leaf from 10 coffee plants in each plot fortnightly. We also collected one mined leaf every 10 meters on each of the four transects. We chose leaves with intact mines to assure that parasitoids had not emerged and that leaf miners had not been attacked by predatory wasps. Each mined leaf was incubated in a separate plastic vial with the petioles inserted in water to maintain turgidity (Pereira, Picanço, et al., 2007, Reis Jr., Lima, et al., 2000). Leaves were kept in the laboratory until the emergence of leaf miners or parasitoids. Emerged parasitoids were stored in 70% ethanol for identification. A total of 2704 mined leaves was sampled and the parasitism rate per coffee plant was assessed.

Death of coffee leaf miner larvae due to predation can be observed because the predators need to tear the mines to reach the miner larvae (Lomeli-Flores, Barrera, et al., 2009, Pereira, Picanço, et al., 2007). A total of 21632 coffee leaves was sampled from plots and transects to assess damage caused by coffee leaf miners (see above).

They were taken to the laboratory, where they were examined for torn mines to assess predation rate.

To assess the proportion of bored berries with natural enemies or other insects, such as secondary pests, bored fruits were collected in plots and transects three times between July and November 2013, after coffee harvesting. These fruits were sampled from the ground because the densities of natural enemies in such fruits may be 90% higher than fruits collected from coffee trees (Jaramillo, Chabi-Olaye, et al., 2009). A total of 1154 berries were dissected under a stereomicroscope to search for natural enemies and secondary pests. All insects found inside the bored berries were stored in 70% ethanol for identification. The proportion of bored berries with natural enemies and secondary pests per plot and per distance was assessed.

To assess the predation of coffee berry borers, 350 non-infested berries were fortnightly collected from the field and taken to the laboratory. The surface of the berries was previously sterilized to avoid fungal contamination. The berries were washed with detergent for 15min, rinsed with tap water, then dipped in a 2% sodium hypochlorite solution for 10 min, rinsed again with sterile distilled water, thereafter soaked in a 2% potassium sorbate solution and finally rinsed with sterile distilled water (Perez, Infante, et al., 2005). Subsequently, the berries were placed in plastic containers and exposed for 24h to 400 female coffee berry borers from a colony maintained at the Pheromone Laboratory of the Federal University of Viçosa. Bored berries were selected and transferred to plastic containers filled with a 3cm layer of a mixture of plaster of Paris and activated charcoal (9:1) (Jaramillo, Chabi-Olaye, et al., 2009). The mixture allows regulation of the relative humidity in the environment, slowing down the dehydration of the berries and preventing them from rotting (Jaramillo, Chabi-Olaye, et al., 2009). Every tree days, the containers were watered to maintain humidity.

Fifteen days after infestation, the bored berries were brought back to the field inside mesh bags. The experimental unit consisted of a pair of mesh bags containing three bored fruits in each bag. One of the bags allowed natural enemies to enter due to the large holes of the mesh. The other bag served as a control for mortality unrelated to natural enemies, and was made of extremely fine polyester that did not allow natural enemies to enter (Larsen and Philpott, 2010) (Figure 2). Three pairs of bags were placed on the soil surface, fixed to the lowest branch of three different coffee plants in each plot. The same methodology was applied in the transects, where one pair of bags was placed on one coffee plant every 10 meters. The berries were placed on the ground because berries on the soil are the main reservoir of natural enemies of coffee berry borers (Jaramillo et al., 2009b). The bags were replaced with new bags with infested berries after 15 days and berries from the old bags were dissected under a stereomicroscope to assess predation of coffee berry borers inside the berries (Larsen and Philpott, 2010). This was repeated until the end of the fruit season. A total of 2304 infested berries was dissected between April and July of 2013. To ensure that predation occurred in bored berries inside open mesh bags, the number of coffee berry borers in open and closed bags was compared. The predation of H. hampei in open bags was compared between monoculture coffee and coffee with Inga trees and along the transects.

Figure 2. Exposed bored coffee fruits used to assess predation of coffee berry borers. A pair of mesh bags, each containing three bored fruits were tied to a low branch of a coffee plant and rested on the soil. One of the bags consisted of course mesh, allowing natural enemies to enter (right). The other bag was made of fine polyester, through which natural enemies could not enter, and served as a control (left).

Statistical analysis

The effect of Inga trees consorted with coffee plants and the effect of distance from Inga trees on the coffee yield, on the damage caused by leaf miners and berry borers, on the parasitism and predation of coffee leaf miners and on the predation of coffee berry borers were analyzed using linear mixed effects models (LME of the package nlme) (Pinheiro et al., 2010). The data were analyzed separately for each of the response variables. When necessary, the response variables were square-root transformed to stabilize variance. When plots with and without Inga were compared, we used the presence of Inga, time and their interactions as factors. When the effect of distance was analyzed, we used distance, time and their interactions as factors. Because coffee plants were sampled within the same blocks over time we used block as a random factor. To assess effect of distance from Inga trees, we used transect as a random factor.

Attack of natural enemies may be correlated with abundance of pests, we therefore included the proportion of mined leaves as an explanatory variable in the analyses of parasitism of coffee leaf miners and the proportion of preyed mines. Because the data of coffee leaf miner parasitism and predation of mines were zero-inflated, we were forced to join data through time for a proper analysis. To assess predation of coffee berry borers, the type of mash bag (course mesh or fine polyester) was included as a factor. Because the proportion of mined leaves showed a seasonal trend, we first fitted a cosine function through the time series (Crawley, 2013) and subsequently used a linear mixed effects model (LME of the nlme package of R) with treatment and the cosine functions as fixed factors.

The proportion of bored coffee fruits with natural enemies or secondary pests was analyzed using general linear mixed effects models (GLMER of the package lme4) with a binomial error distribution (Bates, Maechler, et al., 2014). The data were analyzed separately for each of the response variables. When plots with and without Inga were compared we used the presence of Inga, time and their interactions as fixed factors and block as random factor. When the effect of distance was analyzed, we used distance, time and their interactions as fixed factors and transect as random factor. For all analyses, we formulated a full model and then removed non-significant interactions and factors using the anova function of R (2010). Factor levels were compared through a post-hoc analysis by grouping factor levels (Crawley, 2013). All analyses were performed using R software (R Development Core Team, 2011) and residuals were analyzed to check for the suitability of the models and distributions used (Crawley, 2013).

