ECOLOGIA QUÍMICA DE *Tenuisvalvae notata* (MULSANT) e *Cryptolaemus montrouzieri* MULSANT (COLEOPTERA: COCCINELLIDAE)

por

JENNIFER OBERGER FERREIRA

(Sob Orientação da Professora Christian Sherley Araújo da Silva Torres. UFRPE)

RESUMO

Tenuisvalvae notata (Mulsant), nativa da América do Sul, e *Cryptolaemus montrouzieri* Mulsant, nativa da Austrália, são joaninhas utilizadas no controle de cochonilhas-farinhentas (Pseudococcidae). Quando ocorrem simultaneamente na mesma área podem competir pelas presas e os semioquímicos emitidos por elas podem interferir nessa competição. Diante disso, avaliou-se a influência dos semioquímicos dos rastros e dos voláteis de *T. notata* e *C. montrouzieri* no desenvolvimento, na reprodução, no forrageamento e na capacidade predatória dessas espécies. Observou-se um atraso de 2 dias no tempo de desenvolvimento de *C. montrouzieri* quando expostos aos voláteis de coespecíficos e heteroespecíficos, mas não houve efeito em sua reprodução. Em *T. notata*, houve um aumento na fertilidade e fecundidade quando foram expostas aos voláteis de coespecíficos, mas o período de desenvolvimento não foi alterado. No comportamento predatório, voláteis de coespecíficos e heteroespecíficos alteraram a predação de larvas de instar I-III e adultos de *T. notata* e de larvas de instar I-II e adultos de *C. montrouzieri.* Em áreas com rastros, *C. montrouzieri* adultos reduziram a taxa de predação quando expostos a rastros de *T. notata*, mas não houve alteração na predação de adultos de *T. notata*. Apesar disso, *T. notata* fêmeas evitaram áreas com rastros de heteroespecíficos fêmeas, enquanto *C.*

montrouzieri fêmeas foram atraídas por áreas com rastros de *T. notata* fêmeas. O perfil químico dos rastros e voláteis, composto por monoterpenos, ésteres, hidrocarbonetos, alcoóis, aldeídos e cetonas, é específico de cada espécie e gênero e alteraram o tempo de desenvolvimento de *C. montrouzieri*, a reprodução de *T. notata*, o forrageamento e a taxa de predação dessas joaninhas. Esse é o primeiro estudo que relaciona os semioquímicos de *T. notata* e *C. montrouzieri* a comportamentos e aspectos biológicos dessas espécies, analisando a influência dessas interações no controle biológico de cochonilhas.

PALAVRAS-CHAVE: Controle biológico, joaninhas, comunicação química, semioquímicos.

CHEMICAL ECOLOGY OF *Tenuisvalvae notata* (MULSANT) and *Cryptolaemus montrouzieri* MULSANT (COLEOPTERA: COCCINELLIDAE)

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ABSTRACT

Tenuisvalvae notata (Mulsant), native to South America, and *Cryptolaemus montrouzieri* Mulsant, native to Australia, are ladybeetles used to control mealybug insects (Pseudococcidae). They can compete for prey when they occur in the same area, and the semiochemicals can mediate this competition. Therefore, we evaluate the influence of the semiochemicals of footprints and volatiles of *T. notata* and *C. montrouzieri* on the development, reproduction, foraging, and predatory capacity of these species. There was a 2-day delay in the development time of *C. montrouzieri* when they were exposed to conspecific and heterospecific volatiles, but there was no effect on their reproduction. On the other hand, fertility and fecundity of *T. notata* increased when they were exposed to conspecific volatiles, but the period of development was not altered. Regarding predatory behavior, volatiles of conspecifics and heterospecifics altered the predation rate of instar I-III larvae and adults of *T. notata* and instar I-II larvae and adults of *C. montrouzieri*. In areas with footprints, *C. montrouzieri* adults reduced the predation rate when exposed to *T. notata* footprints, but there was no change in the predation of adults of *T. notata*. Despite this, *T. notata* females avoided areas with heterospecific female's footprints, while *C. montrouzieri* females were attracted to areas with *T. notata* female's footprints. The chemical profile of

the footprints and volatiles, composed of monoterpenes, esters, hydrocarbons, alcohols, aldehydes, and ketones, is species and gender-specific and altered the development time of *C. montrouzieri*, reproduction of *T. notata*, foraging, and predation rate of these ladybeetles. This is the first study that relates the semiochemicals of *T. notata* and *C. montrouzieri* to the behavior and biological aspects of these species, analyzing the influence of these interactions on the biological control of scale insects.

KEY WORDS: Biological control, ladybeetles, chemical communication, semiochemicals.

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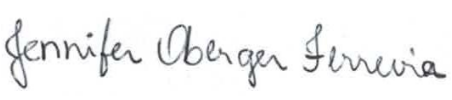

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INTRODUÇÃO

Coccinellidae

Os insetos entomófagos são agentes importantes no equilíbrio populacional de insetos fitófagos. Entre eles, os predadores se destacam pois consomem a presa, impedindo que gerem descendentes (Parra *et al*. 2002). E entre esses predadores, a família Coccinellidae (Coleoptera) chama a atenção devido ao seu impacto significativo no controle biológico (Michaud 2012).

A família Coccinellidae compreende um diverso grupo de besouros distribuídos mundialmente, com 6.000 espécies descritas em 360 gêneros, conhecidos popularmente como joaninhas (Vandenberg 2002). Em geral, os adultos são pequenos, de coloração variada, muitas vezes relacionada com as condições ambientais onde vivem. Podem apresentar dimorfismo sexual, e em média medem de 1 a 18 mm de comprimento, com as fêmeas geralmente maiores que os machos (Guedes 2013). Entre as principais características morfológicas estão o corpo oval, aproximando a esférico e plano ventralmente, cabeça escondida sob o protórax, antenas curtas e capitadas com 8 a 10 segmentos, tarsos com quatro segmentos (3^{o} reduzido e 2^{o} dilatado), asas do tipo élitro de cores vistosas e aparelho bucal mastigador. As principais características biológicas são desenvolvimento holometábolo, reprodução sexuada, ovos elípticos ou alongados, com número variável entre as espécies e larvas campodeiformes (Lixa 2008).

Os coccinelídeos se dividem em sete subfamílias: Coccidullinae, Coccinellinae, Scymninae, Ortaliinae, Chilocorinae, Sticholotidinae e Epilachninae (Kovár 1996). Essas subfamílias são caracterizadas por diferenças morfofisiológicas e pelo comportamento alimentar (Giorgi *et al.* 2009). Os representantes da subfamília Epilachninae, como *Hepilachna varivestis* Mulsant,

seguem uma dieta exclusivamente fitófaga (Fan *et al.* 1992). Existem também as espécies micófagas, como as pertencentes aos gêneros *Psyllobora*, *Halyzia*, *Thea*, *Vibidia* e *Illeis*, da tribo Halyziini (Guedes 2013). Por fim, temos as espécies carnívoras, com representantes nas subfamílias Coccinellinae, predadoras predominantemente de pulgões e psilídeos; Coccidullinae, que predam pulgões, cochonilhas e formigas; Chilocorinae, que predam pulgões e cochonilhas; Ortaliinae, predadores de cigarrinhas, psilídeos e formigas; Sticholotidinae, predadores de pulgões e cochonilhas; e Scymninae, que se alimentam de ácaros, pulgões e cochonilhas (Giorgi *et al.* 2009, Guedes 2013).

Quando sua presa preferida está escassa, as joaninhas predadoras se alimentam de recursos alternativos, tais como secreções açucaradas de pulgões e cochonilhas ("*honeydew*"), néctar floral ou extrafloral, pólen ou podem recorrer ao canibalismo, garantindo sua sobrevivência. Entretanto, muitas espécies de coccinelídeos completam seu desenvolvimento larval e produzem uma progênie viável apenas quando consomem sua presa preferencial, a qual estimula e mantém a produção normal de ovos (Lixa 2008, Khan & Yoldas 2018).

Entre as espécies descritas, 90% são carnívoras, a maioria com um grande potencial predatório e reprodutivo, alto desempenho de forrageamento e um período de sobrevivência acima de 30 dias, em condições favoráveis de temperatura e disponibilidade de alimento (Kindlmann & Dixon 1993, Dreyer *et al*. 1997a, Obrycki *et al*. 2009, Nedvěd & Honěk 2012). Devido a essas características favoráveis, estão sendo cada vez mais usadas em programas de controle biológico aplicado (controle biológico clássico e aumentativo) para reduzir a população de insetos praga.

O primeiro caso de controle biológico clássico bem sucedido envolveu a introdução da joaninha *Rodolia cardinalis* (Mulsant) para controlar *Icerya purchasi* Maskell, em plantas de Citrus na Califórnia durante o final da década de 1880 (Caltagirone & Doutt 1989). Pouco tempo depois, em 1891, a joaninha *Cryptolaemus montrouzieri* Mulsant, nativa da Austrália, foi

introduzida para o controle de *Planococcus citri* (Risso) na Califórnia. Desde então, essa espécie foi introduzida em mais de 40 países nas regiões temperadas e tropicais para o controle de cochonilhas (Both & Pope 1986, Maes *et al*. 2015).

Além da utilização de joaninhas em programas de controle biológico clássico, estas são importantes controladoras naturais, usadas em liberações inundativas nas lavouras ou em casa telada, a fim de controlar rapidamente as pragas, a exemplo das espécies *Hippodamia convergens* (Guerin-Meneville), *Coleomegilla maculata* (DeGeer), *Tenuisvalvae notata* (Mulsant) (= *Hyperaspis notata*), *Eriopis connexa* (Gemar), *Harmonia axyridis* Pallas, entre outras (Parra 1992, Scopel & Roza-Gomes 2011).

Liberações periódicas dessas joaninhas são necessárias para proporcionar a manutenção da população dos inimigos naturais de forma efetiva para o controle das pragas (Marques *et al.* 2015). Entretanto, a ocorrência simultânea de espécies diferentes, em especial a interação entre espécies nativas e não nativas, podem afetar esse controle, por promover comportamentos de competição pelo alimento, canibalismo e predação intraguilda (Oliveira 2020, Li *et al*. 2021). Nessas situações, os adultos da maioria das espécies de joaninhas dispersam do local de liberação, reduzindo a eficiência do controle. Diante disso, estudos que analisam a dinâmica entre espécies diferentes que competem pela mesma presa, em ocorrência simultânea, são imprescindíveis para determinar a metodologia de liberação mais eficiente para o controle biológico.

Existe uma discrepância na ação de predação entre as larvas e os adultos de joaninhas. As larvas, pela restrição de dispersão, exercem predação sobre a praga alvo no sítio de desenvolvimento ou liberação, enquanto os adultos dispersam do local de liberação (Ferran & Dixon 1993). Devido a esse fato, metodologias para a atração e retenção de adultos destes predadores em áreas de liberação vêm sendo alvo de pesquisas, empregando-se atrativos e arrestantes alimentares, como por exemplo no estudo desenvolvido por Zhu & Park (2005), onde

os autores concluíram que em testes de campo, as armadilhas com iscas de salicilato de metila são altamente atraentes para adultos de *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). Portanto, a retenção dos adultos de joaninhas no sítio alvo da liberação tem papel fundamental na dinâmica populacional, pois estes predadores podem se reproduzir e suas larvas, ao se desenvolverem nos mesmos locais, auxiliam no controle biológico das pragas na área alvo.

***Cryptolaemus montrouzieri* Mulsant**

Cryptolaemus montrouzieri é um inimigo natural efetivo das cochonilhas e desempenha um papel fundamental no controle biológico dessas pragas (Xie *et al.* 2017). No Brasil, essa joaninha foi introduzida pelo Laboratório de Entomologia da Embrapa Mandioca e Fruticultura, com apoio do Laboratório Costa Lima da Embrapa Meio Ambiente, proveniente do Instituto de Investigaciones Agricolas – Centro de Entomologia La Cruz- INIA, Chile, como alternativa para o controle biológico de cochonilhas sem carapaça e pulgões (afídeos) em cultivos de importância econômica e, adicionalmente, como forma estratégica de controle biológico clássico da cochonilha-rosada *Maconellicoccus hirsutus* (Green) (Sanches & Carvalho 2010).

O adulto de *C. montrouzieri* tem o corpo moderadamente convexo, com cerdas que dão um aspecto "aveludado", de 4,0 a 5,0 mm de comprimento, com a cabeça e parte posterior do abdômen de cor alaranjada e os élitros pretos (Rahaman & Aniszewsk 2016).

O ciclo de vida dessas joaninhas varia de 22 a 30 dias, dependendo das condições ambientais e da presa consumida (Maes *et al*. 2014, Marques *et al*. 2015). Normalmente apresentam uma proporção sexual de 1:1 e, ao emergirem os adultos, demoram de 5 a 7 dias para atingirem a maturação sexual. Apresentam dimorfismo sexual evidente, com uma diferença na coloração do primeiro par de pernas, onde o primeiro par de pernas das fêmeas é todo preto e nos machos, o fêmur é alaranjado (Babu & Azam 1987). Em temperatura controlada (25˚C a 30˚C)

podem atingir até 110 dias de vida, com uma fecundidade média de 211 ovos por fêmea (Kairo *et al*. 2013). As fêmeas são seletivas na escolha dos locais de oviposição, utilizando principalmente as pistas químicas na seleção do local apropriado. Segundo Merlin *et al*. (1996a), fêmeas ovipositaram mais na presença de ovissacos de *Pulvinaria hydrangeae* (Steinweden) = (*Eupulvinaria hydrangeae)* e em massas de cera de *P. citri*, mas não ovipositaram em algodão com a mesma conformação dos ovissacos. Entretanto, ao inserir uma solução da cera de *P. citri* no algodão, houve oviposição, comprovando que as pistas químicas da presa são importantes na seleção dos locais de oviposição para *C. montrouzieri*.

O potencial de *C. montrouzieri* como agente de controle de cochonilhas foi constatado no controle das cochonilhas *P. citri* e *Phenacoccus solenopsis* Tinsley adultas (Ahi *et al*. 2012, Saljoqi *et al*. 2015), de *Ferrisia virgata* (Cockerell) em plantas ornamentais no Egito (Attia & El-Arnouty 2007) e de *M. hirsutus* na Índia (Mani & Krishnamoorthy 2008).

***Tenuisvalvae notata* (Mulsant)**

A joaninha *T. notata*, nativa da América do Sul, pertence à subfamília Scymninae e é um importante predador de cochonilhas (Hemiptera: Pseudococcidae) (Dreyer *et al.* 1997a, 1997b).

Os adultos de *T. notata* possuem coloração que varia do branco ao amarelo com manchas circulares de coloração marrom escuro a preto (Mulsant 1850). O período de desenvolvimento dura cerca de 36 dias, com longevidade do adulto de mais de 150 dias em condições favoráveis de temperatura e disponibilidade de alimento (Dreyer *et al.* 1997a, Barbosa *et al.* 2014).

Os indivíduos dessa espécie preferem acasalar com parceiros com quem já acasalaram previamente. Os machos precisam de cerca de 4 dias para a primeira cópula, enquanto as fêmeas podem acasalar logo após a emergência, com a maior frequência de cópulas a partir de cinco dias de idade (Santos *et al*. 2017). As fêmeas produzem uma média de 54 descendentes ao longo de 30

dias e têm maior longevidade quando acasaladas apenas uma vez, em comparação com aquelas acasaladas várias vezes. Por outro lado, a fecundidade, a fertilidade e o desenvolvimento da prole não são afetados pelo número de acasalamentos femininos ou história de acasalamento masculino. Ocorre apenas uma diminuição gradual na fecundidade e na fertilidade ao longo do período de oviposição (Túler *et al.* 2017).

Em condições de escassez de alimento, Oliveira *et al.* (2004) constataram que quando esse período é longo, há uma redução significativa na reprodução, mas não na sobrevivência dos adultos. Essa característica favorece a persistência de *T. notata* em áreas de baixa infestação de presas, durante os períodos de colonização inicial ou após a aplicação de medidas alternativas de controle.

Em termos de preferência alimentar, *T. notata* se alimenta de cochonilhas na fase larval e adulta, tendo preferência alimentar por cochonilhas-farinhentas (Hemiptera: Pseudococcidae). Diante disso, Oliveira *et al.* (2004) investigaram o potencial que *T. notata* tem de regular uma população da cochonilha-de-listra, *F. virgata*. Nesse estudo, os autores observaram que o predador poderia aumentar numericamente sua população mais rápido que sua presa, e que 40% dos adultos alimentados com *F. virgata* permanecem vivos após 150 dias de observação. Em outro estudo, Barbosa *et al.* (2014) constataram que *T. notata* pode reduzir significativamente a população de larvas de *Ferrisia dasylirii* (Cockerell) (= *virgata*). Portanto, associando a taxa de predação diária com a fecundidade e a longevidade, *T. notata* é um agente potencial para o controle biológico efetivo da cochonilhas.

As cochonilhas-farinhentas pertencem à família Pseudococcidae e são consideradas pragas em várias culturas agrícolas. Entre elas, *F. dasylirii* foi encontrada em culturas de importância econômica como o algodão, coqueiro, abacaxi, melão, cacau, tomate, citrus, banana, goiaba, uva, gengibre, café e graviola (Kaydan & Gullan 2012, Rondelli *et al*. 2018, Pacheco da Silva *et al*.

2019). As injúrias causadas pela sucção da seiva por *F. dasylirii* são amarelecimento e queda prematura de folhas, botões florais e frutos. Além disso, a excreção do excesso de seiva ingerida (honeydew) sobre a planta favorece o desenvolvimento de fungos oportunistas causadores da fumagina, que interferem nos processos de fotossíntese e respiração. As injúrias pela sucção da seiva e o desenvolvimento dos fungos podem resultar em redução na produção ou mesmo morte das plantas (Gravena 2003, Culik & Gullan 2005, Santa-Cecília *et al*. 2007). Diante disso, o controle efetivo dessa cochonilha é imprescindível para manter uma produção satisfatória e, nesse sentido, as joaninhas coccidófagas *T. notata* e *C. montrouzieri* são eficientes na supressão da população dessas pragas.

Apesar do importante papel das joaninhas coccidófagas na supressão de populações de herbívoros, há poucas pesquisas envolvendo suas interações mediadas por semioquímicos. Em relação ás duas coccidófagas alvo dessa pesquisa, *T. notata* e *C. montrouzieri*, sua coexistência em campo já foi constatada (Peronti *et al*. 2016) e há predação intraguilda entre elas (Oliveira 2020), entretanto, ainda não há relatos da influência da comunicação química entre essas espécies.

Ecologia química de Coccinelídeos

A ecologia química é o estudo dos semioquímicos, substâncias que, dentro de um contexto natural, transmitem informações numa interação entre indivíduos, produzindo uma resposta comportamental ou fisiológica, que pode ser vantajosa ou desvantajosa para os organismos envolvidos (Trigo *et al.* 2000, Sloggett *et al.* 2011).

Em insetos, a comunicação química influencia no comportamento adaptativo dos indivíduos, pois intermedeia as interações intraespecíficas e interespecíficas, afeta a escolha alimentar, o reconhecimento de espécies, a evasão da área em momento de perigo, a localização do parceiro sexual, o comportamento de acasalamento e a escolha do local para oviposição

(Hemptinne & Dixon 2000, Raymond *et al*. 2000, Brown *et al.* 2008, Mishra *et al*. 2013, Ninkovic *et al.* 2013, Bell & Cardé 2013, Pattanayak *et al.* 2015, Urbina *et al*. 2018). Nesse sentido, a análise dos semioquímicos envolvidos nas interações entre espécies é fundamental para compreender a dinâmica que ocorre entre elas, especialmente quando se pretende introduzir ou liberar várias espécies em uma área.

Nas interações com coccinelídeos, os voláteis emitidos por plantas podem ser atrativos e modular o seu comportamento (Maeda *et al.* 2015). Sarkar *et al.* (2015) analisaram a atração da joaninha fitófaga *Epilachna dodecastigma* (Wied.) aos voláteis de *Momordica charantia* L. (Cucurbitales, Cucurbitaceae) danificadas e não danificadas por herbivoria, em 24 e 120 horas após o ataque, e constataram que as joaninhas foram mais atraídas por voláteis de plantas danificadas por herbivoria. Estudos envolvendo joaninhas predadoras também mostraram a existência de interação tri-trófica entre plantas e inimigos naturais. Xiu *et al*. (2019) observaram que *H. axyridis* são atraídas por voláteis florais de *Sophora japonica* e Zhao *et al*. (2020) concluíram que os compostos acetato de isoamila, α-humuleno, *trans*-3-hexen-1-ol, salicilato de metila e β-pineno foram atrativos para *H. axyridis* e *C. septempunctata*. Além dos voláteis florais, Sarmento *et al.* (2008) concluíram que *Cycloneda sanguinea* (Linnaeus) é atraída por odores provenientes de plantas de tomate atacadas pelos herbívoros *Macrosiphum euphorbiae* (Thomas) e *Tetranychus evansi* Baker & Pritchard.