Results

Coffee yield

During both harvests, the average coffee fruit weight was higher in coffee plants with Inga than in monoculture coffee (2013: $F_{1, 75}$= 14.87, P< 0.0001; 2014: $F_{1, 75}$= 5.71, P= 0.02; Figure 3). Coffee fruit weight was higher during the second harvest ($F_{1, 154}$= 115.03, P< 0.0001; Figure 3). Production per coffee plant was higher in the first harvest ($F_{1, 155}$= 102.08, P< 0.0001) and did not differ between monoculture coffee and coffee with trees (2013: $F_{1, 75}$= 0.001, P= 0.97; 2014: $F_{1, 75}$= 0.40, P= 0.53; Figure 4). The distance from Inga trees did not affect coffee fruit weight (2013: $F_{1, 19}$= 0.69, P= 0.42; 2014: $F_{1, 19}$= 0.62, P= 0.44). However, production per coffee plant decreased with distance from Inga trees in the first year, but such trend was not observed in the second year (2013: $F_{1, 19}$= 13.00, P= 0.002; 2014: $F_{1, 11}$= 0.001, P= 0.97, Figure 5).

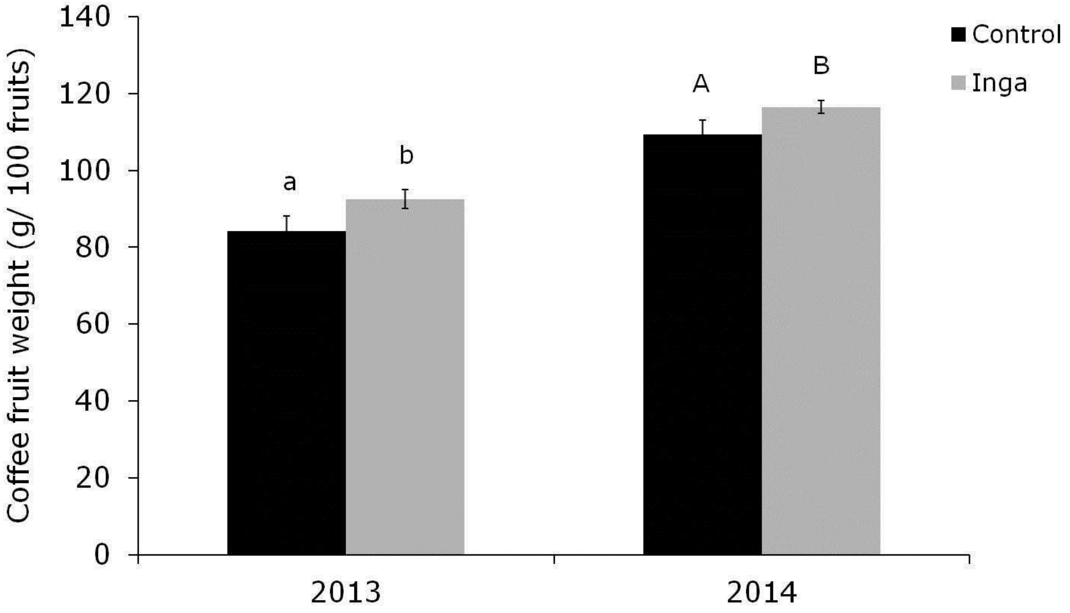

Figure 3. The average (±s.e.) fruit weight from monoculture coffee (black bars) and coffee with Inga trees (grey bars) for two different years. Bars with different letters differ significantly (P < 0.05).

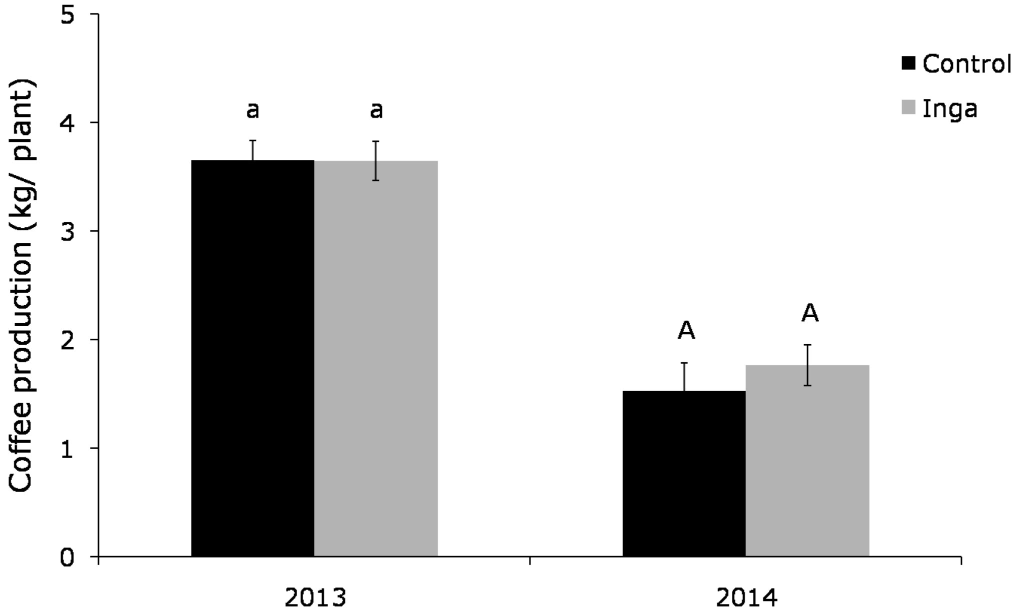

Figure 4. The average (±s.e.) coffee production (kg per coffee plant) from monoculture coffee (black bars) and coffee with Inga trees (grey bars) for two different years. Bars with different letters differ significantly (P < 0.05).