Nas interações entre presa-predador, semioquímicos liberados pelas presas são atrativos para coccinelídeos. O feromônio dos pulgões *E*-β-farneseno por exemplo, representa um estímulo que leva a joaninha *C. septempunctata* para a presa em curtas distâncias (Nault *et al*. 1973, Pettersson *et al.* 2008) e o feromônio sexual das cochonilhas *Pseudococcus viburni* (Signoret) e *Pseudococcus calceolariae* (Maskell) desencadeiam uma resposta atrativa em *C. montrouzieri* (Urbina *et al*. 2018). Por outro lado, as presas geralmente evitam áreas com coccinelídeos; esse

comportamento foi constatado em psilídeos, pulgões e cochonilhas expostos a semioquímicos de coccinelídeos (Ninkovic *et al.* 2013, Seo *et al*. 2018, Pakyari *et al*. 2019).

Os semioquímicos de joaninhas afetam também sua interação com outros inimigos naturais. Os parasitoides *Aphidius eadyi* Stary, Gonzáles & Hall, *Aphidius ervi* Haliday e *Praon volucre* (Haliday) evitaram folhas previamente visitadas por *Adalia bipunctata* (L.) e *C. septempunctata* (Nakashima *et al*. 2006). Além disso, houve uma redução no parasitismo de *Leptomastix dactylopii* Howard na presença de *C. montrouzieri* (Chong & Oetting 2007) e na oviposição do sirfídeo *Sphaerophoria rueppellii* (Wiedemann) na presença de *A. bipunctata* ou de seus rastros (Amorós-Jiménez *et al.* 2015).

Em interações coespecíficas, os semioquímicos auxiliam no comportamento de agregação das espécies *H. axyridis* (Pallas), *A. bipunctata* e *H. convergens* (Durieux *et al.* 2012, Susset *et al*. 2013, Wheeler & Cardé 2013). Além disso, desempenham um importante papel no comportamento sexual de coccinelídeos. Machos são atraídos para as fêmeas e, ao encontrá-las, tocam seus élitros para fazer o reconhecimento químico da potencial parceira sexual e, posteriormente, iniciam o acasalamento (Hemptinne *et al*. 1996, Omkar 2004, D'Ávila *et al*. 2018). Élitros lavados em clorofórmio não desencadeiam o acasalamento (Hemptinne *et al*. 1996) comprovando a atuação dos hidrocarbonetos cuticulares no comportamento sexual de joaninhas.

Por outro lado, interações negativas também ocorrem, já que semioquímicos coespecíficos podem reduzir a oviposição de joaninhas. Ruzicka (2006) observou uma redução na oviposição de *Cheilomenes sexmaculata* (F.) na presença de rastros de larvas coespecíficas. Comportamento semelhante foi identificado em fêmeas de *C. montrouzieri*, conforme anteriormente mencionado (Merlin *et al*. 1996b). Essa redução na oviposição também foi observada em interações entre outras espécies de joaninhas. Ruzicka (2006) constatou que rastros de larvas de *Ceratomegilla undecimnotata* (Schneider) e *Cycloneda limbifer* Caseyarvas também inibiram a oviposição de *C.*

sexmaculata, enquanto Mishra *et al.* (2013) concluíram que a presença das joaninhas *C. septempunctata*, *Coccinella transversalis* Fabricius e *Propylea dissecta* (Mulsant) reduziram ou inibiram a oviposição de *Menochilus sexmaculatus* (F.).

Além de evitar locais com semioquímicos de outros coccinelídeos para reduzir a competição, algumas espécies liberam marcações químicas em seus ovos. Nesse sentido, Katsanis *et al.* (2017) detectaram compostos químicos deixados nos ovos de duas espécies nativas do gênero *Calvia* que reduziram a predação intraguilda por *H. axyridis*, enquanto Pervez & Khan (2020) revelaram que compostos químicos deixados como marcadores em ovos em *P. dissecta* são reconhecidos pelos pais, que evitam predar seus próprios ovos. A redução da oviposição na presença de semioquímicos e a marcação química dos ovos são importantes estratégias das fêmeas para impedir o canibalismo ou a predação intraguilda e assim, aumentar as chances da sua prole atingir a fase adulta (Hemptinne & Magro 2015).

A análise dos semioquímicos emitidos por coccinelídeos revelou compostos, a maioria hidrocarbonetos, encontrados em voláteis, rastros e lipídios cuticulares de joaninhas (Kosaki & Yamaoka 1996, Al Abassi *et al.* 1998, Magro *et al.* 2007). Apesar de alguns compostos serem comuns a várias espécies, a mistura qualitativa e quantitativa dos compostos químicos são espécie e gênero-específicos (Pattanayak *et al.* 2014). Assim, além de determinar padrões comportamentais, o perfil químico pode ser usado como ferramenta de identificação quimiotaxonômica de coccinelídeos.

Esses e outros estudos demonstram a importância dos semioquímicos nas interações entre espécies de coccinelídeos, plantas, suas presas e outros inimigos naturais, e indicam que para alcançar o sucesso no controle biológico com o uso dessas espécies, é necessário compreender essas interações.

Apesar de *T. notata* e *C. montrouzieri* ocorrerem simultaneamente e concorrerem pela mesma presa e da influência dos semioquímicos em espécies de coccinelídeos já ter sido constatada, a influência dos compostos químicos no comportamento e nas interações entre essas duas espécies ainda não foi analisada. Diante disso, o objetivo desse trabalho foi avaliar a influência dos semioquímicos dos rastros e voláteis de *Tenuisvalvae notata* e *Cryptolaemus montrouzieri* no tempo de desenvolvimento, na reprodução, no forrageamento e na capacidade predatória dessas espécies. Para atingir esse objetivo, a tese foi dividida em dois artigos. O primeiro tem como objetivo analisar o efeito dos semioquímicos dos rastros de *T. notata* e *C. montrouzieri* no forrageamento e na capacidade predatória dessas duas espécies, enquanto o segundo artigo analisou a influência dos semioquímicos voláteis na biologia e no comportamento predatório delas.

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CAPÍTULO 2

FOOTPRINTS OF THE LADYBEETLES *Cryptolaemus montrouzieri* AND *Tenuisvalvae notata* AFFECT THEIR FORAGING BEHAVIOR AND PREDATION RATE[1]

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[1]Ferreira, J.O. Footprints of the ladybeetles *Cryptolaemus montrouzieri* and *Tenuisvalvae notata* affect their foraging behavior and predation rate. A ser submetido.

ABSTRACT - Ladybeetles *Tenuisvalvae notata* (Mulsant), and *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), are used in the biological control of mealybugs in many countries, including Brazil. As they walk on plant surfaces, they leave chemical footprints that can affect the interactions between them and other coccinellids in the same environment. Therefore, this study aimed to evaluate the effects of footprints on their foraging behavior and predatory potential. Arena bioassays were conducted to evaluate the effect of the footprints on conspecifics and heterospecifics. In addition, the chemical profiles of these footprints were analyzed by GC-FID and GC-MS. For this, extracts of footprints were obtained from a glass Petri dish where 20 adults were allowed to walk for 24 hours. Behavioral bioassays showed that both species can recognize the footprints of each other. *T. notata* females avoided areas treated with footprints of heterospecific females, whereas *C. montrouzieri* females were arrested on areas with footprints of *T. notata* females. Second instar larvae of both species were a higher predation rate on areas treated with heterospecifics footprints. *T. notata* adults did not change their predation rate when exposed to footprints, but *C. montrouzieri* females captured more prey when exposed to footprints of conspecifics, while males reduced predation rate when exposed to footprints of conspecifics and heterospecifics. The composition of footprint extracts is species and gender-specific and was composed mainly of linear hydrocarbons from C_{20} to C_{33}, with saturated and non-saturated hydrocarbons. This is the first report regarding the effects of footprints on the behavior of these species.

KEY WORDS: Coccinellidae, biological control, semiochemicals, predatory behavior.

OS RASTROS DAS JOANINHAS *Cryptolaemus montrouzieri* E *Tenuisvalvae notata* AFETAM SEU FORRAGEAMENTO E TAXA DE PREDAÇÃO

RESUMO – As joaninhas *Tenuisvalvae notata* (Mulsant), nativas da América do Sul e *Cryptolaemus montrouzieri* Mulsant, nativas da Austrália (Coleoptera: Coccinellidae) são usadas no controle biológico de cochonilhas em diferentes países, incluindo o Brasil. Ao caminhar na superfície das plantas, deixam rastros químicos que podem afetar as interações entre eles e outros coccinelídeos no ambiente. Diante disso, o objetivo desse estudo foi avaliar os efeitos dos rastros no forrageamento e no potencial predatório dessas espécies. Para os testes comportamentais, arenas foram montadas com rastros coespecíficos e heteroespecíficos. Para as análises do perfil químico, foram extraídos extratos dos rastros de uma placa de petri onde 20 adultos caminharam por 24 horas. Os bioensaios comportamentais mostraram que as duas espécies podem reconhecer os rastros uma da outra. Fêmeas de *T. notata* evitaram áreas tratadas com rastros de heteroespecíficas fêmeas, enquanto *C. montrouzieri* fêmeas foram atraídas por áreas com rastros de *T. notata* fêmeas. Larvas de segundo instar das duas espécies aumentaram a predação quando expostas a rastros heteroespecíficos. Adultos de *T. notata* não alteraram a predação quando foram expostos aos rastros, mas *C. montrouzieri* fêmeas capturaram mais presas quando expostas a rastros coespecíficos, enquanto machos reduziram a taxa de predação quando expostos a rastros de coespecíficos e heteroespecíficos. A composição dos extratos dos rastros é específica de cada espécie e gênero e foi composta principalmente por hidrocabonetos lineares de C_{20} a C_{33}, com hidrocarbonetos saturados e insaturados. Este é o primeiro relato sobre a identificação e os efeitos dos rastros no comportamento dessas espécies.

PALAVRAS-CHAVE: Coccinellidae, controle biológico, semioquímicos, predação.

Introduction

The family Coccinellidae (Coleoptera) is a large group of beetles distributed worldwide, commonly known as ladybugs, ladybird beetles or ladybeetles (Hemptinne & Dixon 1997, Pickering *et al*. 2011). Among the described species of coccinellids, about 90% are carnivores, which make them important biological control agents of insects, such as aphids, mealybugs, psyllids, insect eggs, and small caterpillars, in different crops (Chakupurakal *et al*. 1994, Scopel & Roza-Gomes 2011, Sarwar 2016, Qin *et al*. 2019 Rounagh-Ardakani *et al*. 2020).

In classical biological control, there is the introduction/importation of an exotic species of a natural enemy to control an exotic pest species (DeBach 1964). In this context, the ladybeetle, also known as mealybug destroyer, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), which is native to Australia, was introduced in Brazil to control *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) in citrus orchards (Sanches & Carvalho 2010), and later used to control the hibiscus mealybug *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae) (Culik *et al*. 2013, Lima & Santos 2018). This species was also introduced in more than 40 countries in both temperate and tropical regions to control mealybugs (Kairo *et al*. 2013). Another ladybeetle species used in the classical control programs of mealybugs is *Tenuisvalvae notata* (Mulsant) (= *Hyperaspis notata*) (Coleoptera: Coccinellidae). It is native to South America and in the late 80s was introduced in Africa to control the cassava mealybug, *Phenacoccus manihoti* Matille-Ferrero (Hemiptera, Pseudococcidae) (Chakupurakal *et al*. 1994). In Brazil, *T. notata* was found in the north, northeast, midwest and southeast regions preying upon various species of mealybugs (Dreyer *et al*. 1997, Barbosa *et al*. 2014, Peronti *et al*. 2016, Ferreira *et al*. 2020).

Therefore, in agroecosystems, one might have the mutual occurrence of different ladybeetle species, indigenous and introduced, especially if they have similar thermal requirements and

894 geographical distribution as estimated for *T. notata* and *C. montrouzieri* (Ferreira *et al*. 2021).
895 Moreover, those species may compete for the same food niche (mealybugs) or just for space and
896 supplementary food resources such as pollen and nectar. It is known that many ecological
897 interactions among ladybeetle species occurs mostly by chemical communication, and that
898 emission and perception of semiochemicals are important for food foraging, mating, finding
899 refuges and oviposition sites, and defense against predators (Vilela & Pallini 2002, Sarmento *et*
900 *al*. 2008, Zarbin *et al*. 2009, Mishra *et al*. 2013).

901 Hydrocarbons have been found in volatiles, footprints, and cuticular lipids of ladybeetles
902 (Kosaki & Yamaoka 1996, Al Abassi *et al*. 1998, Pattanayak *et al*. 2014). According to
903 Pattanayak *et al*. (2015), the amount and composition of those chemical compounds are affected
904 by the nutrition and habitat of the ladybeetles and can affect the recognition of the species
905 (Hemptinne & Dixon, 2000), the opposite sex (Omkar 2004), aggregations (Wheeler & Cardé
906 2013), mating behavior (Fassotte *et al*. 2016), and oviposition (Mishra *et al*. 2013). Also, those
907 compounds may reduce intraguild predation in ladybeetles (Katsanis *et al*. 2017).

908 Coccinellids leave footprints on the plant surface as they walk around; those footprints are
909 perceived by prey and other natural enemies, and may cause them to exit from the area; hence,
910 interfering with biological control of pests at that site (Magro *et al*. 2007, Mishra *et al*. 2013,
911 Ninkovic *et al.* 2013). Previous studies have shown that footprints of the ladybeetle *Coccinella*
912 *septempunctata Linnaeus*, *Cycloneda limbifer* Casey, and *Semiadalia undecimnotata* (Schneider),
913 are recognized by other species of ladybeetles and reduce the interspecific competition among
914 them (Ruzicka 2001). Insect parasitoids may also use ladybeetle footprints to avoid parasitizing a
915 host that is more vulnerable to predation by ladybeetles (Nakashima *et al.* 2004). *T. notata* and *C.*
916 *montrouzieri* share the same environment and the same source of food, therefore they can
917 compete by sharing the same prey (Peronti *et al.* 2016). Thus, unraveling whether these two

species of ladybeetles recognize heterospecific footprints and how this recognition alters their behavior and predation rate can provide information for biological control programs. Here we evaluate i) whether *T. notata* and *C. montrouzieri* use their footprint to recognize conspecifics, and also heterospecifics, and the influence of the footprints on their predation rate, and ii) the chemistry of footprint extracts of both species to determine the major hydrocarbons present. We hypothesized that the chemical profile of the footprints is species and gender-specific and influences the pattern of locomotion and predation rate.

Materials and methods

The insects used in the bioassays were obtained from the colonies maintained in the Insect Behavior Laboratory, at the Universidade Federal Rural de Pernambuco (UFRPE) (−8.017070°S and −34.944362°W). The conditions for maintenance of insects and execution of bioassays were temperature of 25 ± 2 ºC, 60 ± 10% relative humidity, and a photoperiod of 12h:12h (L:D).

Prey. The colony of *Ferrisia dasylirii* (Cockerell) (Hemiptera: Pseudococcidae) was reared on pumpkins, *Cucurbita moschata* (Duch.), var. jacarezinho, in the initial maturation stage, obtained from the local market, "Centro de Abastecimento Alimentar" (CEASA), Recife-PE, Brazil. After being rinsed and dried, pumpkins were placed in plastic trays (30 x 45 x 4 cm) lined with a paper towel and infested on the petiole with gravid mealybugs, originated from the stock colony.

When the pumpkin was completely infested by mealybugs, it received a clean pumpkin on top, allowing the free movement of nymphs and adults to the new pumpkin. The average time to complete the infestation of a pumpkin is about 30 days, after that, they were used in the colonies of the ladybeetles.

Predators. Colonies of *T. notata* and *C. montrouzieri* were kept apart in the laboratory, but under the same environmental conditions as the prey. Adults of each predator species were placed in acrylic boxes (40 x 25 x 20 cm), with circular lateral openings, covered with a fine mesh to allow ventilation inside the boxes. The bottom of the boxes was lined with a paper towel, where one infested pumpkin was offered to the predators, following Barbosa *et al*. (2014). Ladybeetles were allowed to feed and mate freely in the rearing boxes. New infested pumpkins were offered to predators every 20 days, as prey were consumed from the previously offered pumpkins. Eggs and larvae of the predators were kept in the same rearing cages as the adults.

Walking behavior of ladybeetles exposed to footprints of conspecifics and heterospecifics. Behavioral bioassays were conducted at Universidade Federal Rural de Pernambuco (UFRPE). Virgin adults of *T. notata* and *C. montrouzieri*, 5-10 days old, were used in this test to investigate possible effects of footprints on walking behavior of the ladybeetles. Petri dishes (9 cm diameter) were used as test arenas. Prior to tests, the base and lid of the Petri dish were covered with filter paper discs (grammage 80g/m^2, Celab), of the same diameter, and a group of six ladybeetle adults, of the same sex and species, was released in the arena and allowed to walk freely for 24 hours to obtain the footprints. After this time, insects and filter paper were collected with the help of metal tweezers. Next, the filter paper containing the footprints was cut in half, obtaining two parts per disc. These treated papers were used to prepare the test arenas, which were composed of clean Petri dishes, of the same diameter. Each test arena received one-half of the filter paper containing the footprints while the other half of the Petri dish received a clean filter paper half (control). The wall of the Petri dish was treated with vaseline to prevent the ladybeetles climbing to the lid. After that, one ladybeetle adult (male or female, of each species) was released in the center of the arena, allowed 5 min acclimation, and its behavior was recorded for 10 min using

965 ViewPointTM 288 (ViewPoint Life Sciences Inc., Montreal, Canada). Recorded parameters were
966 walking distance (WD), walking time (WT), walking speed (WS), and the number of stops (NS).
967 Tested treatments were as follows: i) males exposed to footprints of conspecific males vs. control
968 (untreated area); ii) males exposed to footprints of conspecific females vs. control; iii) males
969 exposed to footprints of heterospecific males vs. control; iv) males exposed to footprints of
970 heterospecific females vs. control; v) females exposed to footprints of conspecific females vs.
971 control; vi) females exposed to footprints of heterospecific females vs. control; vii) females
972 exposed to footprints of conspecific males vs. control and viii) females exposed to footprints of
973 heterospecific males vs. control. There were 40 replicates for each treatment combination, and in
974 each replicate a new arena was used. The position of treatments was alternated between trials to
975 avoid any bias in the response of the ladybeetles.
976
977 **Effect of footprints on predation rate of ladybeetles.** In this bioassay, we investigated the
978 predation rate of larvae and adults *T. notata* and *C. montrouzieri* upon *F. dasylirii*. Prey was
979 offered to ladybeetles as follows: 6 mealybug nymphs were offered to 1 first or second instar
980 ladybeetle larva, or 6 adult mealybugs were offered to 1 third or fourth instar larva, and 1 adult of
981 the ladybeetle. This number of mealybugs provided daily to *T. notata* and *C. montrouzieri* was
982 determined based on previous tests, which showed that they consumed an average of less than
983 five mealybugs per day. The effect of footprints on predation rate was measured on a glass Petri
984 dish arena (5.5 cm diameter). Prior to the predation experiment, the Petri dishes received two
985 ladybeetle adults (*C. montrouzieri* or *T. notata*), which were allowed to walk freely inside the
986 arena for 24 hours to leave their footprints. Meanwhile, the first and second instar ladybeetle
987 larvae were starved for 2 hours, and older larvae and adults were starved for 24 hours before the
988 test to equalize hunger level and induce predation (adapted after Sengonca *et al*. 1995).

Predation rate upon mealybugs was measured according to the following treatments: i) I-IV instar larvae of *T. notata* on heterospecific footprints; ii) I-IV instar larvae of *T. notata* on conspecific footprints; iii) I-IV instar larvae of *C. montrouzieri* on heterospecific footprints; iv) I-IV instar larvae of *C. montrouzieri* on conspecific footprints; v) adults (male or female) of *C. montrouzieri* on heterospecific footprints; vi) adults (male or female) of *T. notata* on heterospecific footprints; vii) adults (male or female) of *T. notata* on conspecific footprints; viii) adults (male or female) of *C. montrouzieri* on heterospecific footprints*;* ix) I-IV instar larvae and adults of *T. notata* unpaired and x) I-IV instar larvae and adults of *C. montrouzieri* unpaired. Each treatment had 20 replicates. The number of mealybugs consumed was measured after 24 hours of exposure.

Collection of footprints. Glass Petri dishes (5.5 cm diameter) were used to collect the footprints of the ladybeetles. They were washed in water and acetone and heated at 180 ºC for 12 hours in a convection oven (Ethik, Brazil). Each clean Petri dish received 20 adults, 5 to 10 day-old, of each ladybeetle species, of the same sex . Insects were allowed to walk freely for 24 hours. After that, adults were removed and the Petri dishes were washed with 2 ml of distilled hexane PA for one minute. Footprint extracts were concentrated to 100 µl under a nitrogen flow and stored in a freezer at -20 ºC until further use in chemical analyses. Each treatment (both species and male and female) had 6 replicates.

Chemical analyses. For quantitative analysis, all footprints extracts were analyzed by gas chromatography coupled to a flame ionization detector (GC-FID) [Agilent Technologies 7890A equipped with a DB-5MS column (0.25 mm inner diameter (ID) x 30 m, 0.25 µm film, J&W Scientific, Folsom, CA, USA); the temperature of the detector was adjusted at 270 ºC]. The oven

1013 was programmed to 50° C for 2 min, then to 280° C at 5° C per min, followed by an increase of
1014 10° C per min to 280° C (held for 20 min). Aliquots of 2 µL of each sample were injected using
1015 the splitless mode, with the inlet at 300º C, and helium as the carrier gas. Data were collected
1016 using GC OpenLabsoftware (Agilent, USA). As an internal standard (IS), 1 µL of 16-
1017 hexadecanolide (in distilled hexane) was added to the samples. The quantification was done
1018 comparing the area of the IS with the areas of all compounds in the chromatogram profile. The
1019 response factor for each compound was considered 1.0.

1020 For qualitative analysis selected footprints extracts, were analyzed using a Shimadzu QP
1021 2010 quadruple mass spectrometer equipped with a DB-5MS column (0.32mm ID x 60 m, 1.0 µm
1022 film, Supelco, Bellefonte, PA, USA), a splitless injector, and helium as the carrier gas. Ionization
1023 was by electron impact (70 eV, source temperature at 230 °C). The injector was at 250 °C using
1024 the same temperature programme as in GC-FID analysis. Data were collected with GC-solution
1025 software (version 2.42, Shimadzu, Japan). Identifications were made by comparison of spectra
1026 with mass spectral library databases (NIST, 2008), use of retention indices (RIs), and were
1027 confirmed by overlap of the footprints extracts with authentic standards. The RIs were calculated
1028 by comparison to the retention times of a series of linear hydrocarbon alkanes (C8–C40) analysed
1029 with the same temperature program.

1030 For compounds that authentic standards were not available, identification was based on
1031 comparison with published spectra, their fragmentation pattern, and using retention index
1032 (published at Database of Pheromones and Semiochemicals (Pherobase) and National Institute of
1033 Standards and Technology (NIST): Chemistry WebBook websites).

1034

1035 **Statistics -** Data for walking distance, walking speed, and the number of stops were subjected to a
1036 multivariate analysis of variance (MANOVA), using the PROC GLM of SAS (SAS Institute

2002). Walking time in areas of the arena treated and untreated with footprints was subjected to a chi-square (χ^2) test using the PROC FREQ of SAS (SAS Institute 2002).

Predation data were subjected to the Shapiro Wilk normality test. As they did not assume normality, they were subjected to General Linear Model analysis (GLM) with Poisson error distribution. Due to the overdispersion of data, they were subjected to a Quasipoisson analysis, followed by a contrast analysis to separate the means. These analyses were performed using the software R 4.0.5 (R Development Core Team 2011).

Data on quantitative amounts of chemical compounds identified in footprints of ladybeetles were subjected to Shapiro Wilk normality test to check for ANOVA assumptions. Next, as data of the quantitative amount of compounds present in *T. notata* footprints were normally distributed, they were subjected to (GLM) with normal error distribution (Gaussian). In contrast, data of *C. montrouzieri* were not normally distributed and were subjected to (GLM) with Gamma error distribution. In addition, data for the total amount of compounds present on footprints of ladybeetles were not normally distributed and were $\sqrt{(x + 0.5)}$ transformed to assume a normal distribution. Next, the total amount of compounds was subjected to factorial analysis of variance (ANOVA), with species and sex as factors.

The chemical composition similarity between the footprints of *T. notata* and *C. montrouzieri* male and females was analyzed by principal components analysis (PCA). The dissimilarity was tested by a permutation analysis of variance (PERMANOVA) with 999 permutations, based on the index of similarity of Bray-Curtis, using the R "Vegan" package (R Development Core Team 2011).

Results

Walking behavior of ladybeetles exposed to footprints of conspecifics and heterospecifics - There was no significant effect of footprints on the walking distance (WD), walking speed (WS), number of stops (NS), and walking time (WT) when *C. montrouzieri* were exposed to footprints of conspecific (Table 1) (Fig. 1). There were no significant changes in the walking behavior parameters of *C. montrouzieri* males exposed to footprints of males and females *T. notata* (Table 1) (Fig. 1). In contrast, *C. montrouzieri* females had a higher number of stops in areas treated with *T. notata* male's footprints ($F_{1,79} = 5.53$; $P = 0.02$), augmented the walking distance ($F_{1,79} = 7.19$; $P < 0.01$) (Table 1) and spent more time in areas treated with footprints of *T. notata* females ($\chi^2 = 4.5$; $P = 0.03$) (Fig. 1).

T. notata males ($F_{1,79} = 4.15$; $P = 0.04$) and females ($F_{1,79} = 9.26$; $P < 0.01$) had a significantly higher number of stops in areas treated with footprints of conspecific males (Table 2). Also, *T. notata* males spent more time in areas with footprints of conspecific males ($\chi^2 = 4.32$; $P = 0.03$) (Fig. 2). However, *T. notata* males and females did not show significant change in their walking behavior when exposed to footprints of conspecifics females. *T. notata* males did not show significant change in their walking behavior when exposed to footprints of *C. montrouzieri* males or females. In contrast, *T. notata* females reduced the number of stops ($F_{1,79} = 8.92$; $P < 0.01$) (Table 2) and spent less time in areas treated with footprints of *C. montrouzieri* females ($\chi^2 = 4.31$; $P = 0.03$) (Fig. 2). In addition, *T. notata* females reduced the walking distance ($F_{1,79} = 4.60$; $P = 0.03$) and number of stops ($F_{1,79} = 8.02$; $P < 0.01$) in areas treated with footprints of *C. montrouzieri* males.

Effect of footprints on predation rate of ladybeetles. For second instar larvae there was a higher predation rate on areas treated with footprints of heterospecifics ($F_{2,59}$ =11.02; $P < 0.01$), for all other treatments there was no effect of footprints of conspecific and heterospecific ladybeetles on predation rate of *T. notata* larvae. Moreover, there was no effect of footprints on predation rate of adult *T. notata* females and males (Table 3).

A similar result was observed for the predation rate of *C. montrouzieri* immature when submitted to predation in the presence of heterospecific and conspecific footprints; only second instar larvae ($F_{2,59}$ = 5.33; $P < 0.01$) had a significant increase in predation when exposed to footprints of heterospecifics (Table 3). All other instar larvae of *C. montrouzieri* showed no effect of the heterospecific and conspecific footprints on their predation rate (Table 3).

Among the adults of *C. montrouzieri*, females captured significantly more prey when exposed to footprints of conspecifics ($F_{2,59}$ = 10.36; $P < 0.01$), whereas males reduced predation rate when exposed to footprints of conspecifics and heterospecifics ($F_{2,59}$ = 5.52; $P < 0.01$) (Table 3).

Chemical profile of ladybeetle's footprints. Twenty-four and eighteen saturated and unsaturated hydrocarbons were identified in the hexanic extracts of footprints of males and females *C. montrouzieri* and *T. notata*, respectively (Figs 3 and 4). The total amount of hydrocarbons present in extracts of footprints between both ladybeetle species ($F_{3,23}$ = 187.33; $P < 0.01$) and genders ($F_{3,23}$ = 21.48; $P < 0.01$) were different (Fig 5). There was no difference when comparing the total amount of hydrocarbons obtained from male and female footprint extracts of *C. montrouzieri* (Fig. 5) ($F_{1,11}$ = 1.40; $P = 0.26$), whereas a higher amount of total hydrocarbons was quantified from *T. notata* male footprint extract compared to female conspecific footprint extracts ($F_{1,11}$ = 28.08; $P < 0.01$) (Fig. 5). Footprint extracts of *T. notata* males and females contained a higher

amount of hydrocarbons compared to males ($F_{1,11}$ = 90.88; P < 0.01) and females ($F_{1,11}$ =121.83; P < 0.01) of *C. montrouzieri* (Fig. 5).

When analyzing the compounds individually for *C. montrouzieri*, the hydrocarbon tetradecane was present in higher amounts in female footprint extracts (χ^2 = 4.01; Df = 1; P = 0.04), and 3-tricosene and 7-tricosene were detected only in these extracts. The following compounds were present in higher levels in male footprint extracts: tricosane (χ^2 = 5.59; Df = 1; P = 0.01), tetracosane (χ^2 = 7.61; Df = 1; P < 0.01), 11- heptacosene (χ^2 = 26.47; Df = 1; P < 0.01), 7 - heptacosene (χ^2 = 15.41; Df = 1; P < 0.01), heptacosane (χ^2 = 8.46; Df = 1; P < 0.01), (*Z*)-13 docosenoamide (χ^2 = 20.86; Df = 1; P < 0.01), 11-nonacosene (χ^2 = 19.78; Df = 1; P < 0.01), nonacosane (χ^2 = 4.84; Df = 1; P = 0.02) and 7 - hentriacontene (χ^2 = 7.38; Df = 1; P < 0.01); 9 – pentacosene were found only in male extracts. There was no significant difference between sexes of *C. montrouzieri* in the amount of the following compounds pentadecane, hexadecane, heptadecane, 2-heneicosene, docosane, 2-tricosene, pentacosane, hexacosane, 11-triacontene, 9 - hentriacontene and hentriacontene (Table 4).

There was no qualitative difference between the hydrocarbon profiles between genders of *T. notata*, but there was a significant difference in the amounts of most compounds. For *T. notata* the following compounds were quantified in higher amount in male footprint extracts: tetradecane ($F_{1,11}$ = 22.99; P < 0.01), pentadecane ($F_{1,11}$ = 25.77; P < 0.01), pentacosane ($F_{1,11}$ = 53.52; P < 0.01), 7-heptacosene ($F_{1,11}$ = 7.07; P = 0.02), heptacosane ($F_{1,11}$ = 51.25; P < 0.01), 9-nonacosene ($F_{1,11}$ = 29; P < 0.01), 7- nonacosene ($F_{1,11}$ = 15.01; P < 0.01), nonacosane ($F_{1,11}$ = 36.29; P < 0.01), triacontene ($F_{1,11}$ = 11.74; P < 0.01), hentriacontadiene ($F_{1,11}$ = 11.15; P < 0.01), 11-hentriacontene ($F_{1,11}$ = 15.80; P < 0.01), 7-hentriacontene ($F_{1,11}$ = 13.13; P < 0.01), tritriacontene ($F_{1,11}$ = 13.33; P < 0.01), and 9-tritriacontene ($F_{1,11}$ = 16.27; P < 0.01). There was no significant

1130 difference in the amount of the following compounds: hexadecane, hexacosane, and
1131 pentatriacontene (Table 4).

1132 When we compared the composition and amount of compounds present in the extracts of
1133 footprints between species, nine hydrocarbon compounds were found in both species, but in
1134 higher amounts in *T. notata* footprint extracts. Those compounds were: tetradecane (χ^2 = 14.37;
1135 Df = 3; P < 0.01), pentadecane (χ^2 = 14.47; Df = 3; P < 0.01), hexadecane (χ^2 = 8.73; Df = 3; P =
1136 0.03), pentacosane (χ^2 = 638.30; Df = 3; P < 0.01), hexacosane (χ^2 = 153.57; Df = 3; P < 0.01),
1137 heptacosane (χ^2 = 738.16; Df = 3; P < 0.01), nonacosane (χ^2 = 125.21; Df = 3; P < 0.01), 7 –
1138 heptacosene (χ^2 = 187.71; Df = 3; P < 0.01), and 7-hentriacontene (χ^2 = 18.17; Df = 3; P < 0.01).
1139 *C. montrouzieri* produced less hydrocarbons, but had a higher diversity of compounds, with 15
1140 hydrocarbons identified exclusively in its footprint extracts, such as saturated and unsaturated
1141 hydrocarbons between C_{21} and C_{24}, whereas in *T. notata* footprints were identified as
1142 hydrocarbons with higher molecular weight, both saturated and unsaturated C_{33} and C_{35} (Table 4).

1143 The principal components analysis (PC1 = 66.57% and PC2 = 20.60%) grouped the extracts
1144 of footprints of both ladybeetle species, and between sexes of *T. notata* ($F_{3,23}$ = 57.14; P < 0.01)
1145 (Fig. 6). The PCA graph shows that most compounds are related to *T. notata*, this is because of
1146 the higher levels of hydrocarbons extracted from their footprint extracts compared to *C.*
1147 *montrouzieri* footprints extracts. The compounds pentacosane (C13), heptacosane (C17), 7-
1148 nonacosene (C21), 9-hentriacontene (C27) and tritriacontene (C30) influences the separation of
1149 males and females of *T. notata* and males are related with hydrocarbons with a higher number
1150 of carbons, whereas pentacosane and heptacosane are related with females (Fig. 6).

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Discussion

The biological control of mealybugs is an important strategy to manage these pests in many crops worldwide. The coccidophagous ladybeetles *C. montrouzieri* and *T. notata* have been used to control mealybugs in Brazil and other countries in both tropical and temperate climates (Chakupurakal *et al.* 1994, Maes *et al*. 2015). These ladybeetle species may occur in the same area, as they have similar food preferences and thermal requirements (Ferreira *et al*. 2020), and in cases of prey scarcity, they may compete for food, and have negative interactions such as cannibalism and intraguild predation (Oliveira 2020). To avoid this competition, these ladybeetles can use the chemical clues released in the footprints of possible competitors to avoid places already occupied. This influence of chemical clues from ladybeetle footprints on the behavior of other insect species has already been demonstrated (Ruzicka 2001, Mishra *et al*. 2013, Ninkovic *et al*. 2013, Patel *et al*. 2020) and induced avoidance in some possible competitors.

The behavioral studies showed that *C. montrouzieri* did not alter its walking behavior when exposed to footprints of conspecific individuals. This behavioral response, together with the similarity in the amount of hydrocarbons identified in the male and female extracts, indicates that the footprint may not be used to detect the presence of a conspecific for this species. In contrast, *T. notata* males and females stopped more often when they detected footprints of conspecific males. Previous studies regarding the sexual behavior of *T. notata* showed that females engage in male courtship and try to mate with males (Santos *et al*. 2017). Therefore, it is possible that the divergence in the chemical profile of male footprint is important for the *T. notata* female to recognize and detect the presence of potential mates in the area, while males can use these footprints to avoid competition. The hydrocarbons 7-nonacosene, pentacosane, heptacosane, 9-hentriacontene and tritriacontene stood out in the separation of male *T. notata* extracts. Behavioral

1176 experiments with blends containing these hydrocarbons could help to evaluate if they influence
1177 these interactions.

1178 In the heterospecific interactions, *C. montrouzieri* females altered their walking behavior
1179 when exposed to footprints of *T. notata* females. Previous studies have shown that *C.*
1180 *montrouzieri* is capable of detecting the chemical trails of other species (Finlay-Doney & Walter
1181 2012, Urbina *et al*. 2018). The possible attraction of *C. montrouzieri* to footprints of *T. notata*,
1182 identified in this work, may be related to its capacity to locate the competitor in the same area,
1183 and might indicate possible negative effects of the exotic species *C. montrouzieri* on the
1184 indigenous *T. notata*. Oliveira (2020) showed that when food is scarce, *C. montrouzieri* behaves
1185 as an intraguild predator on *T. notata* immature stages. In contrast, *T. notata* spent less time on
1186 areas treated with the footprints of *C. montrouzieri*, suggesting that *T. notata*, an inferior
1187 competitor compared to *C. montrouzieri*, would tend to avoid such areas to avoid predation.

1188 It is expected that in asymmetric interactions between competitors, the least aggressive
1189 species would use chemical clues from heterospecific individuals to avoid unfavorable situations
1190 such as competition for food and predation upon immature stages. This avoidance behavior is
1191 present in other interactions with ladybeetles. For instance, the aphid parasitoid *Aphidius ervi*
1192 Haliday avoids foraging in the same area where the ladybeetle *C. septempunctata* is, or with
1193 footprints of this predator species (Nakashima *et al*. 2004). Also, Ugine *et al*. (2018) found
1194 that *Coccinella novemnotata* Herbst spent more time foraging in plants with conspecific
1195 individuals than plants with *C. septempunctata*, suggesting that they can recognize visual and/or
1196 olfactory clues left by conspecific and heterospecific individuals, and use these clues to avoid
1197 occupying the same micro-habitat where heterospecific individuals may be found.

1198 Regarding the predatory behavior, *T. notata* adults did not alter their predation rate on
1199 mealybugs when exposed to conspecific and heterospecific ladybeetles footprints. This result

suggests that even though *T. notata* adults can recognize the chemical clues left by *C. montrouzieri*, it does not alter their consumption of prey. Instead, to reduce chances of possible competition or intraguild predation, they reduce the time they spent in areas treated with footprints of *C. montrouzieri*, as we found in the walking behavior bioassay. In contrast, although female *C. montrouzieri* increased the time spent and the number of stops in areas treated with female *T. notata* footprints, their predation rate increased only in areas with conspecific footprints. Males, which did not alter foraging behavior when exposed to conspecific and heterospecific footprints, reduced predation when exposed to conspecific and heterospecific footprints. The ability of *C. montrouzieri* to recognize conspecific and heterospecific semiochemicals has been previously demonstrated (Merlin *et al*. 1996, Finlay-Doney & Walter 2012, Urbina *et al*. 2018). Therefore, even under food abundance, as we tested in this research, by having the ability to recognize the presence of a competitor by semiochemicals left in the substrate, males of *C. montrouzieri* can reduce predation on preferred prey (scale insects). On the other hand, semiochemicals of conspecifics could also indicate the presence of more food, inducing females to increase their predation rate.

Despite having some similar compounds, the chemical profiles of *C. montrouzieri* and *T. notata* footprints, as expected, showed a species-specific blend of footprint hydrocarbons and quantitative differences within species. *C. montrouzieri* presents a higher diversity of hydrocarbons than *T. notata*, and the latter produced higher amounts of hydrocarbons compounds. Another interesting difference is that *C. montrouzieri* presented hydrocarbons with lower weight molecular such as C_{21} and C_{24} (saturated and unsaturated), whereas *T. notata* produced compounds with higher molecular weight such as C_{33} and C_{35}. In both species, the major compound has 29 carbons; in *C. montrouzieri* the compound is 11-nonacosene, and in *T notata* the compound is 7-nonacosene. Further studies could evaluate if these compounds function in

species recognition through footprints left on the plants by conspecifics and their prey. Previous studies have identified that the amount of hydrocarbons of other coccinellids are specific to each species and sex (Nakashima *et al*. 2006, Magro *et al*. 2007, Pattanayak *et al.* 2014). Therefore, the variation in composition of hydrocarbons present in the footprints of ladybeetles is important to species and sex recognition.

The alkanes pentacosane, heptacosane and nonacosane, found in the footprints of *C. montrouzieri* and *T. notata,* were identified in the footprints of other coccinellids, such as *C. septempunctata* and *Harmonia axyridis* Pallas (Kosaki & Yamaoka 1996, Nakashima *et al*. 2004, Durieux *et al*. 2012). The similarity of the chemical profiles of footprints of congeneric coccinellid larvae has already been detected (Magro *et al*. 2007, Pattanayak *et al*. 2015) and suggests that, in addition to determining behavioral traits, the chemical footprint profile can be used for chemotaxonomic purposes.

In conclusion, the chemical profile of hydrocarbon extracted from the footprints of T. *notata* and *C. montrouzieri* are different, and their behavioral responses suggest that the specific blend of hydrocarbons present in their footprints is important to species recognition and decision making regarding dispersal and predation. Behavioral responses of these predators can vary from avoidance of areas with footprints of potential competitors, as observed in *T. notata*, or arrestment, as observed in *C. montrouzieri*, which may lead to changes in preferred prey consumption and intraguild predation upon competitors. This is the first report regarding the effects of footprints of *T. notata* and *C. montrouzieri* on predator walking behavior and predation of mealybugs. Further studies are necessary to elucidate the relative importance of blends or specific compounds in the chemical communication of these species. These results can contribute to pest management decisions regarding the biological control of mealybugs with these ladybeetle species.

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Table 1. Walking distance (WD, cm), walking speed (WS, cm/s) and number of stops (NS) of *Cryptolaemus montrouzieri* adults exposed to conspecific and heterospecific footprints.