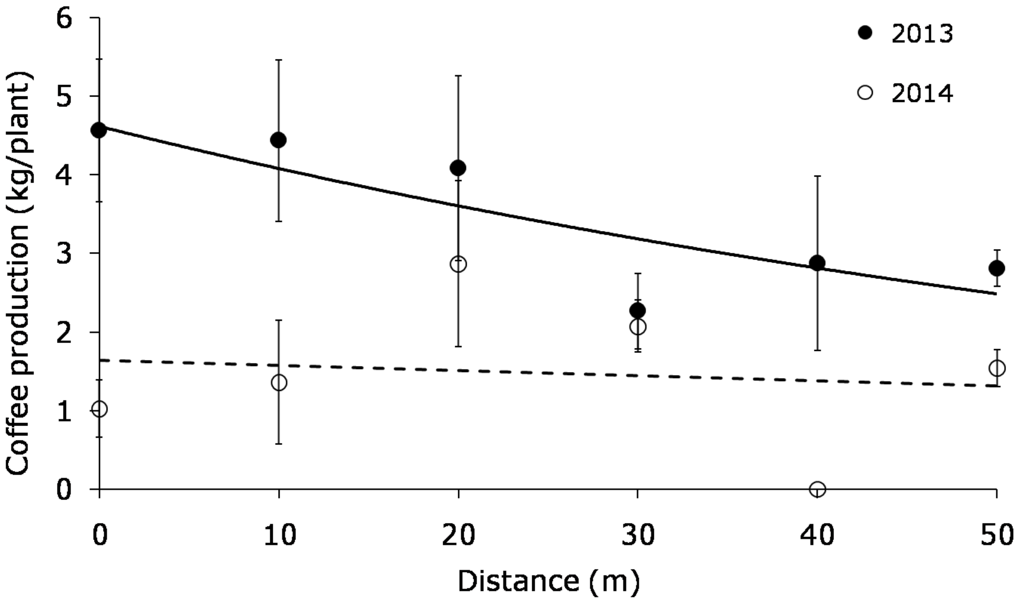

Figure 5. Coffee plant production (kg per plant) in 2013 (closed circles) and 2014 (open circles) as a function of distance to nearby Inga trees. Shown are averages (±s.e.) per distance (2013: $F_{1,19}$= 13.00, P= 0.002; 2014: $F_{1,11}$= 0.001, P= 0.97).

Coffee damage

The proportion of mined leaves showed a seasonal trend (Figure 6). It decreased during the rainy season and increased during the dry period. Especially when leaf miners cause most damage, i.e. during the dry season, the proportion of mined leaves was lower for coffee with Inga ($F_{1,201}$= 13.50, P= 0.0003, Figure 6) and was also affected by distance from the trees ($F_{1,618}$= 4.77, P= 0.03, Figure 7). Coffee plants near trees (0 m) had a lower proportion of mined leaves throughout the experiment than coffee plants beyond 10 m (L.ratio = 10.98, P< 0.0001; Figure 7).

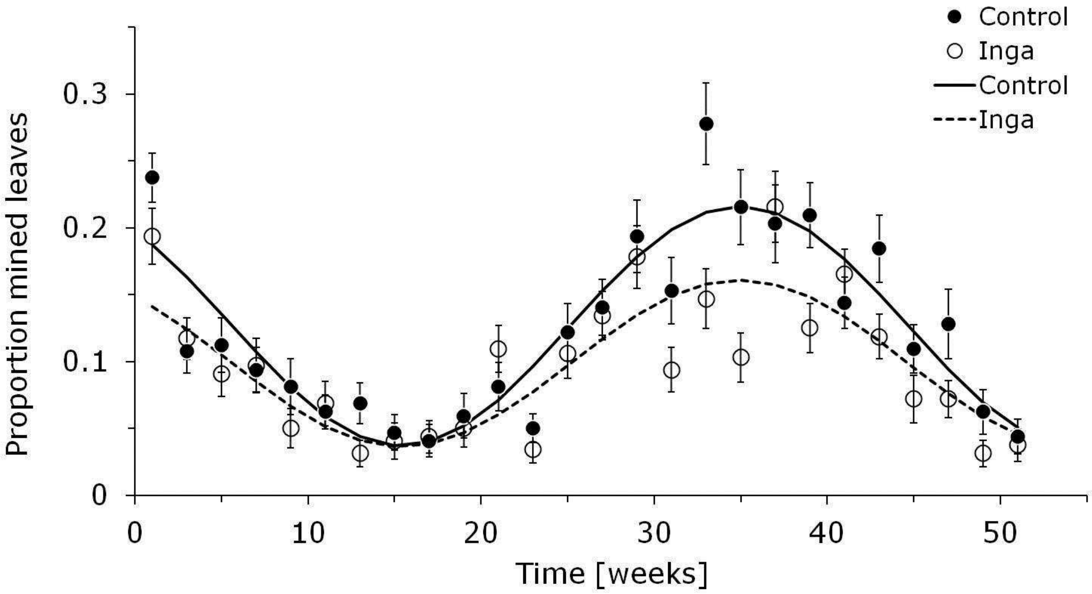

Figure 6. The proportion of mined coffee leaves from coffee monoculture (closed circles) and coffee with Inga trees (open circles) as a function of time ($F_{1,201}$= 13.50, P= 0.0003). Data points are averages (±s.e.) per sampling time. The curve was fitted to these averages for illustrative purposes (y= a + b * (cos (pi * (c* Time + d))), with a= -0.113, b= 0.076, c= 0.051 and d= 0.212).

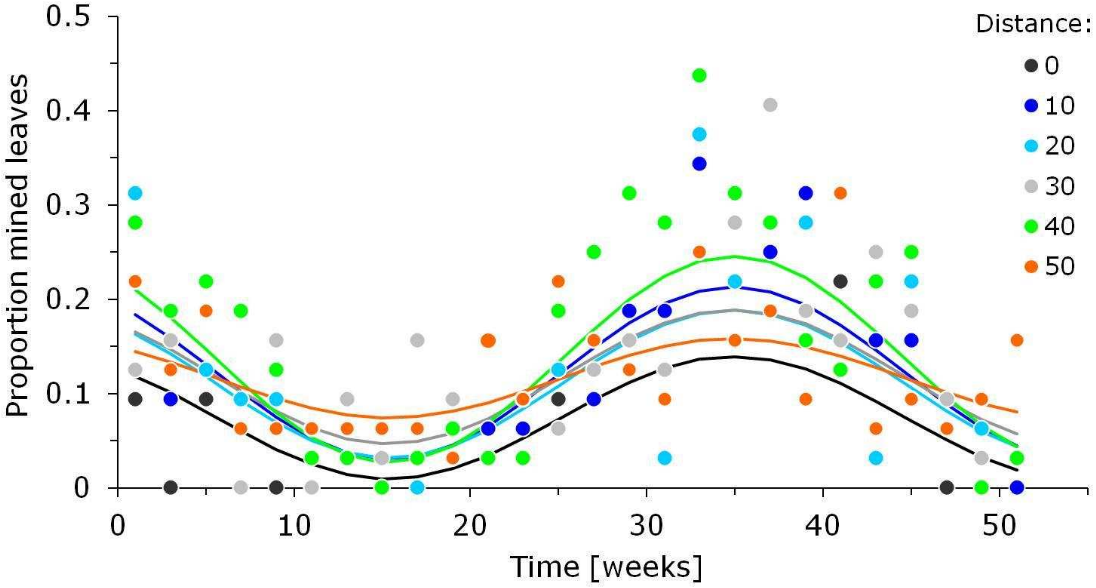

Figure 7. The proportion of mined coffee leaves as a function of time and distance from nearby Inga trees. Shown are averages per sampling time for each distance ($F_{1,618}$= 4.77, P= 0.03). See legend to Figure 4 for further explanations. Coffee plants near trees (0 m) had a lower proportion of mined leaves throughout the experiment than coffee plants beyond 10 m (L.ratio = 10.98, P< 0.0001).

The proportion of bored coffee fruits was also lower for coffee with trees ($F_{1,\ 8}$= 12.74, P= 0.0006, Figure 8) and was higher for coffee plants distant from the trees ($F_{1,\ 259}$= 8.43; P= 0.004, Figure 9). The proportion of bored fruits was also recorded after harvesting in both years. A higher infestation of coffee berry borers was observed in the second year ($F_{1,\ 153}$= 485.44, P< 0.0001). However, coffee plants that were consorted to Inga trees were less damaged in the first year ($F_{1,\ 74}$= 5.54, P= 0.02, Figure 10) and the same trend was found in the subsequent year, although the difference was marginally significant ($F_{1,\ 75}$= 3.07, P= 0.08, Figure 10). We did not observe an effect of distance on coffee damage for sampling done after coffee harvesting (2013: $F_{1,\ 19}$= 1.11, P= 0.31; 2014: $F_{1,\ 19}$= 0.01, P= 0.92).

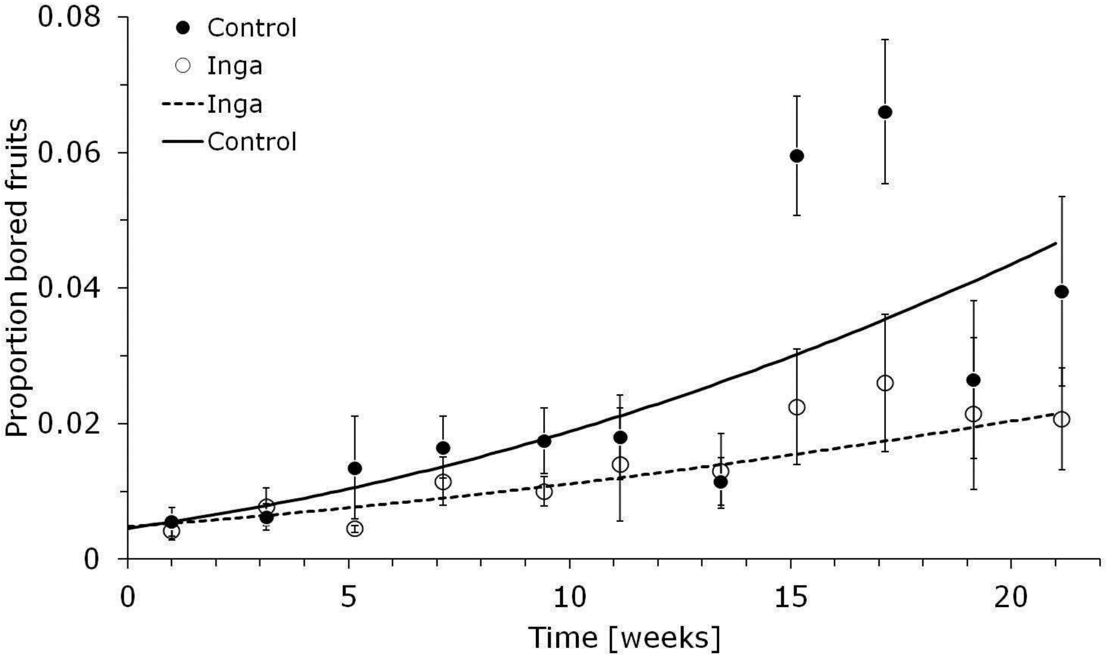

Figure 8. The proportion of bored coffee fruits from monoculture coffee (closed circles) and from coffee with Inga trees (closed circles) as a function of time. Shown are averages (±s.e.) per sampling time ($F_{1,\ 8}$= 12.74, P= 0.0006).