	***Cryptolaemus montrouzieri* female**			***Cryptolaemus montrouzieri* male**		
	A1 (treated area); A2 (untreated area)			A1 (treated area); A2 (untreated area)		
Sex	WD (cm)	WS (cm/s)	NS	WD (cm)	WS (cm/s)	NS
			Conspecific			
Female footprint	A1: 75.3 ± 9.78 a A2: 87.6 ± 9.63 a $F_{1,79}$ = 0.8; P = 0.37	A1: 0.48 ± 0,03 a A2: 0.48 ± 0.03 a $F_{1,79}$ = 0.01; P = 0.92	A1: 252.8 ± 33.11 a A2: 314 ± 38.41 a $F_{1,79}$ = 1.46; P = 0.23	A1: 82.7 ± 9.60 a A2: 90.3 ± 10.94 a $F_{1,79}$ = 0.27; P = 0.60	A1: 0.46 ± 0.03 a A2: 0.49 ± 0.03 a $F_{1,79}$ = 0.44; P = 0.50	A1: 283.7 ± 35.67 a A2: 285.1 ± 40.19 a $F_{1,79}$ = 0.0 P = 0.98
Male footprint	A1: 89.7 ± 8.46 a A2: 83.3 ± 10.16 a $F_{1,79}$ = 0.23; P = 0.63	A1: 0.42 ± 0.02 a A2: 0.49 ± 0.03 a $F_{1,79}$ = 1.23; P = 0.27	A1: 297.7 ± 30.13 a A2: 266 ± 26.73 a $F_{1,79}$ = 0.62; P = 0.43	A1: 95.2 ± 10.09 a A2: 84.5 ± 8.93 a $F_{1,79}$ = 0.63; P = 0.42	A1: 0.47 ± 0.02 a A2: 0.53 ± 0.03 a $F_{1,79}$ = 1.91; P = 0.10	A1: 259.2 ± 29.33 a A2: 232.8 ± 30.83 a $F_{1,79}$ = 0.38; P = 0.53
			Heterospecific			
			Tenuisvalvae notata			
Female footprint	A1: 121.1 ± 8.43 a A2: 92.5 ± 6.52 b $F_{1,79}$ = 7.19; P < 0.01	A1: 0.54 ± 0.03 a A2: 0.57 ± 0.03 a $F_{1,79}$ = 0.38; P = 0.53	A1: 321.6 ± 29.51 a A2: 247.4 ± 26.44 a $F_{1,79}$ = 3.51; P = 0.06	A1: 105.7 ± 11.92 a A2: 164.6 ± 38.03 a $F_{1,79}$ = 2.18; P = 0.14	A1: 0.58 ± 0.04 a A2: 0.59 ± 0.07 a $F_{1,79}$ = 0.03; P = 0.80	A1: 253.8 ± 43.52 a A2: 236 ± 39.53 a $F_{1,79}$ = 0.09; P = 0.76
Male footprint	A1: 100.5 ± 7.76 a A2: 86.6 ± 7.77 a $F_{1,79}$ = 1.60; P = 0.21	A1: 0.46 ± 0.01 a A2: 0.49 ± 0.02 a $F_{1,79}$ = 1.01; P = 0.31	A1: 354.3 ± 37.53 a A2: 242.2 ± 29.37 b $F_{1,79}$ = 5.53; P = 0.02	A1: 104.4 ± 14.40 a A2: 100 ± 9.09 a $F_{1,79}$ = 0.07; P = 0.79	A1: 0.54 ± 0.03 a A2: 0.52 ± 0.03 a $F_{1,79}$ = 0.28; P = 0.50	A1: 236.5 ± 39.61 a A2: 198.3 ± 27.81 a $F_{1,79}$ = 0.62; P = 0.43

Means followed by the same lower-case letter within the column are not statistically different by the F test (α = 0.05%).

Table 2. Walking distance (WD, cm), walking speed (WS, cm/s) and number of stops (NS) of *Tenuisvalvae notata* adults exposed to conspecific and heterospecific footprints.

	***Tenuisvalvae notata* female**			***Tenuisvalvae notata* male**		
	A1 (treated area); A2 (untreated area)			A1 (treated area); A2 (untreated area)		
Sex	WD (cm)	WS (cm/s)	NS	WD (cm)	WS (cm/s)	NS
Conspecific						
Female footprint	A1: 69.8 ± 7.15 a A2: 62.2 ± 9.63 a $F_{1,79}$ = 0.64; P = 0.42	A1: 0.45 ± 0.02 a A2: 0.46 ± 0.02 a $F_{1,79}$ = 0.03; P = 0.86	A1: 347.8 ± 38.68 a A2: 254.1 ± 31.26 a $F_{1,79}$ = 3.55; P = 0.06	A1: 90.1 ± 15.59 a A2: 75.4 ± 8.75 a $F_{1,79}$ = 0.67; P = 0.41	A1: 0.47 ± 0.03 a A2: 0.46 ± 0.04 a $F_{1,79}$ = 0.01; P = 0.92	A1: 317.7 ± 36.85 a A2: 258.1 ± 40.63 a $F_{1,79}$ = 1.18; P = 0.28
Male footprint	A1: 77.4 ± 7.44 a A2: 60.6 ± 7.74 a $F_{1,79}$ = 2.44; P = 0.12	A1: 0.42 ± 0.01 a A2: 0.44 ± 0.03 a $F_{1,79}$ = 0.27; P = 0.60	A1: 365.3 ± 33.26 a A2: 221.4 ± 33.58 b $F_{1,79}$ = 9.26; P < 0.01	A1: 77.9 ± 8.37 a A2: 58.5 ± 7.56 a $F_{1,79}$ = 2.94; P = 0.09	A1: 0.41 ± 0.02 a A2: 0.38 ± 0.03 a $F_{1,79}$ = 0.67; P = 0.41	A1: 367.5 ± 41.57 a A2: 253.8 ± 37.28 b $F_{1,79}$ = 4.15; P = 0.04
Heterospecific						
Cryptolaemus montrouzieri						
Female footprint	A1: 56.5 ± 8.46 a A2: 75.2 ± 8.01 a $F_{1,79}$ = 2.56; P = 0.11	A1: 0.39 ± 0.03 a A2: 0.45 ± 0.03 a $F_{1,79}$ = 1.45; P = 0.23	A1: 201.3 ± 31.29 b A2: 345.2 ± 36.65 a $F_{1,79}$ = 8.92; P < 0.01	A1: 98.4 ± 14.47 a A2: 85.2 ± 8.97 a $F_{1,79}$ = 0.62; P = 0.43	A1: 0.47 ± 0.04 a A2: 0.52 ± 0.03 a $F_{1,79}$ = 0.94; P = 0.3	A1: 276.1 ± 32.95 a A2: 253.6 ± 33.22 a $F_{1,79}$ =0.23; P = 0.63
Male footprint	A1: 72.9 ± 7.78 b A2: 103.8 ± 12.14 a $F_{1,79}$ = 4.60; P < 0.01	A1: 0.55 ± 0.07 a A2: 0.53 ± 0.04 a $F_{1,79}$ = 0.04; P = 0.83	A1: 213 ± 30.82 b A2: 356.3 ± 40.15 a $F_{1,79}$ = 8.02; P < 0.01	A1: 85.8 ± 10.62 a A2: 99.1 ± 17.41 a $F_{1,79}$ = 0.42; P = 0.51	A1: 0.48 ± 0.03 a A2: 0.51 ± 0.04 a $F_{1,79}$ = 0.31; P = 0.58	A1: 218.5 ± 34.28 a A2: 260.5 ± 31.94 a $F_{1,79}$ = 0.80; P = 0.37

Means followed by the same lower-case letter within the column are not statistically different by the F test ($\alpha = 0.05\%$).

Table 3. Daily consumption (±SE) of *Ferrisia dasylirii* nymphs by larvae (LI-LIV) and adults male (M) and female (F) of the ladybeetles *Tenuisvalvae notata* (T) and *Cryptolaemus montrouzieri* (C) subjected or not to footprints of conspecific and heterospecific couples.

Focal species/paired treatment	Average (±SE) nymphs preyed	Focal species/paired treatment	Average (±SE) nymphs preyed
T L1I unpaired	0.8 ± 0.11 a	C LI unpaired	1.2 ± 0.14 a
T LI x Tn	0.7 ± 0.10 a	C LI x Cm	1.3 ± 0.13 a
T LI x Cm	1.0 ± 0.10 a	C LI x Tn	1.5 ± 0.11 a
Statistics	$F_{2,59} = 1.90$; $P = 0.15$	Statistics	$F_{2,59} = 1.33$; $P = 0.27$
T LII unpaired	1.8 ± 0.13 b	C LII unpaired	2.7 ± 0.19 b
T LII x Tn	1.9 ± 0.15 b	C LII x Cm	2.6 ± 0.18 b
T LII x Cm	2.8 ± 0.18 a	C LII x Tn	3.4 ± 0.15 a
Statistics	$F_{2,59} = 11.02$; $P < 0.01$	Statistics	$F_{2,59} = 5.33$; $P < 0.01$
T LIII unpaired	2.0 ± 0.19 a	C LIII unpaired	2.6 ± 0.16 a
T LIII x Tn	2.5 ± 0.11 a	C LIII x Cm	2.6 ± 0.13 a
T LIII x Cm	2.2 ± 0.09 a	C LIII x Tn	3.1 ± 0.19 a
Statistics	$F_{2,59} = 2.45$; $P = 0.09$	Statistics	$F_{2,59} = 2.75$; $P = 0.07$
T LIV unpaired	2.7 ± 0.16 a	C LIV unpaired	3.8 ± 0.18 a
T LIV x Tn	3.1 ± 0.15 a	C LIV x Cm	4.1 ± 0.16 a
T LIVx Cm	3.0 ± 0.19 a	C LIVx Tn	4.1 ± 0.15 a
Statistics	$F_{2,59} = 1.49$; $P = 0.23$	Statistics	$F_{2,59} = 0.95$; $P = 0.39$
TF unpaired	1.2 ± 0.16 a	CF unpaired	1.7 ± 0.14 b
TF x Tn	1.6 ± 0.16 a	CF x Cm	2.5 ± 0.15 a
TF x Cm	1.7 ± 0.14 a	CF x Tn	1.5 ± 0.18 b
Statistics	$F_{2,59} = 2.13$; $P = 0.12$	Statistics	$F_{2,59} = 10.36$; $P < 0.01$
TM unpaired	1.1 ± 0.10 a	CM unpaired	2.5 ± 0.15 a
TM x Tn	1.1 ± 0.12 a	CM x Cm	1.9 ± 0.15 b
TM x Cm	0.8 ± 0.13 a	CM x Tn	1.8 ± 0.13 b
Statistics	$F_{2,59} = 2.29$; $P = 0.11$	Statistics	$F_{2,59} = 5.52$; $P < 0.01$

Means followed by the same lower-case letter within the column are not statistically different by the F test ($\alpha = 0.05\%$). Tn = couple of *T. notata*; Cm = Couple of *C. montrouzieri*.

1454 Table 4. Mean (±SE) amount and RI calculated using a DB-5MS column of footprints compounds (µg/insect), collected from 24

1455 hours of the adult ladybeetles *Tenuisvalvae notata* (T) and *Cryptolaemus montrouzieri* (C).

Compound	RI	T male	T female	Statistics F; P	C male	C female	Statistics χ^2; P
Hydrocarbons							
Tetradecane*	1400	33.7 ± 1.81 a	23.5 ± 1.11 b	$F_{1,11}$ = 22.99; P < 0.01	13.6 ± 4.38 b	26.5 ± 1.88 a	χ^2 = 4.01; P = 0.04
Pentadecane*	1500	10.9 ± 0.55 a	7.4 ± 0.38 b	$F_{1,11}$ = 25.77; P < 0.01	4.4 ± 1.41 a	8.4 ± 0.58 a	χ^2 = 3.82; P = 0.05
Hexadecane*	1600	1.7 ± 0.09 a	1.4 ± 0.19 a	$F_{1,11}$ = 2.91; P = 0.11	0.8 ± 0.27 a	1.4 ± 0.11 a	χ^2 = 2.69; P = 0.10
Heptadecane*	1702	----------	----------	----------	0.9 ± 0.26 a	1.0 ± 0.23 a	χ^2 = 0.10; P = 0.74
2-Heneicosene	2021	----------	----------	----------	0.2 ± 0.20 a	0.3 ± 0.19 a	χ^2 = 0.24; P = 0.62
Docosane*	2200	----------	----------	----------	0.03 ± 0.03 a	0.3 ± 0.05 a	χ^2 = 3.70; P = 0.05
2-Tricosene	2224	----------	----------	----------	0.9 ± 0.56 a	0.7 ± 0.55 a	χ^2 = 0.03; P = 0.86
3-Tricosene	2230	----------	----------	----------	----------	0.7 ± 0.22	----------
7-Tricosene	2279	----------	----------	----------	----------	0.1 ± 0.04	----------
Tricosane*	2299	----------	----------	----------	5.6 ± 0.82 a	3.3 ± 0.56 b	χ^2 =5.59; P = 0.01
Tetracosane*	2400	----------	----------	----------	0.9 ± 0.42 a	0.2 ± 0.01 b	χ^2 = 7.61; P < 0.01
9-Pentacosene	2492	----------	----------	----------	0.3 ± 0.26	----------	----------
Pentacosane*	2500	85.2 ± 4.71 a	42.9 ± 3.34 b	$F_{1,11}$ = 53.52; P <0.01	1.7 ± 0.22 a	1.8 ± 0.33 a	χ^2 = 0.04; P = 0.82
Hexacosane*	2600	3.8 ± 0.48 a	4.8 ± 0.97 a	$F_{1,11}$ = 0.76; P = 0.40	0.4 ± 0.08 a	0.5 ± 0.06 a	χ^2 = 0.13; P = 0.71
11-Heptacosene	2670	----------	----------	----------	1.9 ± 0.38 a	0.5 ± 0.08 b	χ^2 = 26.47; P < 0.01
7-Heptacosene	2683	6.8 ± 0.69 a	4.2 ± 0.68 b	$F_{1,11}$ = 7.07; P = 0.02	0.8 ± 0.21 a	0.3 ± 0.02 b	χ^2 = 15.41; P < 0.01
Heptacosane*	2700	45.9 ± 3.13 a	20.9 ± 1.53 b	$F_{1,11}$ = 51.25; P < 0.01	0.9 ± 0.15 a	0.5 ± 0.07 b	χ^2 = 8.46; P < 0.01
(*Z*)-13 Docosenoamide	2771	----------	----------	----------	0.9 ± 0.20 a	0.2 ± 0.05 b	χ^2 = 20.86; P < 0.01
9-Nonacosene	2866	3.1 ± 0.30 a	1.3 ± 0.13 b	$F_{1,11}$ = 29.00; P < 0.01	----------	----------	----------
11-Nonacosene	2871	----------	----------	----------	38.4 ± 9.03 a	10.8 ± 1.57 b	χ^2 = 19.78; P < 0.01

7-Nonacosene	2885	105.2 ±10.23a	60.2 ± 5.46 b	$F_{1,11} = 15.01$; $P < 0.01$	----------	----------	----------
Nonacosane*	2900	9.1 ± 0.66 a	4.7 ± 0.32 b	$F_{1,11} = 36.29$; $P < 0.01$	2.1 ± 0.34 a	1.2 ± 0.23 b	$\chi^2 = 4.84$; $P = 0.02$
Triacontene	2960	2.0 ± 0.22 a	1.1 ± 0.12 b	$F_{1,11} = 11.74$; $P < 0.01$	----------	----------	----------
11-Triacontene	2970	----------	----------	----------	2.3 ± 0.50 a	1.5 ± 0.18 a	$\chi^2 = 2.96$; $P = 0.08$
Hentriacontadiene	3051	16.3 ± 1.36 a	9.9 ± 1.32 b	$F_{1,11} = 11.15$; $P < 0.01$	----------	----------	----------
11-Hentriacontene	3066	71.9 ± 6.32 a	43.3 ± 3.46 b	$F_{1,11} = 15.80$; $P < 0.01$	----------	----------	----------
9-Hentriacontene	3071	----------	----------	----------	18.0 ± 3.59 a	13.9 ± 2.08 a	$\chi^2 = 1.06$; $P = 0.30$
7-Hentriacontene	3085	13.1 ± 1.36 a	7.3 ± 0.85 b	$F_{1,11} = 13.13$; $P < 0.01$	13.8 ± 2.97 a	6.7 ± 1.01 b	$\chi^2 = 7.38$; $P < 0.01$
Hentriacontene	3100	----------	----------	----------	0.4 ± 0.14 a	0.5 ± 0.12 a	$\chi^2 = 0.04$; $P = 0.83$
Tritriacontene	3252	59.2 ± 6.05 a	30.9 ± 4.87 b	$F_{1,11} = 13.33$; $P < 0.01$	----------	----------	----------
9-Tritriacontene	3263	19.0 ± 2.35 a	8.6 ± 0.99 b	$F_{1,11} = 16.27$; $P < 0.01$	----------	----------	----------
Pentatriacontene	3451	9.4 ± 2.77 a	8.3 ± 1.61 a	$F_{1,11} = 3.09$; $P = 0.10$	----------	----------	----------
Ester							
Methyl hexadecanoate	1910	5.9 ± 3.29a	1.3 ± 0.11a	$F_{1,11} = 1.99$; $P = 0.18$	2.8 ± 0.86 a	1.5 ± 0.55 a	$\chi^2 = 1.67$; $P = 0.19$

Means within a row followed by the same letter are not significantly different. *Identified by authentic standards.

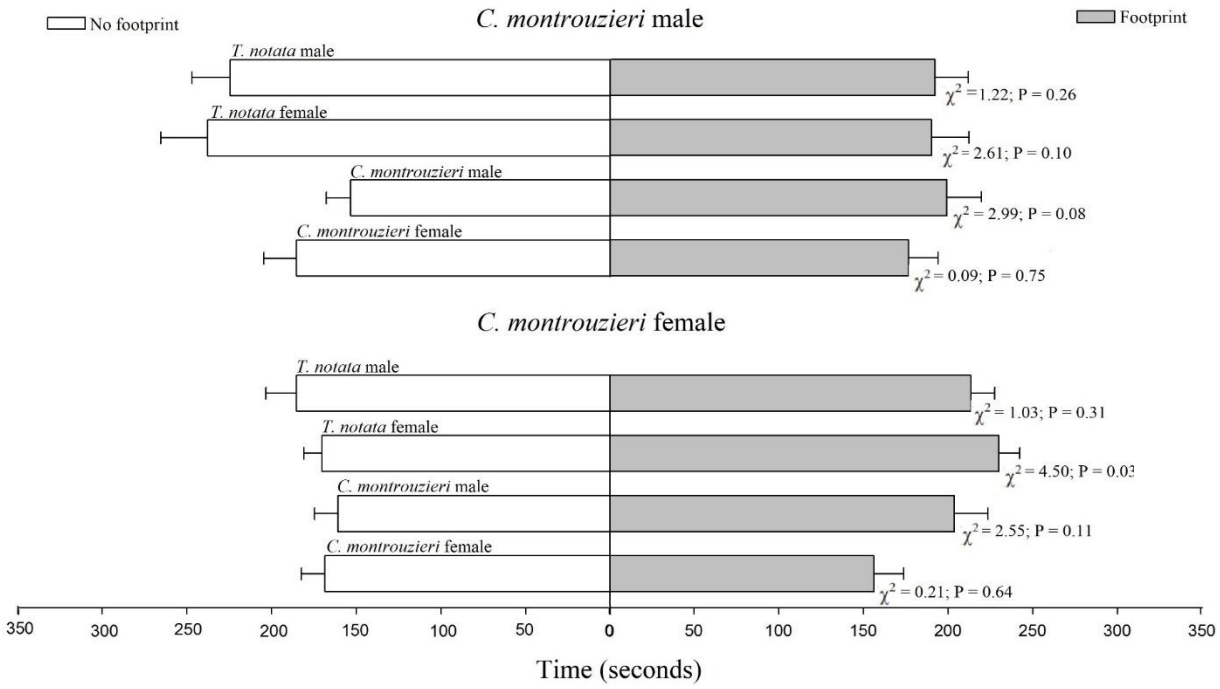

Figure 1. Average (± SE) walking time (WT, seconds) of *Cryptolaemus montrouzieri* male and female exposed to conspecific and heterospecific footprints on treated and untreated area in a 10 minutes trial.

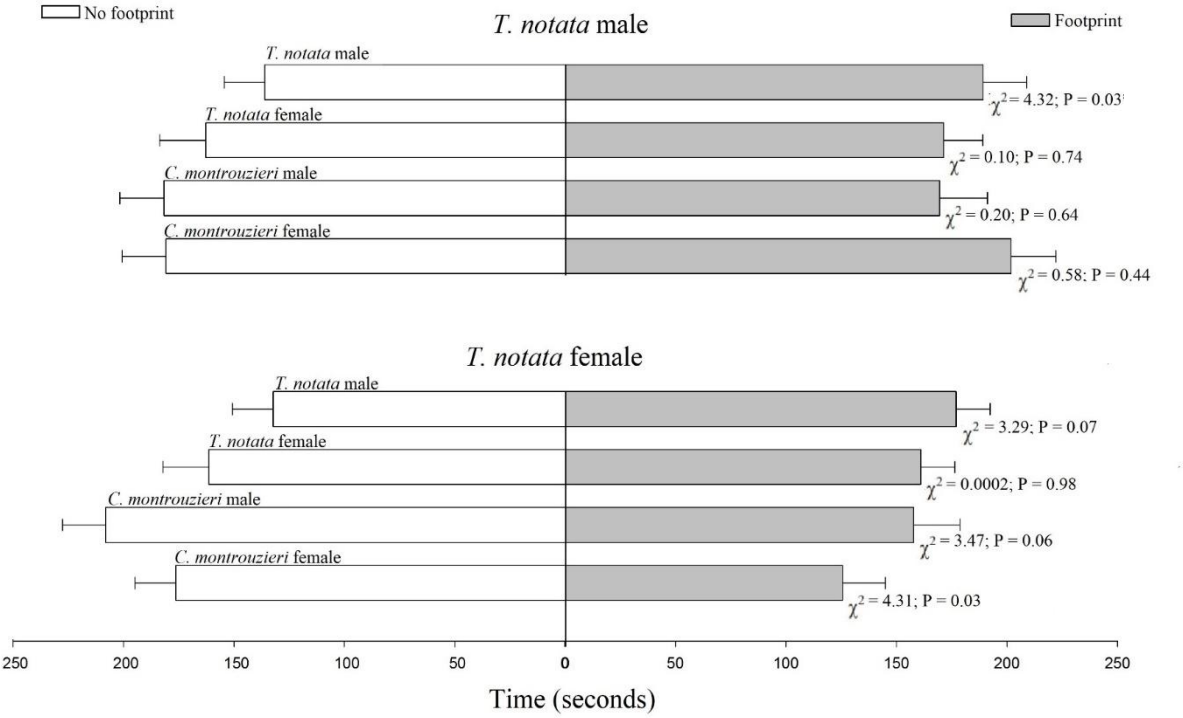

Figure 2. Average (± SE) walking time (WT, seconds) of *Tenuisvalvae notata* male and female exposed to conspecific and heterospecific footprints on treated and untreated area in a 10 minutes trial.