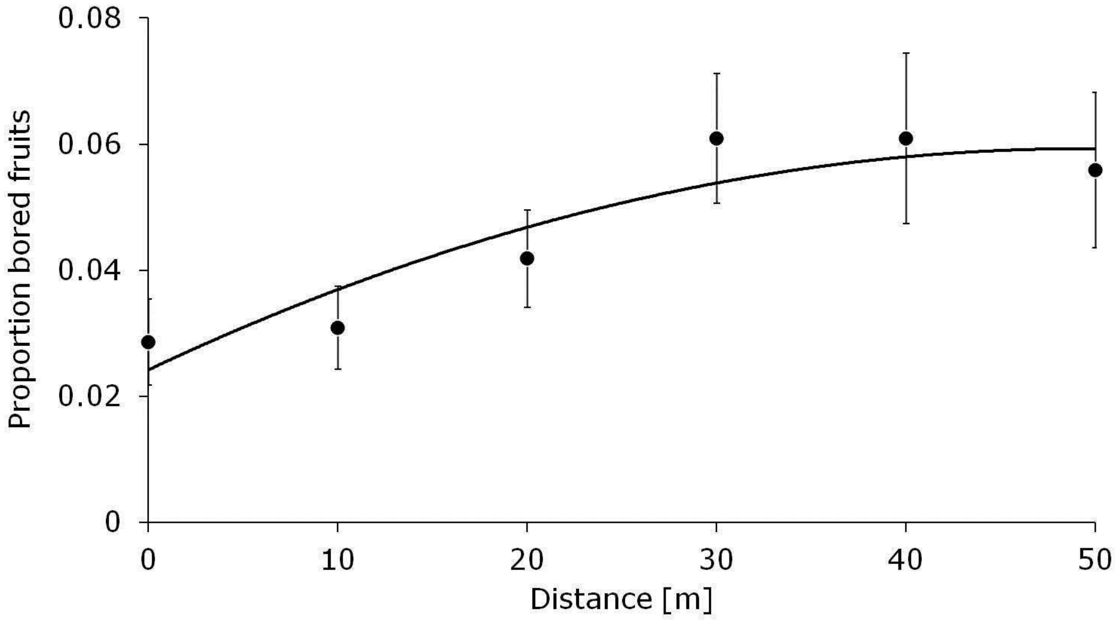

Figure 9. The proportion of bored coffee fruits as a function of distance from nearby Inga trees. Shown are averages (±s.e.) per distance ($F_{1,\,259}$= 8.43; P= 0.004).

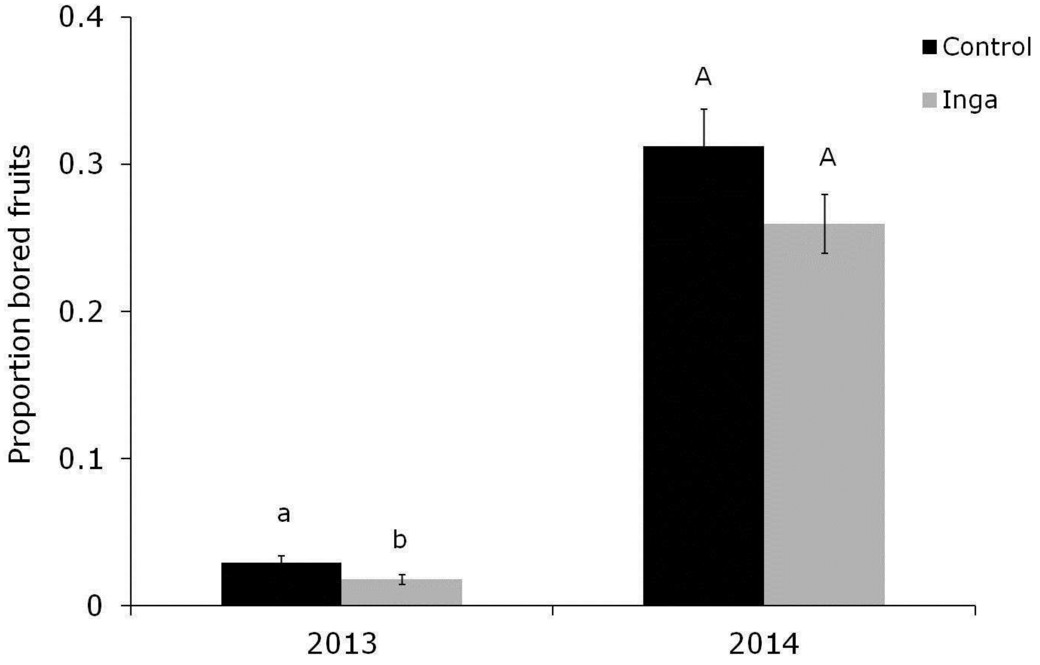

Figure 10. The average (±s.e.) proportion of bored coffee fruits from monoculture coffee (black bars) and coffee with Inga trees (grey bars) for two years. Bars with different letters differ significantly ($P < 0.05$).

Natural enemies

A total of 1365 parasitoids of the coffee leaf miner emerged from mined leaves (Table 1). In monoculture coffee systems 37.53% of leaf miners were parasitized, contrasting to 41.74% when coffee was consorted with Inga trees. However, the parasitism rate of coffee leaf miners did not differ between monoculture coffee and Inga shaded coffee ($F_{1, 203}$= 1.68, P= 0.20). Also, parasitism of coffee leaf miners did not decrease with distance from the trees ($F_{1, 19}$= 0.00, P= 0.98). The proportion of mines predated by wasps was not different between monoculture and Inga shaded coffee ($F_{1, 197}$= 0.07, P= 0.78). However, the predation rate tended to decrease with distance from the trees ($F_{1, 19}$= 3.90, P= 0.06).

Table 1. Identity and number of parasitoids emerged from mined coffee leaves.

Taxa	Abundance			Total
	Monoculture	Consorted coffee	Transects	
EULOPHIDAE				
Entedoninae				
Closterocerus coffeellae Ihering	41	46	15	102
Horismenus sp.	210	190	77	477
Proacrias coffeae Ihering	43	28	23	94
BRACONIDAE				
Miracinae				
Centistidea striata Penteado-Dias	109	133	85	327
Orgilinae				
Orgilus sp.	49	74	24	147
Rogadinae				
Stiropius reticulatus Penteado-Dias	28	40	28	96
Unidentified species	43	46	33	122
Total	523	557	285	1365

Natural enemies and herbivores (secondary pests) were recorded inside 12.55% of total bored coffee fruits sampled from the ground. Secondary pests were recorded inside 7.75% of bored berries sampled from monocultures and in 5.84% of berries from coffee consorted with Inga trees. Predators were found in 8.14% of the berries from monoculture and in 9.12% of berries from coffee with trees. Ants accounted for the majority of predators (91.30%) and non identified beetles (Staphylinidae) represented the remaining predators. The parasitoid Prorops nasuta Wat. (Hymenoptera: Bethylidae) was recorded inside 1.16% of bored berries from monoculture contrasting to 4.74% of berries from coffee consorted with Inga trees. Considering predators and parasitoids together, the proportion of bored coffee fruits with natural enemies inside did not differ between berries sampled from coffee monoculture and coffee consorted (χ^2= 0.19, Df= 1, P= 0.66), neither did the proportion of fruits with secondary pests (χ^2=1.61, Df= 1, P= 0.20). However, the proportion of natural enemies was higher near Inga trees than at distances beyond 10 m (χ^2= 28.69; Df= 1; P< 0.0001, Figure 11).

Herbivores (secondary pests) inside bored berries also decreased with distance from Inga trees (χ^2= 4.39; Df= 1; P= 0.04, Figure 11).

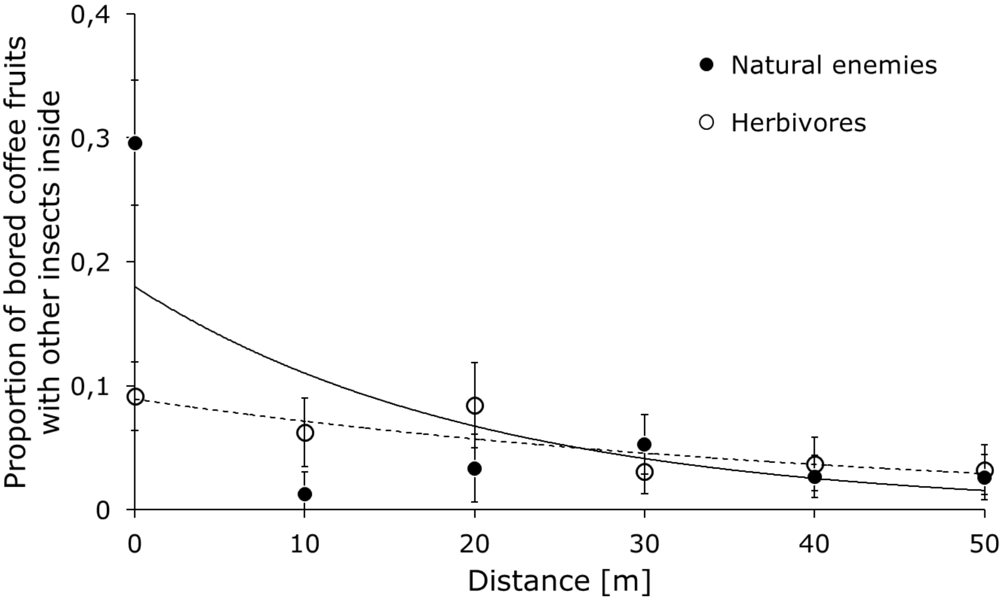

Figure 11. The proportion of bored coffee fruits with natural enemies (closed circles) or herbivores (open circles) as a function of distance from nearby Inga trees. Shown are averages (±s.e.) per distance (Natural enemies: χ^2= 28.69; Df= 1; P< 0.0001; Herbivores: χ^2= 4.39; Df= 1; P= 0.04).

Predation of coffee berry borers inside bags that allowed for natural enemies to enter did not differ between coffee monoculture and coffee with Inga trees ($F_{1,122}$= 0.61; P= 0.43). The distance from Inga trees also did not affect the number of coffee berry borers inside coffee fruits ($F_{1, 371}$ = 2.61; P= 0.11). In the transects, the number of coffee berry borers inside open bags was lower than in closed bags ($F_{1, 372}$ = 5.62; P= 0.02). Hence, the bags did work as expected along the transects, but the number of coffee berry borers did not differ between open and control bags in the plots ($F_{1,121}$ = 0.05; P= 0.81).

Discussion

Through manipulative field experiments, we found that natural enemies of coffee pests were affected by the proximity of Inga trees and that coffee consorted with Inga trees were less damaged by pests and produced heavier fruits. To our knowledge, this is the first long-term field experiment showing that associated plants bearing extrafloral nectaries enhance natural pest control and fruit weight. The major bottleneck in implementation of plant diversification strategies for pest control by farmers is that these practices do not always translate into increased yield (Poveda, Isabel Gomez et al., 2008). Most studies shows that increased diversification can lead to lower crop production (Poveda, Isabel Gomez et al., 2008; Letourneau, Armbrecht, Rivera et al., 2011). However, the decreased yield in some diversified systems compared to monocultures can be a direct cause of reduced density of the main crop due to the presence of non-crop plants (Letourneau, Armbrecht, Rivera et al., 2011). Also, diversified systems can produce secondary products associated with non-crop plants, such as fruits and timber, that can play a important role in terms of both use and exchange value (Rice, 2008; 2011). Finally, farming diversification has to be designed to support functional biodiversity and provide intended ecosystem services (Landis, Wratten et al., 2000; Kremen, Iles et al., 2012). Therefore, it is important to assess functionally important plants to shape agricultural systems to specifically reduce pest pressure and enhance production (Poveda, Isabel Gomez et al., 2008). Our results show that Inga species are important elements of diversity in coffee systems to enhance pest control and coffee production.