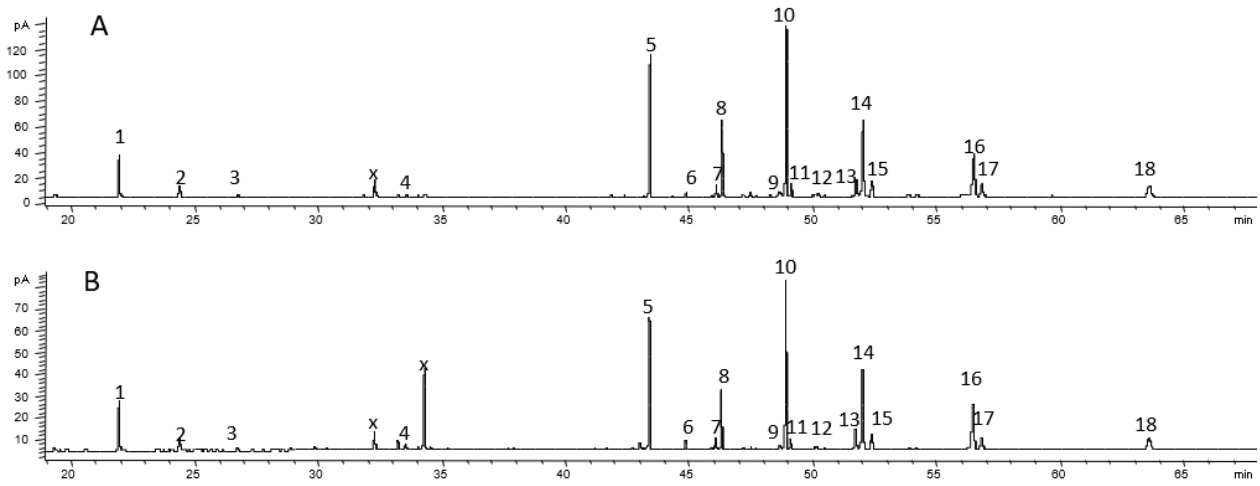

Figure 3. Chromatographic profile of hexanic extracts of *Tenuisvalvae notata* males (A) and females (B) footprints. Compounds: 1) tetradecane, 2) pentadecane, 3) hexadecane, 4) methyl hexadecanoate, 5) pentacosane, 6) hexacosane, 7) 7-heptacosene, 8) heptacosane, 9) 9-nonacosene, 10) 7- nonacosene, 11) nonacosane, 12) triacontane, 13) hentriacontadiene, 14) 11-hentriacontene, 15) 7-hentriacontene, 16) tritriacontene, 17) 9-tritriacontene, 18) pentatriacontene, x) not idetified.

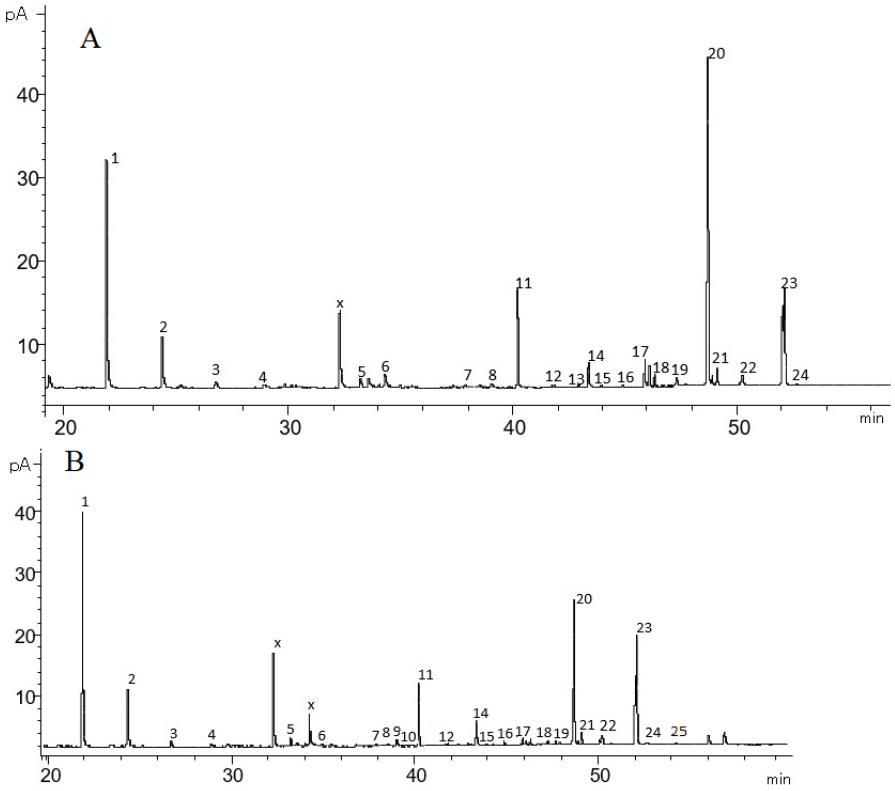

Figure 4. Chromatographic profile of hexanic extracts of *Cryptolaemus montrouzieri* males (A) and females (B) extracts footprints. Compounds: 1) tetradecane, 2) pentadecane, 3) hexadecane, 4) heptadecane, 5) methyl hexadecanoate, 6) 2-heneicosane, 7) docosane, 8) 2-tricosene, 9) 3-tricosene, 10) 7-tricosene, 11) tricosane, 12) tetracosane, 13) 9-pentacosene, 14) pentacosane, 15) hexacosane, 16) 11-heptacosene, 17) 7-heptacosene, 18) heptacosane, 19) (*Z*)-13-docosenoamide, 20) 11-nonacosene, 21) nonacosane, 22) 11-triacontene, 23) 9-hentriacontene, 24) 7-hentriacontene, 25) hentriacontene, x) not identified.

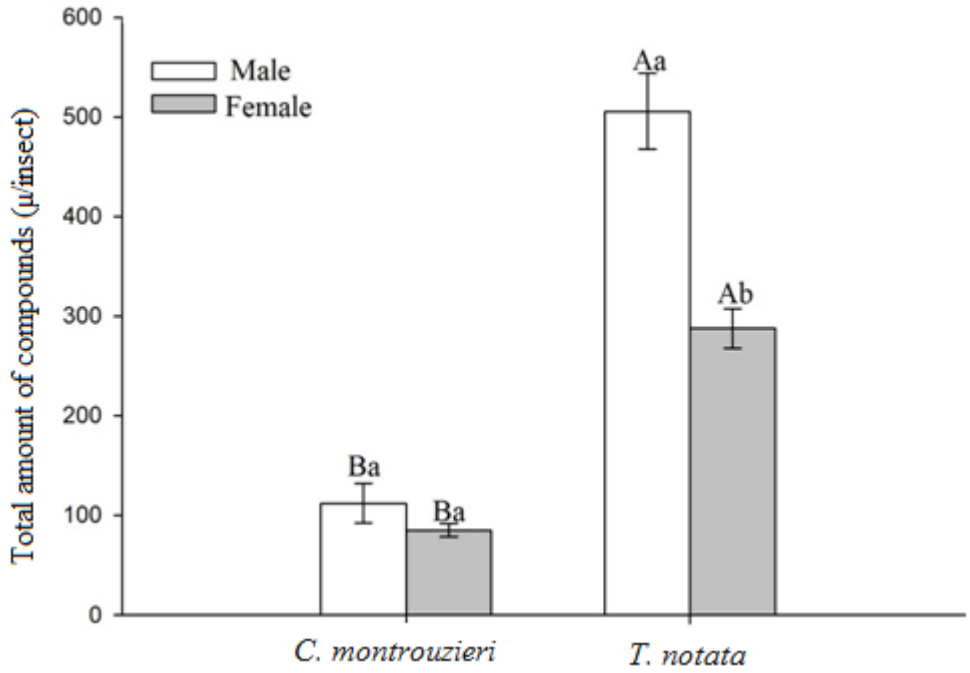

Figure 5. Total compounds quantified in extracts of *Cryptolaemus montrouzieri* and *Tenuisvalvae notata* male and female footprints. Uppercase letters indicate significant difference between species and lowercase between genders.

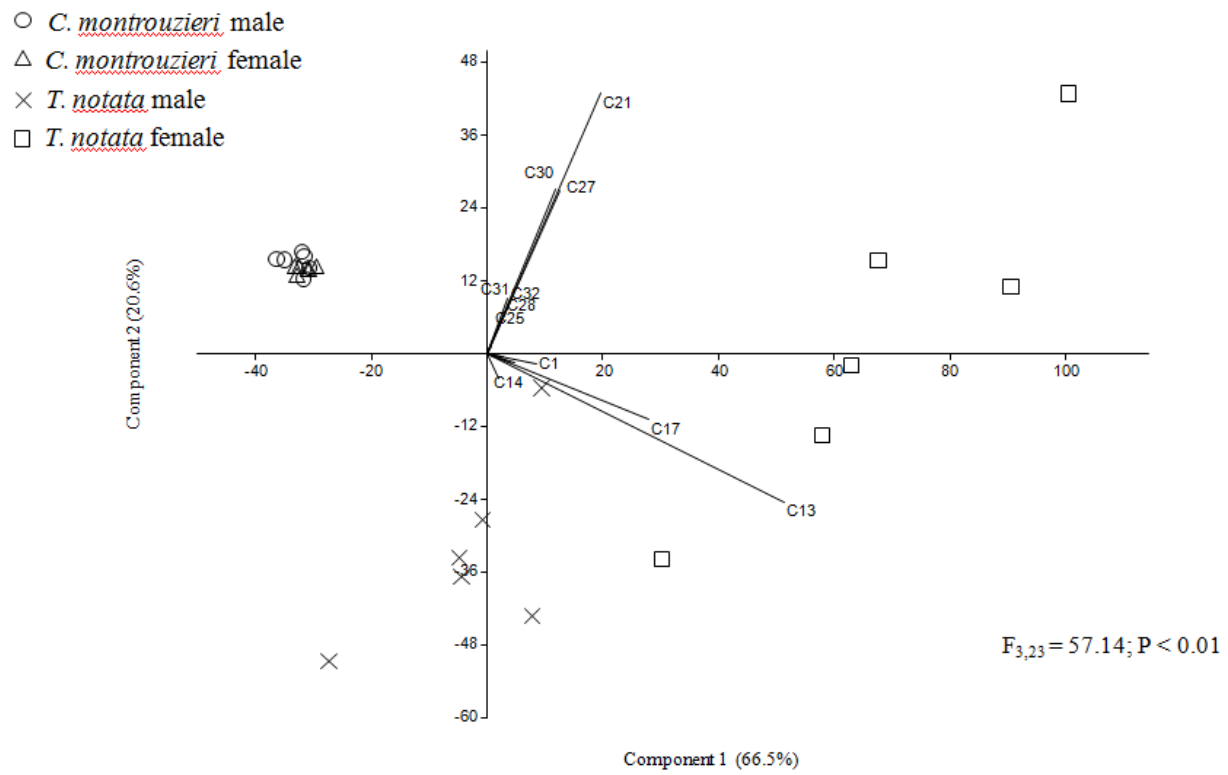

Figure 6. Principal component analysis (PCA) of hydrocarbons found in extracts of footprints of *Tenuisvalvae notata* and *Cryptolaemus montrouzieri* males and females. C1 (tetradecane), C2 (pentadecane), C3 (hexadecane), C4 (heptadecane), C5 (2-heneicosene), C6 (docosane), C7 (2-tricosene), C8 (3-tricosene), C9 (7-tricosene), C10 (tricosane), C11 (tetracosane), C12 (9-pentacosene), C13 (pentacosane), C14 (hexacosane), C15 (11-heptacosene), C16 (7-heptacosene), C17 (heptacosane), C18 (*Z*)-13docosenoamide), C19 (9-nonacosene), C20 (11-nonacosene), C21 (7-nonacosene), C22 (nonacosane), C23 (triacontene), C24 (11-triacontene), C25 (hentriacontadiene), C26 (11-hentriacontene), C27 (9-hentriacontene), C28 (7-hentriacontene), C29 (hentriacontene), C30 (tritriacontene), C31 (9-tritriacontene), C32 (pentatriacontene).

CAPÍTULO 3

DO SEMIOCHEMICALS OF LADYBEETLES AFFECT THEIR DEVELOPMENT, SURVIVAL, REPRODUCTION, AND PREDATORY BEHAVIOR?[1]

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[1]Ferreira, J.O. Do semiochemicals of ladybeetles affect their development, survival, reproduction, and predatory behavior? A ser submetido.

ABSTRACT- *Tenuisvalvae notata* (Mulsant), indigenous from South America, and *Cryptolaemus montrouzieri* Mulsant, which originated from Australia, are predators of mealybugs. The semiochemicals produced by either specie can be altered the interactions between them, with possible effects on their behavior. Therefore, this study measured ladybeetles' developmental times, fecundity, fertility, and predation upon mealybugs when predators were exposed to volatiles of conspecific and heterospecific individuals. There was no effect of volatiles on the developmental times of *T. notata.* However, there were increases in fecundity and fertility for females exposed to conspecific volatile. For *C. montrouzieri*, there was a 2-day delay in developmental times when they were exposed to volatiles conspecific and heterospecific, but with no effects on reproduction. For predatory behavior, I-II instar larvae and adults of *T. notata* increased predation rate when exposed to volatiles of conspecific and heterospecific. In *C. montrouzieri*, I-II instar larvae also increased predation rate when exposed to volatiles of conspecific and heterospecific, females increased predation rate when exposed to volatiles of conspecific females, whereas males reduced predation in this condition. The chemical analysis of volatiles air-entrainment extracts from both species identified seven compounds specific for *T. notata* and eight specifics for *C. montrouzieri*, with qualitative and quantitative differences between male and female extracts. The results showed that the volatiles blends emitted by ladybeetles affect their developmental times, reproduction, and competition for prey, affecting their potential as biological control agents of mealybugs.

KEY WORDS: Biological control, predators, chemical communication, organic volatile compounds

OS SEMIOQUÍMICOS DE JOANINHAS AFETAM SEU DESENVOLVIMENTO, SOBREVIVÊNCIA, REPRODUÇÃO E PREDAÇÃO?

RESUMO – *Tenuisvalvae notata* (Mulsant), nativa da América do Sul, e *Cryptolaemus montrouzieri* Mulsant, originário da Austrália, são predadores de cochonilhas. Os semioquímicos produzidos por essas espécies podem alterar a interação entre elas, com possíveis efeitos sobre seu comportamento. Portanto, neste estudo medimos o tempo de desenvolvimento, a fecundidade, a fertilidade e a predação de cochonilhas quando esses predadores foram expostos a voláteis de indivíduos coespecíficos e heteroespecíficos. Não houve efeito de voláteis sobre o tempo de desenvolvimento de *T. notata*. No entanto, houve aumento na fecundidade e fertilidade quando as fêmeas foram expostas a voláteis coespecíficos. Para *C. montrouzieri*, houve um atraso de dois dias no tempo de desenvolvimento quando foram expostos a voláteis coespecíficos e heteroespecíficos, mas sem efeitos na reprodução. No comportamento predatório, larvas de instar I-II e adultos de *T. notata* aumentaram a taxa de predação quando expostos a voláteis de coespecífico e heteroespecífico. Em *C. montrouzieri*, larvas de instar I-II aumentaram a taxa de predação quando expostas a voláteis de coespecífico e heteroespecífico, fêmeas aumentaram a taxa de predação quando expostas a voláteis de fêmeas coespecíficas, enquanto os machos reduziram a predação nesta condição. Foram identificados sete compostos específicos nos extratos de voláteis de *T. notata* e oito nos extratos de *C. montrouzieri*, com diferenças qualitativas e quantitativas entre os extratos de machos e fêmeas. Os resultados mostraram que as misturas de voláteis emitidas por joaninhas afetam seus tempos de desenvolvimento, reprodução e competição por presas, afetando seu potencial como agentes de controle biológico de cochonilhas.

PALAVRAS-CHAVE: Controle biológico, predadores, comunicação química, compostos orgânicos voláteis

Introduction

The ladybeetles (Coleoptera: Coccinellidae) are known biological control agents of aphids, psyllids, scales, mealybugs, whiteflies, mites, and other insects (Hodek *et al.* 2012). Due to their efficiency as predators, they have been used in various classical biological control programs worldwide. For instance, the ladybeetle *Cryptolaemus montrouzieri* Mulsant, native to Australia and commonly known as the mealybug destroyer, was introduced in more than 40 countries to control mealybugs (Booth & Pope 1986). Also, *Tenuisvalvae notata* (Mulsant), indigenous to South America, was introduced in the 1980s in Africa to control the cassava mealybug, *Phenacoccus manihoti* Matille-Ferrero (Hemiptera: Pseudococcidae) (Chakupurakal *et al.* 1994, Dreyer *et al.* 1997). Similarly, other species such as *Coccinella septempunctata* L., *Coccinella undecimpunctata* L., *Harmonia axyridis* (Pallas), *Hyperaspis pantherina* Fürsch, and *Rodolia cardinalis* (Mulsant) were introduced in different countries to manage pests throughout times (Kenis *et al*. 2017, Kundoo & Khan 2017).

The efficiency of ladybeetles as biological control agents has been questioned due to some of their behaviors such as evasion of releasing sites after satiation, cannibalism, intraguild predation, competition, possible displacement of native species, and interference in the behavior of other natural enemies (Elliott *et al.* 1996, Michaud 2002, Mishra *et al.* 2013, Amorós-Jiménez *et al.* 2015, Crookes *et al*. 2019). Those behaviors are the result of the interactions on the food web the ladybeetles are inserted. In this context, *C. montrouzieri* and *T. notata* prefer to prey upon mealybugs, can be found in the same tropical areas (Ferreira 2019), and they might interact in the same food web as shown by Peronti *et al*. (2016) in colonies of *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae) in São Paulo State, Brazil. This simultaneous occurrence can cause competition for prey, but also cannibalism and intraguild predation when prey is scarce (Oliveira 2020).

Insect communication is multimodal, with the chemical communication being the most common among them (Córdoba-Aguilar *et al*. 2018). Moreover, the semiochemicals are chemical signals that when used between individuals of the same species are called pheromones and with different species are called allelochemicals (Pettersson 2012). Previous studies have shown that semiochemicals produced by *C. montrouzieri* affect their oviposition behavior, can be detected by their prey, which evades the same area, and interfere with the foraging behavior of other natural enemies (Merlin *et al*. 1996, Chong & Oetting 2007, Pakyari *et al*. 2019). Also, *C. montrouzieri* has been reported as a better competitor than other ladybeetle species, which could have a negative impact on native species (Gkounti *et al*. 2015, Oliveira 2020).

Even though *C. montrouzieri* has been introduced in Brazil about 20 years ago (Sanches *et al*. 2002), only recently there are some reports regarding the potential of *C. montrouzieri* to interfere in ecological interactions with native species such as *T. notata* (Ferreira *et al*. 2020, Ferreira *et al*. 2021, Oliveira 2020). In this context, we hypothesized that the semiochemicals emitted by *C. montrouzieri* and *T. notata* are species-specific and may affect their interactions, biology and predatory capacity. Therefore, the objective of this study was to evaluate the effects of their volatiles semiochemicals on their developmental times, reproduction, survival, and predatory behavior.

Material and Methods

The insects used in the bioassays were obtained from the colonies maintained in the Insect Behavior Laboratory, at the Universidade Federal Rural de Pernambuco (UFRPE) (−8.017070°S and −34.944362°W). The conditions for maintenance of insects and execution of bioassays were temperature of 25 ± 2 ºC, 60 ± 10% relative humidity, and a photoperiod of 12h:12h (L:D).

Prey. The colony of *Ferrisia dasylirii* (Cockerell) (Hemiptera: Pseudococcidae) was reared on pumpkins, *Cucurbita moschata* (Duch.), var. jacarezinho, in the initial maturation stage, obtained from the local market, "Centro de Abastecimento Alimentar" (CEASA), Recife-PE, Brazil. Shortly, after being rinsed and dried, pumpkins were placed in plastic trays (30 x 45 x 4 cm) lined with a paper towel and infested on the petiole with gravid mealybugs, originated from the stock colony.

When the pumpkin was completely infested by mealybugs, it received a clean pumpkin on top, allowing the free movement of nymphs and adults to the new pumpkin. The average time to complete the infestation of a pumpkin is about 30 days, after that, they were used in the colonies of the ladybeetles.

Predators. Colonies of *T. notata* and *C. montrouzieri* were kept apart in the laboratory, but under the same environmental condition as the prey. Adults of each predator species were placed in acrylic boxes (40 x 25 x 20 cm), with circular lateral openings, covered with a fine mesh to allow ventilation inside the boxes. The bottom of the boxes was lined with a paper towel, where one infested pumpkin was offered to the predators, following Barbosa *et al*. (2014a). Ladybeetles were allowed to feed and mate freely in the rearing boxes. New infested pumpkins were offered to predators as prey were consumed from previously offered pumpkins. Eggs and larvae of the predators were kept in the same rearing cages with the adults.

Effect of volatile semiochemicals on development, reproduction and survival. This test investigated the possible interference of volatile semiochemicals produced by conspecific and heterospecific on the development and reproduction of ladybeetles. Thus, eggs of *T. notata* and *C. montrouzieri* were collected from the colonies, kept in Petri dishes (3.5 cm of diameter), and

monitored daily until larval eclosion. After that, first instar larvae (< 24h-old) were subjected to the following treatments: i) one *T. notata* larva paired with one *T. notata* larva; ii) one *T. notata* larva paired with one *C. montrouzieri* larva; iii) one *C. montrouzieri* larva paired with one *C. montrouzieri* larva; iv) one *C. montrouzieri* larva alone; and v) one *T. notata* larva alone. Each treatment had 40 replicates and mealybug nymphs were offered *ad libitum* as prey.

Pairings of ladybeetles happened in arenas made of a couple of acrylic Petri dishes (5.5 cm in diameter) placed on top of each other. A small square (4x4 cm) was cut on the lids of each Petri dish and covered with *voil* fabric to allow ventilation inside the arena. In each paired treatment (i-iii), the dishes were placed on top of each other, fixed by a rubber band, and with the part of the *voil* fabric in the middle, allowing for gas exchange between the arenas. Therefore, the arena worked as a double chamber, with volatile exchange between the sides, but did not allow for the direct contact of ladybeetles placed on each side. Each side of the arena held only one larva, according to the treatments (i-iii). For treatments iv and v, the arena was only one Petri dish not paired, working as controls for the development of the ladybeetles without the exposition to volatiles of other individuals.

The duration of development from the first instar to adult was observed daily, and changes of each instar were noted by the presence of the exuvia. After emergence, adults were transferred to new Petri dishes of the same size, and couples were allowed to mate for 12h. Next, females were individualized and again subjected to the treatments (i-v) previously described, to follow daily oviposition, egg viability, and survival over 60 consecutive days (Túler *et al*. 2018). Prey was offered *ad libitum* during the evaluation period.

Effect of volatile semiochemicals on predatory behavior. The predation rate of larvae and adults of *T. notata* and *C. montrouzieri* upon nymphs of *F. dasylirii* was investigated in the arenas

previously described. Ladybeetle larvae and adults were subject to the following treatments: i) one I-IV instar larvae of *T. notata* paired with one conspecific larvae of the same age; ii) one I-IV instar larvae of *T. notata* paired with one *C. montrouzieri* of the same age; iii) one I-IV instar larvae of *C. montrouzieri* paired with one conspecific larvae of the same age; iv) one I-IV instar larvae of *T. notata* not paired/alone; v) one I-IV instar larvae of *C. montrouzieri* not paired/alone; vi) one male *C. montrouzieri* paired with one male *T. notata*; vii) one male *C. montrouzieri* paired with one female *T. notata*; viii) one female *C. montrouzieri* paired with one male *T. notata*; ix) one female *C. montrouzieri* paired with one female *T. notata*; x) one male *T. notata* paired with one male *T. notata*; xi) one female *T. notata* paired with one female *T. notata*; xii) one female *T. notata* paired with one male *T. notata*; xiii) one male *C. montrouzieri* paired with one male *C. montrouzieri*; xiv) one female *C. montrouzieri* paired with one female *C. montrouzieri*; xv) one male *C. montrouzieri* paired with one female *C. montrouzieri*; xvi) one male *C. montrouzieri* not paired/alone; xvii) one female *C. montrouzieri* not paired/alone; xviii) one male *T. notata* not paired/alone; xix) one female *T. notata* not paired/alone. Each treatment had 40 replicates.

Ladybeetles were deprived of food before bioassay to balance hunger levels according to age. Larvae of I and II instars were starved for 2 hours, whereas III-IV instar larvae and adults were starved for 24h before tests. Next, ladybeetles assigned to each treatment were offered six mealybugs: II instar nymphs for ladybeetle larvae of I and II instars; whereas adult mealybugs were offered for ladybeetle larvae of III-IV instar and adults (Barbosa *et al*. 2014a). After 24h exposition, the number of preys consumed was recorded.

Ladybeetle's volatile collections. The collection of volatiles and chemical analyses of volatile extracts obtained from the aeration of *C. montrouzieri* and *T. notata* adults were conducted in the Semiochemicals laboratory at Embrapa Recursos Genéticos e Biotecnologia- CENARGEN,

Brasília-DF, Brazil. Thus, virgin adults of each ladybeetle species, 5 to15-days old, were separated into groups of 30 individuals of the same sex and species, and placed in a glass chamber (vol. 1L) for aeration. Each treatment had 6 replicates. Each chamber had three holes (5 cm diameter) on the top part; two holes allowed the air into and out of the chamber, respectively, whereas the third hole was stopped by a perfectly fit glass lid, used to introduce the insects in the chamber. In the hole where the air exits the system, one glass tube (15 cm length x 5 mm diameter) containing 100 mg of the adsorbent Porapak-Q (80-100 mesh, Sigma Aldrich, USA) was connected. The adsorbent was kept in the glass tube by a small portion of glass wool on both sides. Previously to aeration, the tubes containing the adsorbent were conditioned in a constant nitrogen flow at 250 ºC for 12 hours. After use, the tubes with the adsorbent were subjected again to a constant nitrogen flow at 180 ºC for 2 hours. In the aeration system, the tubes containing the adsorbent were connected to the chamber by a silicon tube. The air entering the chamber was previously filtered by a filter of activated charcoal (20-40 mesh, Sigma Aldrich), to guarantee that the air entering the system was purified. Volatile samples were collected for 24 hours at 27 ºC and 60% relative humidity. After aeration, the tubes containing the adsorbent were disconnected from the chamber and washed with 500 µL of hexane PA, and volatile extracts were kept in 2 mL glass vials in the freezer at -20 ºC until further chemical analyses.

Chemical analyses. Samples were analyzed by a gas chromatographer (GC-FID) [Agilent Technologies 7890A, DB-5MS column, 0.25 mm inner diameter (ID) x 30 m, 0.25 µm film, J&W Scientific, Folsom, CA, USA, equipped with a flame ionization detector at 270 ºC]. The column oven was programmed to 40 °C for 2 min, then to 180 °C at 5 °C per min, held for 0.1 min, followed by an increase of 10 °C per min to 280 °C (held for 5 min). Aliquots of 2 µL of each sample were injected using the splitless mode, at 250 ºC, with helium as carrier gas. Each run

lasted for 45 min total. Data were collected using Shimadzu Class GC software and were analyzed using Excel software (Microsoft, 2007). Then, the quantification was done using the internal standard (IS) method, comparing the area of the IS with the areas of all compounds in the chromatogram profile. For this each sample was spkied with 1 μL of the internal standard (IS) hexadecanolide (1mg/mL), at a final concentration of 0.01 mg/mL.

To proceed with qualitative analysis, selected aeration extracts were analysed by GC-MS (gas chromatographer-mass spectrometer). Thus, aliquots of 2 μL of each sample were injected in a Shimadzu QP 2010 quadruple mass spectrometer equipped with a DB-5MS column (0.32mm ID x 60 m, 1.0 μm film, Supelco, Bellefonte, PA, USA), and a splitless injector, with helium as carrier gas. Ionization was done by electron impact (70 eV). The temperature program and column were the same as previously described for the GC-FID. Data were collected with GC solution software (version 2.42, Shimadzu, Japan). Identifications were made by comparison of spectra with library databases (Software NIST-Wiley database, version 2.0, 2008) and were confirmed by overlap of the volatiles extracts with authentic standards when available.

The compounds (*Z*)-3-hexen-1-ol, 2-hexanone, 6-methyl-5-hepten-2-one, benzylic alcohol, α-pinene, β-pinene, benzaldehyde, benzothiazole, hexanal, myrcene, octanal, octanol, nonane, undecane, dodecane, hexadecane, and tetradecane were confirmed using authentic standards. For compounds that authentic standards were not available, identification was done by comparison with published spectra and using kováts index (published at Database of Pheromones and Semiochemicals (Pherobase) and National Institute of Standards and Technology (NIST): Chemistry WebBook websites).

Statistics – Data of development duration (from the first instar to adult), number of eggs per female (fecundity), egg viability (fertility), daily oviposition and predation rate were subjected to

normality tests (Shapiro Wilk) to access for normal distribution. Daily oviposition was subjected to ANOVA. Data of development duration, fecundity, fertility and predation rate did not meet the ANOVA assumptions, then were subjected to a general linear model analysis (GLM) with Quasipoisson distribution, and fertility was subjected to a GLM with Quasibinomial distribution and $\alpha = 0.05\%$. When the analyses showed significant effects of treatments, means were compared using contrast analyses. The survival curves for adult females subjected to different pairing treatments in the first bioassay were determined using the Kaplan-Meier method (Klein & Moeschberger, 2003), and were compared using the log-rank test. Data of the amount of each volatile compound emitted by adults *T. notata* and *C. montrouzieri* were subjected to Shapiro Wilk normality test, and even with transformation, data did not assume normality. Therefore, data were subjected to GLM analysis with a Gamma distribution, comparing the quantity of each volatile compound between species and sex. Data of the total amount of volatiles released assumed normality after the $\sqrt{(x + 0.5)}$ transformation and were subjected to analysis of variance (ANOVA), with species and sex as factors. In addition, to analyze the similarity between compounds released by *T. notata* and *C. montrouzieri* males and females, there was a separation in compounds by chemical group (monoterpenes, esters, hydrocarbons, alcohols, and ketones). Next, data were subjected to a cluster analysis, based on the Bray-Curtis similarity index, using the R package "Vegan". The dissimilarity between compounds was evaluated by the analysis of permutation variance (PERMANOVA) with 999 permutations. All analyses were conducted using the software R 4.0.5 (R Development Core Team 2011).

Results

Effect of semiochemicals on development, reproduction and survival – the average duration of development (± SE) for *T. notata* varied from 28.3 ± 0.44 to 28.5 ± 0.45 days, when it was

exposed to volatiles of conspecifics and heterospecifics, with no significant changes in developmental duration (Table 1). On the other hand, for *C. montrouzieri*, there was a 2-day delay in developmental time when exposed to volatiles of other individuals regardless of the species ($F_{2,159} = 8.62$; $P < 0.01$, Table 1). The duration of development for *C. montrouzieri* when not paired was 24.6 ± 0.28 days, and when exposed to volatiles of other individuals it was 26 ± 0.33 days with conspecifcs, and 26.9 ± 0.29 days with heterospecifics (Table 1).

There was a significant increase in the number of eggs laid ($F_{2,79} = 6.93$; $P < 0.01$, Table 2) when *T. notata* females were exposed to volatiles of conspecifics. Oviposition was higher in the first 20 days after emergence, and decreased along the time. There was no significant difference in the daily oviposition when females where alone or exposed to volatiles of other individuals (Fig. 1). Moreover, egg viability also increased significantly when paired with conspecifics ($F_{2,79} = 6.16$; $P < 0.01$) (Table 2).

For *C. montrouzieri* there was no effect of volatiles of conspecifics and heterospecifics on the number of eggs laid by females (Table 2). Similarly to *T. notata*, oviposition was higher in the first 20 days of observation (Fig. 2), decreasing along the time, but with no difference among the treatments. Egg viability for *C. montrouzieri* was also not affected by volatiles from conspecifics and heterospecifics (Table 2).

Female *T. notata* survival was not affected ($\chi^2 = 2.65$; Df = 2; P = 0.26) by volatiles of other individuals regardless of the species. Similarly, in *C. montrouzieri*, there was no difference in female survival, and all females were alive after 60 days of observation period ($\chi^2 = 0{,}0$; Df = 2; P = 1).

Effect of semiochemicals on predatory behavior – There was a significant increase in predation rate by first instar larvae of *T. notata* when exposed to volatiles of conspecifics and heterospecifics of same age ($F_{2,159}$ = 9.02; P < 0.01, Table 3). Similarly, there was a significant increase in predation rate by second instar larvae of *T. notata* when paired with same age larvae of *C. montrouzieri* ($F_{2,159}$ = 6.51; P < 0.01, Table 3). For third instar larvae of *T. notata* there was a significant decrease in the predation rate when they were paired with conspecifics ($F_{2,159}$ = 3.98; P = 0.02, Table 3). Finally, there was no effect of volatiles of conspecifics and heterospecifics on predation rate of by fourth instar *T notata* larvae.

For adults *T. notata*, there was a significant effect of volatiles on predation rate of males ($F_{4,239}$ = 8.23; P < 0.01, Table 3) and females ($F_{4,239}$ = 4.35; P < 0.01, Table 3). Predation was significantly higher when *T. notata* females were paired with conspecifics and heterospecifics females in comparison to those not exposed to volatiles of others (Table 3). For *T. notata* males, there was a significant increase in predation rate when they were exposed to volatiles of conspecifics males (Table 3).

Regarding first instar larvae of *C. montrouzieri*, there was an increase in predation rate when exposed to volatiles of conspecifics and heterospecifics ($F_{2,159}$ = 4.62; P = 0.01, Table 3). Similarly, second instar larvae increased predation when exposed to volatiles of heterospecifics ($F_{2,159}$ = 17.87; P < 0.01, Table 3). On the other hand, there was no significant effect of volatiles on predation by third, and fourth instar larvae.

For *C. montrouzieri* adult females, there was a significant increase in predation rate when exposed to volatiles of conspecific females ($F_{4,239}$ = 40.85; P < 0.01, Table 3). On the other hand, for *C. montrouzieri* males there was a significant decrease in predation when exposed to volatiles of conspecific females ($F_{4,239 =}$ 9.35; P < 0.01, Table 3).

Chemical analysis – The interaction between the total amount of compounds present in the ladybeetles extracts volatiles was different ($F_{3,22} = 6.09$; P = 0.02) for species and genders (Fig. 3). A higher amount of total compounds was quantified from *C. montrouzieri* male volatile extract compared to female conspecific extracts ($F_{1,11} = 5.36$; P = 0.04), whereas there was no difference when comparing the total amount of compounds obtained from males and females volatiles extracts of *T. notata* (Fig. 3) ($F_{1,11} = 1.62$; P = 0.23) (Fig. 3). Volatiles extracts of *C. montrouzieri* males presented a higher amount of compounds in their extracts compared to *T.notata* males ($F_{1,11} = 6.56$; P = 0.02), but there were no differences between *T. notata* and *C. montrouzieri* females ($F_{1,11} = 0.79$; P = 0.39) (Fig. 3).

There were 30 compounds present in the extracts of volatiles of *T. notata* females and 30 compounds in the extracts of males (Fig 4). The ester methyl-9-octadecenoate ($\chi^2 = 4.95$; Df = 1; P = 0.02), the aldehydes decanal ($\chi^2 = 8.04$; Df = 1; P < 0.01) and tridecanal ($\chi^2 = 7.89$; Df = 1; P < 0.01), and the alcohols (*Z*)-3–hexanol ($\chi^2 = 5.99$; Df = 1; P = 0.01) and hexadecanol ($\chi^2 = 4.41$; Df = 1; P = 0.03) were quantified in higher amount in *T. notata* female extracts. Whereas, heptanal ($\chi^2 = 6.30$; Df = 1; P = 0.01) was quantified in higher amounts in *T. notata* male extracts, and the compound methyl 9-oxononanoate, undecanal, 2,2-dimethyl pentanol, and benzaldehyde were quantified only on *T. notata* male extracts (Table 4). The compound hexadecanoic acid was identified in *T. notata* female extracts in traces amount, therefore it was not quantified.

Regarding the volatiles identified from the air entrainment extracts of *C. montrouzieri* males and females the following compounds were quantified in higher amounts on male extracts: methyl-9-octadecanoate ($\chi^2 = 5.29$; Df = 1; P = 0.02), hexadecane ($\chi^2 = 6.46$; Df = 1; P = 0.01), nonanal ($\chi^2 = 4.70$; Df = 1; P = 0.03), and α-terpineol ($\chi^2 = 8.80$; Df = 1; P < 0.01) (Table 4). The compounds methyl-9-oxononanoate and benzothiazole was found only on *C. montrouzieri* male

extracts. The (*E*)-2-nonenal and octen-3-ol were identified but released in amounts lower than the limit of confidence of the GC-FID used in this analysis, thus they could not be quantified (Fig 5).

There was no significant difference between ladybeetle species and sexes regarding the monoterpenes ($F_{3,22} = 1.50$; $P = 0.11$), esters ($F_{3,22} = 1.25$; $P = 0.28$), and ketones ($F_{3,22 =} 1.07$; $P = 0.41$). However, there was a significant difference between species in the hydrocarbons ($F_{3,22} = 1.90$; $P = 0.01$), the aldehydes ($F_{3,22} = 2.97$; $P < 0.01$), and the alcohols ($F_{3,22} = 2.63$; $P < 0.01$) quantified, dividing the compounds in divergent groups (Fig. 6). In addition, the myrcene, 1,2-dichlorobenzene, tetradecanal, hexadecanal, octadecanal, 2,2-dimethyl pentanol, and hexadecanol were found only on extracts of *T. notata*, whereas (*E.E*)-2,4-nonadienal, heptanol, octanol, 2,7-dimethyl octanol, α-terpineol, and phenoxy-2-propanol were found only on extracts of *C. montrouzieri*.

Discussion

The ladybeetles *T. notata* and *C. montrouzieri* are predatory species that prefer to prey on mealybugs (Hemiptera, Pseudococcidae). They may occur in similar tropical areas with food availability and favorable weather, therefore they can occur in the same food web (Peronti *et al*. 2016, Ferreira *et al*. 2020, Ferreira *et al*. 2021). Thus, these ladybeetle species can show behaviors that reflect the competition between them, such as cannibalism and intraguild predation (Oliveira 2020).

It is known that semiochemicals released by ladybeetles can affect their interaction with prey and other natural enemies (Kajita *et al.* 2006, Chong & Oetting 2007, Meisner *et al*. 2011, Finlay-Doney & Walter 2012, Hodek *et al*. 2012, Ninkovic *et al*. 2013, Susset *et al*. 2013, Kumar *et al*. 2014, Amorós-Jiménez *et al*. 2015, Pervez & Yadav 2018, Urbina *et al*. 2018, Canovai *et al*. 2019, Pakyari *et al*. 2019). Therefore, we expected that volatiles released by *C. montrouzieri* and

T. notata could affect their development and predatory capacity. This is the first study showing that *C. montrouzieri* and *T. notata* produce and release volatile organic compounds that are species-specific and can be detected by heterospecific individuals and have the potential to impair their development, reproduction, and predatory behavior. Results of such research are important to preview possible effects of the interaction of those ladybeetle species in more natural settings and the impacts on their biological control of mealybugs.

Interestingly, the duration of developmental period of the native species *T. notata* was not affected by the presence of volatile semiochemicals released by the introduced species *C. montrouzieri*. On the other hand, *C. montrouzieri* had a delay in about 2 days in its development when subjected to volatiles released by conspecific and by the native competitor. Previous studies have shown that in optimal environmental conditions, the average duration of development for *T. notata* varied from 27-30 days and for *C. montrouzieri* varied from 22-30 days (Barbosa *et al*. 2014b, Maes *et al*. 2014, Marques *et al*. 2015, Ferreira *et al*. 2020), similarly to what we found for development without the exposition to semiochemical volatiles. It is possible that *C. montrouzieri* can detect the compounds released by conspecifics and heterospecifics, and as a reflex of the possible competition, it has a delay in development. Moreover, the capacity of *C. montrouzieri* to recognize semiochemicals in the same area was previously demonstrated (Merlin *et al*. 1996, Finlay-Doney & Walter 2012, Urbina *et al*. 2018). Similar results were found for *C. septempunctata* and *Coccinella transversalis* F when exposed to semiochemicals of competitor species (Kumar *et al*. 2014). The same is not the case for *T. notata*, since its development was not affected by compounds of *C. montrouzieri*.

Regarding reproduction, *T. notata* had an increase in its fitness in terms of oviposition and fertility when subjected to volatiles of conspecific individuals. Previous studies have also shown similar results with other coccinellids exposed to semiochemicals of conspecific individuals

(Kajita *et al*. 2006). Thus, semiochemicals released by conspecific individuals were detected by *T. notata*, which could signal to foundresses an area with food availability for young and resulted in higher oviposition. Conspecific semiochemicals can attract ladybeetle or can generate a tolerance of coccinellids to remain at places with conspecifics, compared to sites with heterospecific (Susset *et al*. 2013, Ugine *et al*. 2018). These compounds could be an indication of food availability, inducing an increase in oviposition. In ladybeetles, parental care by adult females towards larvae is not common, and as larvae have limited locomotion capacity in comparison to adults that can flight and evade areas of danger or competition, the selection of oviposition sites by foundresses affects directly the survival of immature stages (Mishra *et al*. 2013). Based on our findings, regarding development time and reproduction, it seems that the native species *T. notata* is not affected by semiochemicals released by *C. montrouzieri.* Therefore, further studies of olfaction and electrophysiology are necessary to better understand these aspects in *T. notata* adults.