Here, coffee production differed between years and in the first year, when coffee production was higher, plants near Inga trees had higher yield, but such a trend was not observed in the subsequent year. Differences in production between years is explained by the biennial production trend of coffee, alternating between high and low flowering, which leads to significant differences in crop production from one year to another (Damatta, 2004). In contrast to our findings, other studies showed that Inga-shaded coffee produces lower yield compared to intensive monoculture coffee (Haggar, Barrios et al., 2011). However, biennial fluctuations of crop yields occur predominantly in unshaded coffee (Damatta, 2004). Therefore, it is suggested that the higher yields per harvest in unshaded plantations might be compensated by the larger number of more regular crop harvests in shaded plantations (Damatta, 2004). In agreement with our results, other studies have also shown increases in bean weight and bean size in the shade (Muschler, 2001; Vaast, Bertrand et al., 2006; Somporn, Kamtuo et al., 2012). Here, increased coffee fruit weight in coffee with Inga can be primarily subscribed to decreased damage of coffee plants associated with trees. Damage caused by coffee berry borers can significantly reduce coffee fruit weight and quality (Damon, 2000). Also, coffee leaf miners decrease photosynthesizing foliar area, which also reduces the weight of coffee beans (Pereira, Eliseu José G., Picanço, Marcelo C. et al., 2007). Damage caused by coffee pests, however, may be not the only factor influencing coffee fruits. Shade can also affect abiotic conditions and other factors, such as soil nutrient content and pollination, which might affect coffee yield (Damatta, 2004; Lin, 2009; Badano and Vergara, 2011; Classen, Peters et al., 2014).

Irrespective of the seasonal effects on mined leaves, damage caused by coffee leaf miners was lower in coffee with Inga trees throughout the year, as well as damage caused by coffee berry borers. Many studies have reported seasonal fluctuations in coffee leaf miner densities, as was found here (Nestel, Dickschen et al., 1994; Pereira, E.J.G., Picanço, M.C. et al., 2007; Lomeli-Flores, Barrera et al., 2010). Rainfall is one of the main mortality factors of coffee leaf miners and this might explain most of the variation in the proportion of mined leaves during the rainy season (Pereira, E.J.G., Picanço, M.C. et al., 2007). Also, the abundance of natural enemies can vary among seasons. For example, ants can be more abundant in the wet season, which could also contribute to decreased damage of coffee (Philpott, Perfecto et al., 2006). In the dry season, when coffee leaf miners caused highest damage, the difference in proportion of mined leaves between the coffee systems was pronounced. Mortality rates of coffee leaf miners during dry season are mainly addressed to natural enemies (Pereira, E.J.G., Picanço, M.C. et al., 2007). Therefore, enhancement of natural enemies due to provision of sugar-rich food (Chapter II and Chapter III) might explain the decreased damage in coffee plants with Inga trees. Other factors influenced by the trees, such as temperature, humidity and pathogens may also affect damage caused by coffee pests (Baker, Rivas et al., 1994; Pereira, E.J.G., Picanço, M.C. et al., 2007; Teodoro, Mein et al., 2008; Lomeli-Flores, Barrera et al., 2009; Moreira, 2012), but they were not assessed here.

Survival enhancement of natural enemies of coffee leaf miners through nectar feeding (Chapter III) did not result in a significant increase of parasitism and predation of coffee leaf miners in field. However, we observed the proper trends. For example, predation of leaf miners by wasps tended to decrease with increasing distance from the Inga trees. Other studies show that parasitoids can be attracted by nectaries and increase the number of parasitized hosts near patches providing food (Tylianakis, Didham et al., 2004; Balmer, Pfiffner et al., 2013; Jamont, Dubois-Pot et al., 2014). Wasps are also known to feed on extrafloral nectar and benefit the nectary-bearing plants due to predation of its herbivores (Cuautle and Rico-Gray, 2003; Alves-Silva, Baronio et al., 2013). In well developed coffee agroforestry systems, the availability of nectar in Inga trees increased parasitism of coffee leaf miners (Rezende, Venzon et al., 2014). Therefore, we must consider that coffee plants evaluated here were recently intercropped with Inga trees and maybe not all aspects of natural pest control would increase by the presence of small trees.

Natural enemies of coffee berry borers were found more often in berries near Inga trees, but there was no difference in the proportion of berries with natural enemies between shaded coffee and monoculture. Dissection of bored berries revealed that the natural enemies most frequently found inside berries were ants and the parasitoid P. nasuta. Ants are the most important predator of coffee berry borers and they provide substantial natural control of this pest, especially in shaded coffee (Philpott and Armbrecht, 2006; Armbrecht and Gallego, 2007; Larsen and Philpott, 2010; Gonthier, Ennis et al., 2013). They can enter bored berries and feed on larvae and adults inside, and also prevent berry borers from entering new berries (Larsen and Philpott, 2010; Gonthier, Ennis et al., 2013). Ant predation inside berries fallen on the ground can be very important because berries that remain in the field after harvesting can be a population reservoir of coffee berry borers (Damon, 2000). Ants are closely associated with extrafloral nectar-bearing plants, including Inga trees, and increased plant protection against herbivores is often attributed to their presence. (Koptur, 1984; Rosumek, Silveira et al., 2009; Trager, Bhotika et al., 2010). The parasitoid P. nasuta is the most common parasitoid of coffee berry borers, however, parasitism rates in the field are usually low (Infante, Mumford et al., 2001). Laboratory studies have shown that P. nasuta survives longer when it feeds on nectar (Damon, Pacheco et al., 1999). Therefore, ants and parasitoids attracted to extrafloral nectaries may also feed on coffee berry borers and provide protection to coffee plants near Inga trees.

We did not observe predation inside berries placed in mesh bags. The low rates of berries with natural enemies (sampled from the ground) suggest that natural enemies provide low control through predation of borers already inside the berries. It might explain why no differences in predation rates were observed. Perhaps, natural enemies are more efficient in preventing coffee berry borers to enter the berries (Gonthier, Ennis et al., 2013). In fact, Inga-shaded coffee had less bored coffee fruits. Secondary pests were also recorded more often inside bored berries near Inga trees. Nectar can benefit herbivores and studies considering the edibility of nectar sources to herbivores must be done before introducing a nectary-bearing plant to enhance crop protection (Geneau, Wackers et al., 2012). Surveys in agroforestry coffee systems found herbivores feeding on extrafloral nectaries of Inga plants, but none of them were identified as coffee pests (Rezende, Venzon et al., 2014). Also, even when herbivores profit from the plant-provided food, plants may still gain protection (Van Rijn, Van Houten et al., 2002). For example, higher density of pest species may result in a strong numerical response of predators, which could lead to enhanced pest control (Messelink, Maanen et al., 2008; Messelink, Van Maanen et al., 2010). Therefore, increased secondary pests near Inga trees could decrease populations of coffee berry borers through apparent competition.