Predation exerted by first and second instar larvae of both the ladybeetle species showed a tendency to increase when larvae were exposed to volatiles of conspecific and heterospecific individuals, in comparison to predation when larvae were not exposed to semiochemicals of other individuals. This was not the case for older larvae. Previous studies have shown that *H. axyridis* and *C. septempunctata* larvae also release semiochemicals, and despite begin less agile than adults, are capable of escaping from areas treated with footprints of conspecific and heterospecific individuals (Meisner *et al*. 2011, Lucas 2012, Dinesh & Venkatesha 2014, Pervez & Yadav 2018), probably to reduce the risk of cannibalism or intraguild predation. Also, older ladybeetle larvae, of third and fourth instar, act more often as intraguild predators than younger ones (Lucas 2012), and younger larvae are more susceptible than older ones to cannibalism and intraguild predation (Oliveira 2020). This could explain the increase in prey consumption in younger larvae due to the higher number of encounters with available prey in the arena. So, when younger larvae detected

semiochemicals of other individuals, they increased movement rate (e.g., walking time and speed) trying to escape from a possible intraguild predator, at the same time, this increased the chances of finding prey nearby resulting in higher predation. In this context, further studies that evaluate the walking behavior of ladybeetle larvae in the presence of semiochemicals (e.g., volatiles or footprints) of conspecific and heterospecific individuals, and the consumption rate in such conditions, would help to understand the effects of those compounds in their escape behavior and predatory capacity.

As for the adults, we observed higher predation of *T. notata* and *C. montrouzieri* males and females, when exposed to volatiles of conspecifics. Adult coccinellids are better at searching and catching prey than larvae, probably due to their experience in the immature stage and higher mobility. In contrast, adult coccinellids spend more time start prey searching, probably to detect cues present in the foraging area (Pervez & Yadav 2018, Canovai *et al*. 2019). The capacity of responding to a large amount of information and cues from the environment is important to the ecological success of ladybeetles (Hodek & Michaud 2008). Thus, volatile compounds released by conspecific and same-sex individuals were detected first in the environment.

The compounds identified in the extract of volatiles of *T. notata* and *C. montrouzieri* male and females showed gender and species specificity in chemical profiles. From the 42 identified compounds, 27 were released by both species, whereas seven compounds were specific to *T. notata* and 8 of *C. montrouzieri*. Monoterpenes, esters, and ketones identified in both species were similar. Previous studies indicate an effect of food in the production of compounds (Hautier *et al*. 2008, Sloggett *et al*. 2009). In our study, the ladybeetles were reared on the same species of prey, *F. dasylirii*, and environmental condition; hence the similarities in the chemical profiles could be related to the prey. On the other hand, hydrocarbons, alcohols, and aldehydes were different

between the species, indicating chemical specificity in the profiles of *T. notata* and *C. montrouzieri*, which can affect their interaction with conspecific and heterospecific individuals.

Although some studies report changes in the behavior of *C. montrouzieri* against intraspecific and interspecific semiochemicals, there are still no reports on the behavior of this species for individual compounds, as well as for *T. notata*. The compounds α-pinene, β-pinene, nonanal, decanal, and benzaldehyde, found in this study, were attractive for other coccinellids (Han & Chen 2002, James 2005, Zhang *et al*. 2009, Xie *et al*. 2013, Zhao *et al*. 2020). In this work, nonanal, decanal, and 2-ethylhexanol were emitted in large quantities, both by *T. notata* and by *C. montrouzieri*, suggesting that they may be important for the communication of these ladybeetles.

Among the identified alcohols, α-terpineol was the major compound in male *C. montrouzieri* extracts. Electroantennogram studies revealed that males and females *Coleomegila maculata* (DeGeer) (Coleoptera: Coccinellidae) had a higher threshold response to this compound, which was also present in the volatiles of the host plant (Baker *et al*. 2003). Therefore, α-terpineol could also elicit a positive response in *C. montrouzieri* adults, and further electrophysiological and behavioral studies may look into that hypothesis.

Hydrocarbons are present in most insect species, but their qualitative and quantitative profiles are species and gender-specific (Howard & Blomquist 2005, Pattanayak *et al*. 2015, Fassotte *et al*. 2016). Tricosane, tetracosane, pentacosane, and heptacosane hydrocarbons have been related to the aggregation behavior of *H. axyridis* (Durieux *et al*. 2012). There are still no reports of aggregation behavior and sexual pheromones emitted by *T. notata* and *C. montrouzieri*. However, the presence of compounds related to the attraction of coccinellids and the behavioral responses of this study, such as the increase in oviposition in interactions with conspecifics of *T.*

notata and the increase in adult predation in conspecific interactions, raises the hypothesis that there is a recognition of conspecific volatiles by these species.

Although male *C. montrouzieri* emitted a greater amount of compounds than the others, the native species did not respond. This result confirms that insects respond to a specific concentration of odors, and higher concentrations may inhibit the response. (Bell & Cardé 1984, Xiu *et al*. 2019, Zhao *et al*. 2020).

In conclusion, Coccinellid adults search for prey, stay feeding, and reproduce in areas of high prey abundance (Pettersson *et al*. 2005, Pan *et al*. 2020). The presence of semiochemicals of conspecifics in the searching area can be an indication of prey availability, thus inducing the reproduction and predation rates of the ladybeetle species investigated in this research. In contrast, semiochemicals of heterospecifics indicate the presence of a potential competitor or intraguild predator in the same area. The introduced ladybeetle species *C. montrouzieri* was able to detect the semiochemicals of the native species *T. notata*, altering its developmental time and predation rate, whereas *T. notata* did not show any evident response to semiochemicals of *C. montrouzieri*. The information mediated by semiochemicals contribute to understand aspects related to the behavior and ecological interactions, such as competition and reproduction in insects (Pettersson 2012).

Based on our results, further studies can also investigate which of the compounds released by *T. notata* and *C. montrouzieri* have the potential of attracting individuals of the same (e.g., sex or aggregation pheromones), or different species (allelochemicals) in laboratory and field trials. The identification of such compound (s) could lead to the use of synthetic lures to attract and keep the ladybeetles in target areas to improve the biological control of mealybugs.

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Table 1. Mean (± SE) duration of development (from first instar to adult emergence) of the ladybeetles *T. notata* (T) and *C. montrouzieri* (C) subjected or not to volatiles released by conspecific and heterospecific individuals.

Focal species/ Paired treatment	Average (± SE) days	Statistics F_{df}. P
T. notata		
T unpaired	28.3 ± 0.44 a	$F_{2,159} = 0.07$. P = 0.92
TxT	28.5 ± 0.23 a	
TxC	28.5 ± 0.45 a	
C. montrouzieri		
C unpaired	24.6 ± 0.28 b	$F_{2,159} = 8.62$; P < 0.01
CxC	26 ± 0.33 a	
CxT	26.9 ± 0.29 a	

Means followed by the same lower-case letter within the column are not statistical difference among treatments in the column, by the F test ($\alpha = 0.05$).

Table 2. Fecundity (number of eggs) and fertility (egg viability) of the ladybeetles *T. notata* (T) and *C. montrouzieri* (C) subjected or not to volatiles released by conspecific and heterospecific individuals along 60 days observation period.

Focal species / Paired treatment	Average (± SE)	Statistics F_{df}. P	Average (± SE)	Statistics $F_{df;}$ P
T. notata	Fecundity		Fertility	
T unpaired	68.5 ± 14.99 b	$F_{2,59} = 6.93$;	56.6 ± 13.15 b	$F_{2,59} = 6.16$;
TxT	141.0 ± 11.69 a	P < 0.01	119.5 ±10.34 a	P < 0.01
TxC	90.9 ± 18.42 b		73.0 ± 15.80 b	
C. montrouzieri				
C unpaired	83.5 ± 23.10 a	$F_{2,59} = 0.46$;	55.8 ± 16.47 a	$F_{2,59} = 0.33$;
CxC	96.3 ± 15.41 a	P = 0.63	73.1 ± 13.32 a	P = 0.71
CxT	69.6 ± 24.55 a		53.8 ± 19.27 a	

Means followed by the same lower-case letter within the column are not statistical difference among treatments in the column, by the F test ($\alpha = 0.05$).

Table 3. Daily consumption (±SE) of *Ferrisia dasylirii* nymphs by larvae (LI-LIV) and adult males (M) and females (F) of the ladybeetles *Tenuisvalvae notata* (T) and *Cryptolaemus montrouzieri* (C) subjected or not to volatiles of conspecific and heterospecific.

Focal species/paired treatment	Average (±SE) nymphs preyed	Focal species/paired treatment	Average (±SE) nymphs preyed
T LI unpaired	0.7 ± 0.08 b	C LI unpaired	1.4 ± 0.11 b
T LI x T LI	1.1 ± 0.07 a	C LI x C LI	1.8 ± 0.06 a
T LI x C LI	1.2 ± 0.09 a	C LI x T LI	1.8 ± 0.11 a
Statistics	$F_{2,159} = 9.02$; $P < 0.01$	Statistics	$F_{2,159} = 4.62$; $P = 0.01$
T LII unpaired	1.7 ± 0.11 b	C LII unpaired	2.2 ± 0.14 b
T LII x T LII	2.0 ± 0.07 b	C LII x C LII	2.3 ± 0.13 b
T LII x C LII	2.3 ± 0.10 a	C LII x T LII	3.5 ± 0.18 a
Statistics	$F_{2,159} = 6.51$; $P < 0.01$	Statistics	$F_{2,159} = 17.87$; $P < 0.01$
T LIII unpaired	2.3 ± 0.13 a	C LIII unpaired	2.6 ± 0.11 a
T LIII x T LIII	1.9 ± 0.08 b	C LIII x C LIII	2.5 ± 0.08 a
T LIII x C LIII	2.3 ± 0.12 a	C LIII x T LIII	2.7 ± 0.13 a
Statistics	$F_{2,159} = 3.98$; $P = 0.02$	Statistics	$F_{2,159} = 0.41$; $P = 0.66$
T LIV unpaired	2.7 ± 0.10 a	C LIV unpaired	3.6 ± 0.14 a
T LIV x T LIV	2.8 ± 0.08 a	C LIV x C LIV	3.9 ± 0.10 a
T LIVx C LIV	3.0 ± 0.11 a	C LIVx T LIV	4.0 ± 0.16 a
Statistics	$F_{2,159} = 1.21$; $P = 0.29$	Statistics	$F_{2,159} = 1.53$; $P = 0.21$
TF unpaired	1.4 ± 0.10 b	CF unpaired	1.7 ± 0.09 c
TF x TF	2.0 ± 0.09 a	CF x CF	3.3 ± 0.10 a
TF x TM	1.5 ± 0.13 b	CF x CM	1.5 ± 0.13 c
TF x CF	1.8 ± 0.12 a	CF x TF	2.4 ± 0.11 b
TF x CM	1.6 ± 0.09 b	CF x TM	2.9 ± 0.15 ab
Statistics	$F_{4,239} = 4.35$; $P < 0.01$	Statistics	$F_{4,239} = 40.85$; $P < 0.01$
TM unpaired	1.1 ± 0.09 b	CM unpaired	2.0 ± 0.12 a
TM x TM	1.6 ± 0.11 a	CM x CM	2.2 ± 0.12 a
TM x TF	1.2 ± 0.13 b	CM x CF	1.2 ± 0.13 c
TM x CF	1.2 ± 0.10 b	CM x TF	1.9 ± 0.13 a
TM x CM	0.9 ± 0.08 b	CM x TM	1.7 ± 0.12 ab
Statistics	$F_{4,239} = 8.23$; $P < 0.01$	Statistics	$F_{4,239 =} 9.35$; $P < 0.01$

Means followed by the same lower-case letter within the column are not statistically different by the F test ($\alpha = 0.05\%$).

2288 Table 4: Mean (±SE) amount and retention index (RI) calculated using a DB-5MS column of volatile organic compounds

2289 (µg/insect), collected from 24 hours aeration of the adult ladybeetles *Tenuisvalvae notata* (T) and *Cryptolaemus montrouzieri* (C).

Compounds	RI	T male	T female	χ^2; P	C male	C female	χ^2; P
Monoterpenes							
α-Pinene*	937	12.0 ± 6.50 a	18.8 ± 7.77 a	χ^2 = 0.40; P = 0.52	19.7 ± 6.42 a	11.7 ± 3.09 a	χ^2 = 1.52; P = 0.21
β-Pinene*	979	15.6 ± 6.49 a	34 ± 14.97 a	χ^2 =1.63; P = 0.20	26.1 ± 8.86 a	11.8 ± 2.94 a	χ^2 = 3.47; P = 0.06
Myrcene*	992	15.6 ± 9.18 a	21.1 ± 11.20 a	χ^2 = 0.14; P = 0.70	----------	----------	----------
Terpinolene	1090	1.6 ± 1.60 a	0.8 ± 0.80 a	χ^2 = 0.19; P = 0.65	4.0 ± 1.51 a	1.5 ± 0.95 a	χ^2 = 1.73; P = 0.18
Esters							
Methyl-9-oxononanoate	1430	0.5 ± 0.56	----------	----------	3.5 ± 2.36	----------	----------
Isopropyl hexadecanoate	2025	4.2 ± 1.23 a	2.2 ± 1.43 a	χ^2 = 0.87; P = 0.34	9.4 ± 4.03 a	5.5 ± 1.94a	χ^2 = 0.91; P = 0.33
Methyl-9-Octadecenoate	2102	2.6 ± 0.89 b	11.6 ± 6.85 a	χ^2 = 4.95; P = 0.02	15.0 ± 3.18 a	7.0 ± 1.71 b	χ^2 = 5.29; P = 0.02
Hydrocarbons							
2,4-Dimethyl-1-heptene	846	17.0 ± 9.77 a	15.7 ± 11.13 a	χ^2 = 0.007; P = 0.93	24.1 ± 9.87 a	13.1 ± 3.74 a	χ^2 = 1.47; P = 0.22
Nonane*	900	9.1 ± 5.01 a	15.4 ± 14.98 a	χ^2 = 0.24; P = 0.62	16.6 ± 6.52 a	14.5 ± 5.21 a	χ^2 = 0.06; P = 0.79
1,2-Dichlorobenzene	1011	12.0 ± 4.32 a	25.1 ± 11.94 a	χ^2 = 1.60; P = 0.20	----------	----------	----------
Undecane*	1098	10.7 ± 6.19 a	11.2 ± 7.76 a	χ^2= 0.002; P = 0.96	25.8 ± 7.41 a	12.2 ± 3.25 a	χ^2 = 3.58; P = 0.05
Dodecane*	1200	10.2 ± 5.36 a	25.2 ± 13.38 a	χ^2 = 1.42; P = 0.23	32.8 ±8.15 a	17.7 ± 4.23 a	χ^2 = 2.68; P = 0.10
Tetradecane*	1400	7.2 ± 4.05 a	10.1 ± 6.48 a	χ^2 = 0.15; P = 0.68	19.1 ± 5.86 a	10.4 ± 3.45 a	χ^2 = 1.77; P = 0.18
Hexadecane*	1600	----------	----------	----------	107.1 ± 66.61 a	17.7 ± 4.23 b	χ^2 = 6.46; P = 0.01
Aldehydes							
Hexanal*	805	34.1 ±10.07 a	32.0 ±21.44 a	χ^2 = 0.008; P = 0.92	80.8 ± 31.94 a	22.8 ± 16.02 a	χ^2 = 2.31; P = 0.12
Heptanal	903	29.5 ±14.54 a	3.3 ±2.06 b	χ^2 = 6.30; P = 0.01	10.3 ± 4.94 a	5.2 ± 2.76 a	χ^2 = 0.89; P = 0.34
(*E*.*E*)-2,4-Nonadienal	999	----------	----------	----------	6.7 ± 2.05 a	2.9 ± 1.09 a	χ^2 = 2.85; P = 0.09
Octanal*	1003	6.7 ± 4.69 a	6.7 ± 2.38 a	χ^2 < 0.02; P = 0.99	19.7 ± 3.69 a	12.0 ± 2.97 a	χ^2 = 2.53; P = 0.11
Nonanal	1102	35.8 ±17.99 a	131.9 ±51.61 a	χ^2 = 3.81; P = 0.05	108.9 ± 26.44 a	35.8 ±15.68 b	χ^2 = 4.70; P = 0.03
Decanal	1205	19.8 ± 6.99 b	92.2 ±36.60 a	χ^2 = 8.04; P < 0.01	60.3 ±8.89 a	40.3 ± 9.48 a	χ^2 = 2.09; P = 0.14
Undecanal	1306	1.8 ± 1.20	----------	----------	30.8 ± 9.94 a	20.9 ± 5.71 a	χ^2 =0.83; P = 0.36
Dodecanal	1401	3.2 ± 2.12 a	15.3 ± 9.79 a	χ^2 = 2.71; P = 0.09	8.3 ±3.32 a	0.8 ± 0.87 a	χ^2 = 3.70; P = 0.05
Tridecanal	1511	0.3 ± 0.35 b	15.5 ± 5.44 a	χ^2 = 7.89; P < 0.01	14.4 ± 6.11 a	8.8 ± 3.06 a	χ^2 = 0.80; P = 0.37
Tetradecanal	1613	10.0 ± 4.14 a	4.5 ± 2.95 a	χ^2 = 1.08; P = 0.29	----------	----------	----------

Hexadecanal	1819	18.6 ± 4.67 a	31.1 ± 8.10 a	$\chi^2 = 1.99$; P = 0.15	----------	----------	----------
Octadecanal	1921	6.0 ± 2.37 a	3.2 ± 3.26 a	$\chi^2 = 0.36$; P = 0.54	----------	----------	----------
Alcohols							
(*Z*)-3-Hexanol*	801	7.3 ± 0.76 b	16.1 ± 5.54 a	$\chi^2 = 5.99$; P = 0.01	7.3 ± 2.83 a	7.7 ± 1.10 a	$\chi^2 = 0.01$; P = 0.91
2,2-Dimethyl pentanol	962	4.7 ± 3.39	----------	----------	----------	----------	----------
Heptanol	972	----------	----------	----------	1.6 ± 0.75 a	1.9 ± 0.89 a	$\chi^2 = 0.09$; P = 0.75
2-Ethylhexanol	1031	50.8 ± 27.59 a	65.7 ± 52.75 a	$\chi^2 = 0.07$; P = 0.78	121.6 ± 39.24 a	80.8 ± 20.11 a	$\chi^2 = 0.99$; P = 0.31
Benzyl alcohol*	1037	1.4 ± 1.42 a	8.5 ± 8.53 a	$\chi^2 = 1.46$; P = 0.22	1.2 ± 0.97 a	8.3 ± 5.59 a	$\chi^2 = 3.01$; P = 0.08
Octanol*	1072	----------	----------	----------	4.8 ± 2.29 a	0.8 ± 0.81 a	$\chi^2 = 2.33$; P = 0.12
2,7-Dimethyl octanol	1077	----------	----------	----------	8.1 ± 2.67 a	3.4 ±1.29 a	$\chi^2 = 2.88$; P = 0.08
α-Terpineol	1193	----------	----------	----------	124.5 ± 41.94 a	8.6 ± 6.29 b	$\chi^2 = 8.80$; P < 0.01
Phenoxy-2-propanol	1248	----------	----------	----------	12.7 ± 8.24 a	1.4 ± 1.48 a	$\chi^2 = 2.78$; P = 0.09
Hexadecanol	1882	1.6 ± 1.39 b	33.9 ± 33.98 a	$\chi^2 = 4.41$; P = 0.03	----------	----------	----------
Octadecanol	2085	2.9 ± 2.30 a	30.6 ± 30.66 a	$\chi^2 = 3.08$; P = 0.07	4.4 ± 3.64 a	2.2± 2.27 a	$\chi^2 = 0.26$; P = 0.60
Ketones							
3-Hexanone	785	6.5 ± 2.37 a	3.0 ± 1.94 a	$\chi^2 = 1.13$; P = 0.29	6.7 ± 2.18 a	2.6 ± 1.33 a	$\chi^2 = 2.35$; P = 0.12
2-Hexanone*	786	1.0 ± 1.01 a	2.3 ± 1.41 a	$\chi^2 = 0.43$; P = 0.50	2.6 ± 1.37 a	2.4 ± 1.50 a	$\chi^2 = 0.15$; P = 0.90
6-Methyl-5-hepten-2-one*	989	6.6 ± 5.20 a	3.3 ± 3.38 a	$\chi^2 = 0.27$; P = 0.59	14.6 ± 3.94 a	9.4 ± 3.17 a	$\chi^2 = 1.01$; P = 0.31
Aromatics							
Benzaldehyde*	964	6.1 ± 4.58	----------	----------	5.4 ± 3.14 a	2.5 ± 1.25 a	$\chi^2 = 0.95$; P = 0.32
Benzothiazole*	1228	----------	----------	----------	3.9 ± 3.25	----------	----------

Means within a row followed by the same letter are not significantly different. *Identified by authentic standards.