Uncovering the effect of individual plant species on pest regulation will help in understanding the mechanisms which enhance pest control in diversified systems. It can also help in the design of pest-suppressive coffee systems (Staver, Guharay et al., 2001). Diversified coffee systems provide a more stable income due to the provision of fruits and timber, require fewer inputs and enhance functional biodiversity, carbon sequestration, soil fertility, drought resistance and weed and pest control (Jha, Bacon, et al., 2011; Souza, Goede, et al., 2012). Therefore, Inga trees should be used to enhance crop protection in concert with other plant species that will provide other ecosystem services. Our findings strengthen the hypothesis that indirect plant defenses provided by extrafloral nectaries can indeed decrease herbivory on neighboring plants and sustain the use of extrafloral nectary-bearing plants for crop protection.

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General conclusion

Inga trees bearing extrafloral nectaries enhanced natural pest control of pests and production in coffee crops. Fruits from coffee consorted with Inga trees were heavier than fruits from monoculture coffee. This effect on production can be attributed to decreased pest damage on coffee plants intercropped with the trees. Coffee with Inga trees had lower proportion of mined leaves and lower proportion of bored fruits comparing to coffee monoculture. One of the mechanisms proposed to explain decreased damage of coffee is the enhancement of natural enemies due to extrafloral nectar feeding on Inga trees. In fact, parasitoids and a predator of coffee leaf miner that fed on extrafloral nectar of Inga trees increased survival. Moreover, a coffee berry borer predator (Trybomia sp.) also profited from the sugar-rich food resembling nectar. Survival enhancement is expected to lead a numerical response of natural enemies in field, and increased population of parasitoids and predators often lead to increased parasitism and predation rates.

Nectar availability from Inga trees enhanced parasitism of coffee leaf miners, but this could be observed only in well developed agroforestry systems. However, we observed the proper trends in predation and parasitism rates of leaf miners in a field experiment with young Inga trees. Predation of coffee berry borers, on the other hand, was not influenced by the presence of the nectary-bearing trees. However, we only assessed predation inside the berries. Since coffee plants consorted to Inga trees were indeed less damaged, we suggest that natural enemies might play a more important role in preventing pests from attacking coffee plants.

Assessing the effect of individual plant species on pest regulation can guide the selection of functional biodiversity for diversification of agroecosystems. We showed that Inga trees are an important element of diversity in coffee systems to enhance pest control and coffee production. Our results confirm that plants bearing extrafloral nectaries can extend protection to neighboring plants and can be used to enhance natural pest control and crop production in agricultural systems.

Appendix A. Identity and number of hymenopteran parasitoids collected feeding on extrafloral nectar of Inga trees in agroforestry systems (n=5).

Taxa	Species distribution[1]	Abundance
Bethylidae		
Bethylidae sp. 1	1	1
Braconidae		
Braconidae sp. 1	1	1
Braconidae sp. 2	1	2
Chalcididae		
Brachymeriini sp.1	1	1
Chalcidini sp. 1	1	1
Chalcidini sp. 2	1	2
Chalcidini sp. 3	1	1
Crabronidae		
Crabronidae sp. 1	2	6
Crabronidae sp. 4	1	1
Diapriidae		
Diapriidae sp. 1	1	1
Dryinidae		
Dryinidae sp. 1	1	1
Encyrtidae		
Encyrtidae sp. 1	1	1
Encyrtidae sp. 2	1	1
Eulophidae		
Entedoninae sp. 1	2	3
Galeopsomyia sp. 1	1	1
Tetrastichinae sp.1	1	1
Eurytomidae		
Eurytominae sp. 1	3	10
Eurytominae sp. 2	1	1
Evaniidae		
Evaniidae sp. 1	1	1
Evaniidae sp. 2	1	1
Figitidae		
Eucoilinae sp. 1	1	1
Figitidae sp. 1	1	1

Taxa	Species distribution[1]	Abundance
Mymaridae		
Mymaridae sp. 1	2	2
Mymaridae sp. 2	1	1
Platygastridae		
Platygastridae sp. 1	1	1
Pteromalidae		
Pteromalidae sp. 1	1	2
Total abundance		46

[1] The number of agroforestry systems (n=5) in which the species occurred.

Appendix B. Identity and number of predators collected feeding on Inga extrafloral nectaries in agroforestry systems (n=5).

Taxa	L. coffeella[1]	H. hampei[1]	Species distribution[2]	Abundance
Arachnida				
Arachnida sp.1	x[3]	x[7]	1	1
Blattodea				
Blattodea sp.1	x[3]		1	1
Coleoptera				
Coccinelidae sp.1			1	1
Diptera				
Dolichopodidae sp.3			2	2
Hymenoptera				
Formicidae				
Brachymyrmex sp.1		x[8]	1	8
Brachymyrmex sp.2		x[8]	1	2
Camponotus rufipes			3	32
Camponotus sp.1	x[3]		1	2
Camponotus sp.2	x[3]		1	1
Camponotus sp.3	x[3]		2	5
Camponotus sp.4	x[3]		1	1
Crematogaster sp.	x[3]	x[8,10]	4	21
Pachycondyla sp.			1	1
Pheidole sp.		x[8,10,11]	1	4
Procryptocerus sp.			2	5
Pseudomyrmex sp.1	x[3]	x[9]	3	13
Pseudomyrmex sp.2	x[3]	x[9]	3	7
Solenopsis sp.	x[3]	x[8,10,11]	2	2
Vespidae				
Vespidae sp.1	x[3,4,5]		1	1
Neuroptera				
Crysoperla sp.	x[6]		1	1
Thysanoptera				
Trybomia sp.		x	1	2
Total abundance				113

[1] Crosses indicate that the arthropod collected was reported on literature as a predator of coffee leaf-miner (Leucoptera coffeella) or coffee berry borer (Hypothenemus hampei).

[2] The number of agroforestry systems (n=5) in which the species occurred.

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