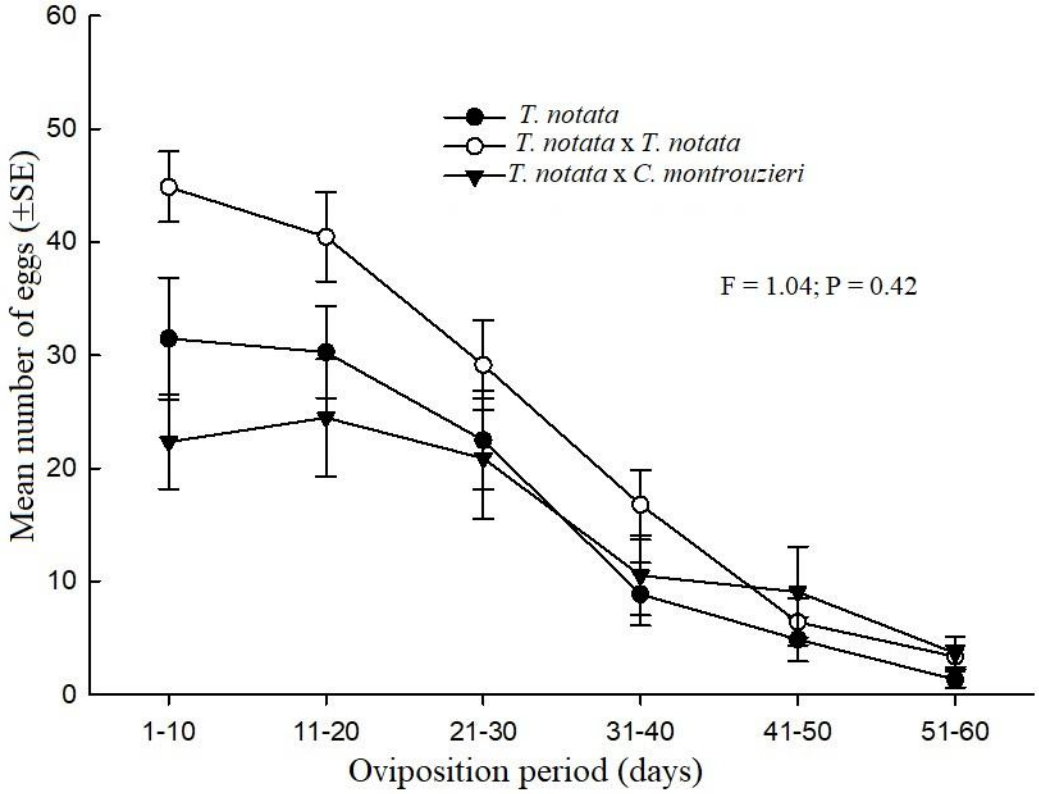
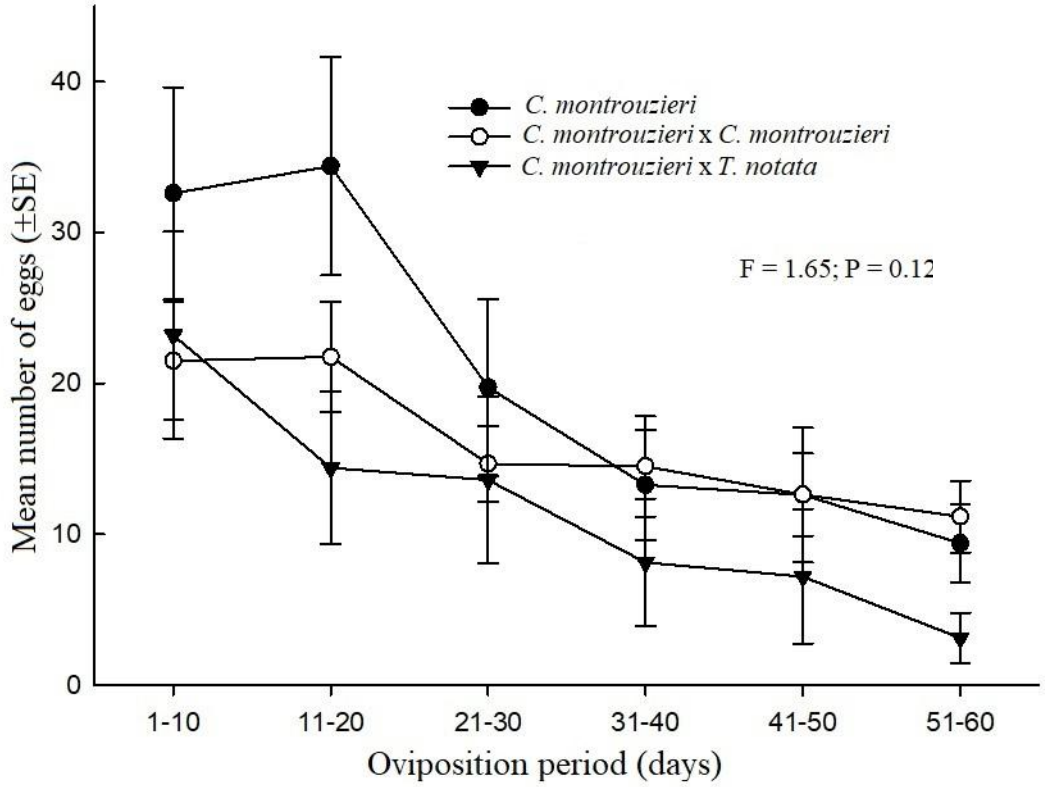


Figure 1. Average (± SE) oviposition by females *Tenuisvalvae notata* subjected to volatiles of conspecific and heterospecific (*Cryptolaemus montrouzieri*) individuals during a 60 days observation period.



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2305 Figure 2. Average (±SE) oviposition by females *Cryptolaemus montrouzieri* subjected to volatiles

2306 of conspecific and heterospecific (*Tenuisvalvae notata*) individuals along 60 days observation

2307 period.

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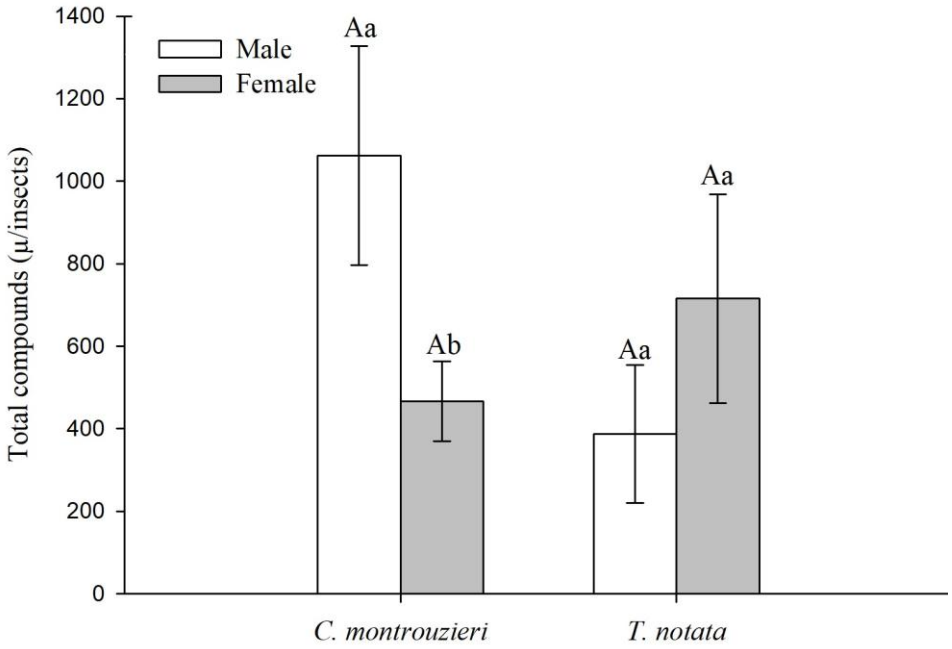

Figure 3. Total amount of volatiles quantified in extracts of *Cryptolaemus montrouzieri* and *Tenuisvalvae notata* males and females. Uppercase letters indicate significant difference between species and lowercase between genders.

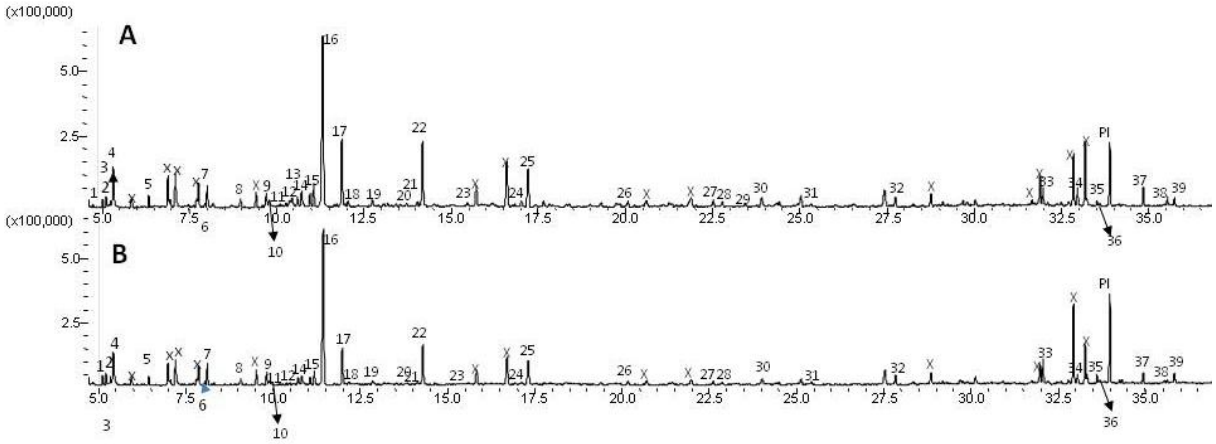

Figure 4: Chromatographic profile of volatile hexanic extracts of *Tenuisvalvae notata* females (A) and males (B). Compounds: 1) (*Z*)-3-hexanol, 2-hexanone, 3) 3-hexanone, 4) hexanal, 5) 2,4 dimethyl-1-heptene, 6) nonane, 7) heptanal, 8) α-pinene, 9) not identified, 9) 2,2-dimethyl pentanol, 10) benzaldeyde, 11) β-pinene, 12) hexadecanoic acid, 13) 6-methyl-5-hepten-2-one, 14) mircene, 15) octanal, 16) 1,2 dichlorobenzene, 17) 2-ethyl-hexanol, 18) benzyl alcohol, 20) terpinolene, 21) undecane, 22) nonanal, 24) dodecane, 25) decanal, 26) undecanal, 27) tetradecane, 28) dodecanal, 29) methyl -9-oxononanoate, 30) not identified, 31) undecanal, 32) tetradecanal, 33) hexadecanal, 34) heptadecanal, 35) octadecanal, 36) methyl hexadecanoate, 37) isopropyl hexadecanoate, 38) octadecanol, 39) methyl-9-octadecenoate.

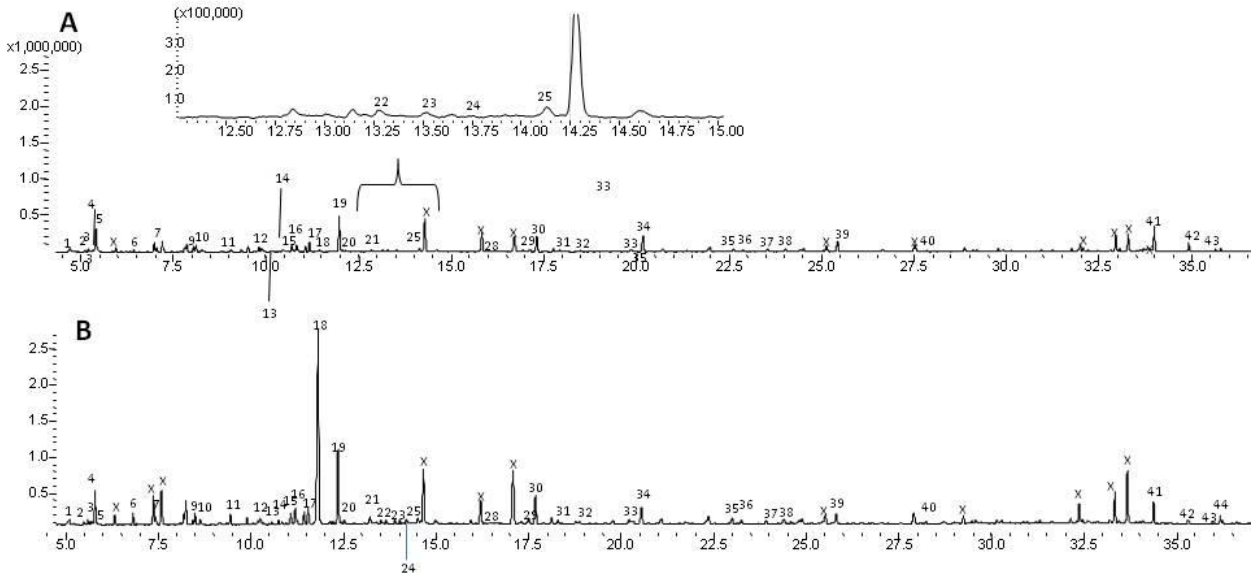

Figure 5: Chromatographic profile of volatile hexanic extracts of *Cryptolaemus montrouzieri* females (A) and males (B). Compounds: 1) not identified, 2) 3-hexanone, 3) 2-hexanone, 4) (*Z*)-3-hexanol, 5) hexanal, 6) 2,4 dimethyl-1-heptene, 7) not identified, 8) (*E*)-2-nonenal, 9) nonane, 10) heptanal, 11) α-pinene, 12) benzaldehyde, 13) heptanol, 14) β-pinene, 15) 6-methyl-5-hepten-2-one, 16) (*E,E*) – 2,4-nonadienal, 17) octanal, 18) not identified, 19) 2-ethyl hexanol, 20) benzyl alcohol, 21) not identified, 22) octanol, 23) 2,7 dimethyl octanol, 24) terpinolene, 25) undecane, 26) nonanal , 28) α-terpineol, 29) dodecane, 30) decanal, 31) benzothiazole, 32) phenoxy-2-propanol, 33) tridecane, 34) undecanal, 35) tetradecane, 36) dodecanal, 37) methyl-9-oxononanoate, 39) tridecanal, 40) hexadecanal, 42) isopropyl hexadecanoate, 43) octadecanol, 44) methyl-9-octadecenoate, x) not identified.

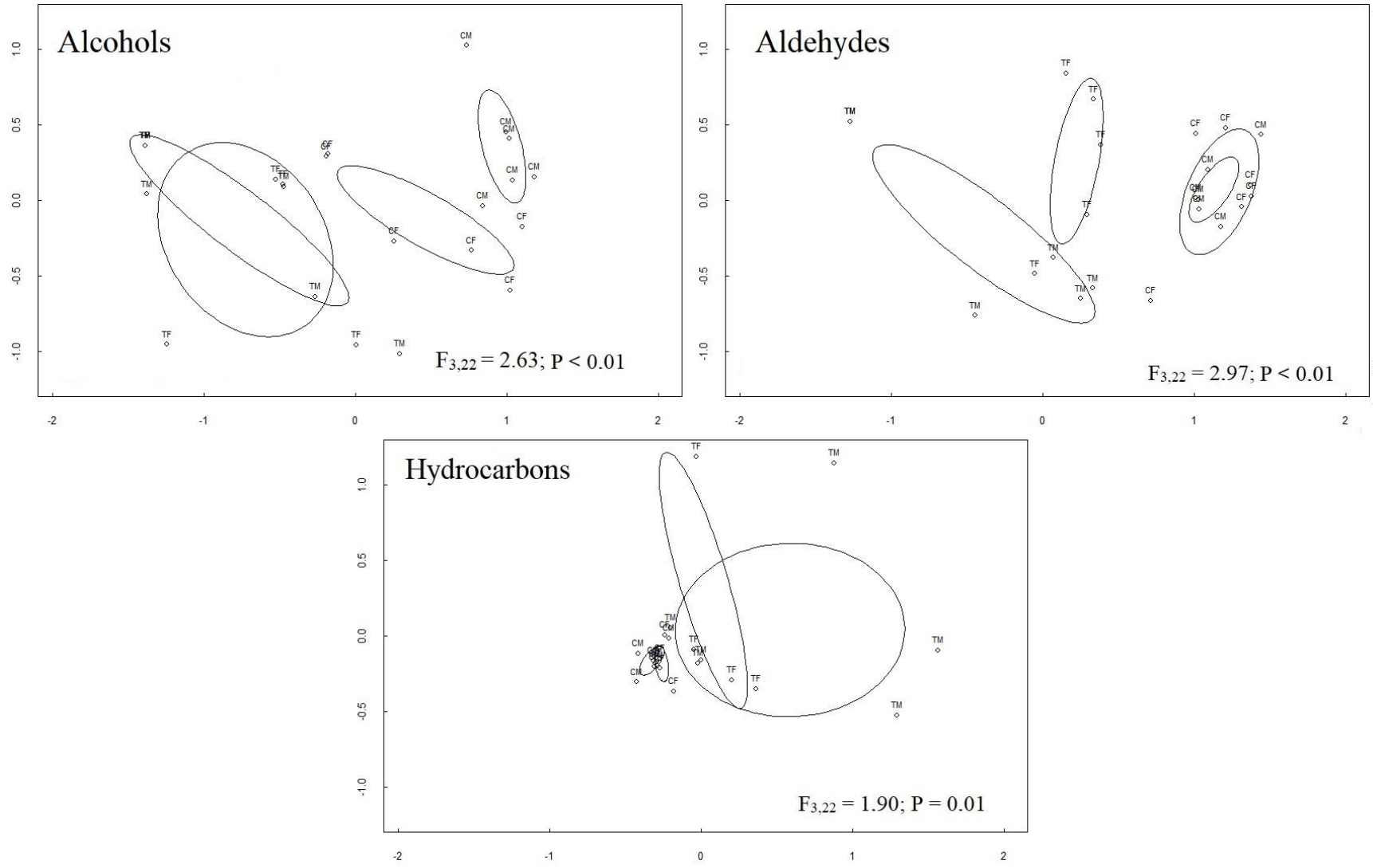

Figure 6: Cluster analysis of hydrocarbons, aldehydes and alcohols from female (F) and males (M) *Tenuisvalvae notata* (T) and *Cryptolaemus montrouzieri* (C).

CAPÍTULO 4

CONSIDERAÇÕES FINAIS

O controle biológico utilizando joaninhas predadoras representa alto potencial para manter as populações de diversas pragas sob controle, uma vez que estas podem reduzir a população de cochonilhas, pulgões, psilídeos, moscas-brancas, algumas pequenas lagartas, entre outras presas. Entre as espécies de joaninhas importantes nos agroecossistemas do Brasil estão *Tenuisvalvae notata* (Mulsant) e *Cryptolaemus montrouzieri* Mulsant, sendo *T. notata* pouco utilizada em programas aplicados de controle biológico com maior contribuição no controle natural, devido à falta de tecnologia e conhecimento básico para criações massais dessa espécie. Por outro lado, *C. montrouzieri* já é amplamente conhecida, estudada e produzida massalmente, tendo sido empregada em vários locais em programas de controle biológico clássico.

Para introduzir uma espécie de coccinelídeo em um ambiente é necessário conhecer aspectos de sua biologia e ecologia, e assim definir como a presença desses indivíduos pode alterar a constituição do ambiente. O comportamento de forrageamento é um importante aspecto ecológico na interação das joaninhas. O nosso estudo constatou que os rastros e voláteis emitidos por *C. montrouzieri* e *T. notata* afetaram o comportamento predatório dessas espécies, seus rastros alteraram o forrageamento delas e seus voláteis influenciaram no tempo de desenvolvimento e na reprodução.

Apesar de *T. notata* ter evadido das áreas com rastros de *C. montrouzieri* enquanto a espécie introduzida foi atraída pelas áreas com rastros de *T. notata*, as respostas mais evidentes foram encontradas em interações com coespecíficos. Os semioquímicos coespecíficos aumentaram a taxa de predação das duas espécies e aumentaram a oviposição de *T. notata*. Diante disso,

bioensaios com frações dos compostos podem ser feitos para confirmar a atratividade dessas joaninhas para áreas com esses extratos. Além disso, a eletroantenografia com esses extratos poderiam identificar para quais compostos as antenas das joaninhas apresentam receptores de odor.

Alguns dos compostos identificados neste estudo foram atrativos para outras espécies de coccinelídeos. Assim, bioensaios com os compostos sintéticos individuais poderiam revelar qual, ou quais compostos estão desencadeando os comportamentos observados. A partir dai, testes de campo podem definir substâncias atrativas para essas joaninhas, o que irá favorecer o controle biológico das cochonilhas.

O perfil químico dos voláteis e rastros das duas espécies foi específico para cada espécie e gênero. Diante disso, a identificação química dos voláteis e rastros poderia ser uma importante ferramenta para estudos quimiotaxonômicos dessas espécies.

Apesar das joaninhas coccidófagas serem importantes agentes do controle biológico de cochonilhas, as pesquisas envolvendo suas interações mediadas por semioquímicos ainda são escassas. As coccidófagas alvo dessa pesquisa, *T. notata* e *C. montrouzieri*, podem ocorrer simultaneamente em uma mesma área e competir por presas, entretanto, ainda não há relatos da influência da comunicação química entre essas espécies. Os resultados obtidos nessa pesquisa podem contribuir com futuras pesquisas de desenvolvimento de compostos que possam manter ou atrair essas joaninhas predadoras em campo, visando o controle biológico de cochonilhas.