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FLEBOTOMÍNEOS (PSYCHODIDAE: PHLEBOTOMINAE) EM PERIDOMICÍLIOS RURAIS: COMO FATORES AMBIENTAIS, ANIMAIS E LIMPEZA DO PERIDOMICÍLIO INFLUENCIAM NA ABUNDANCIA DESSES DÍPTEROS

GUSTAVO ALMEIDA BRITO

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Dissertação apresentada ao Programa de Pós-Graduação em Biodiversidade e Conservação da Universidade Federal do Maranhão, como requisito parcial para obtenção do título de Mestre em Biodiversidade e Conservação.

Orientador: Prof. Dr. José Manuel Macário Rebêlo.

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Aprovada em / /

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Sinto-me como o coiote atrás do papa-léguas, talvez eu nunca consiga pegá-lo, mas jamais desistirei.

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SUMÁRIO

LISTA DE SIGLA E ABREVIATURAS

A	Absence
AIC	Akaike information criteria
An	Animals
C	Cleaning
CDC	Control disease center
DDT	Diclorodifeniltricloroetano
Dist. water	Trap distance to water bodies
GLMM	Generalized linear mixed models
HP	Hoover Pugedo
IBGE	Instituto Brasileiro de geografia e estatística
L.	*Lutzomyia*
LEDs	Lights emitting diode
WHO	World Health Organization
P	Presence
SINAN	Sistema de informação de agravos de notificação
VEG. 50	Vegetation cover in the 50 m radii zone around trap

RESUMO

A propagação das leishmanioses é um problema mundial que afeta mais de 80 países. Uma das formas de combate à doença é o controle do vetor (flebotomíneo). Para entender como a abundância desse díptero é influenciada por variáveis ambientais, pela limpeza do peridomicílio e por animais domésticos ao redor das residências rurais fez-se um estudo na região do Parque Nacional dos Lençóis Maranhense. Foram realizadas coletas em 112 peridomicílios rurais com armadilha CDC. Como resultados foram capturados 3.169 flebotomíneos de 11 espécies. As variáveis que aumentaram a abundância de flebotomíneos foram: a presença de animais, a proximidade aos corpos d'água, a pouca cobertura da vegetação. A limpeza do peridomicílio contribuiu para abundancia de machos de flebotomíneos. Além disso, três animais contribuíram para a atração desses dípteros para o peridomicílio: galinhas, equinos e suínos.

Palavras-chave: Inseto vetor; Controle de pragas; Manejo ambiental.

ABSTRACT

The spread of leishamniasis is a worldwide problem that affects more than 80 countries. One way to combat the disease is by controlling the vector (Phlebotomine). To understand how the abundance of this diptera is influenced by environmental variables, by the cleanness of the peridomicile and by domestic animals around the rural residences. There were collected samples in 112 rural peridomiciles with a CDC trap in cities that are part of the Lençóis Maranhenses National Park. As a result, 3169 phlebotomines were captured and they were from 11 species. The variables that increased the abundance of phlebotomines were: animals presence, bodies of water proximity, and low vegetation cover. The cleaning of the peridomicile contributed to the abundance of males phlebotomines. Besides, three animals contributed to the inducement of these diptera to the peridomicile: chickens, horses and pigs.

Key-words: Vector; Ecology; Control; Environmental management.

CAPÍTULO I

1.1 APRESENTAÇÃO

Os flebotomíneos são pequenos dípteros responsáveis pela disseminação de protozoários do gênero *Leishmania* em várias partes do mundo (WHO, 2010). No Brasil, anualmente milhares de novos casos são reportados pela vigilância sanitária (SINAN 2016). Nesse contexto, o Maranhão se destaca negativamente como um dos estados brasileiros com maior número de casos de leishmaniose tegumentar e visceral (SINAN 2016). Isso se deve à complexidade de interações entre parasitos, hospedeiros e vetores (WHO, 2010). Como exemplo, no estado do Maranhão, existem oito espécies de flebotomíneos vetores e, pelo menos, quatro espécies de *Leishmania* circulando na população (MINISTÉRIO DA SAÚDE DO BRASIL, 2007).

Para combater as leishmanioses é necessária a integração de várias práticas: tratamento de casos clínicos humanos, combate ao vetor e controle de reservatórios (WHO, 2010). O vetor é o foco dessa dissertação, que muito embora seja bastante estudado, ainda é de difícil controle. A prática mais usada no mundo para o combate do vetor é o uso de várias famílias de inseticidas (WHO, 2010). No entanto, essa prática é muito árdua, pois exige grande investimento e uma equipe grande trabalhando por um longo período de tempo (ALEXANDER; MAROLI, 2003). Quando essas práticas falham, ocorrem resistências dos flebotomíneos aos inseticidas, que atualmente é o maior problema para o combate do vetor (ALEXANDER et al., 2009).

Pensando nessa problemática, testamos uma forma de combate ao vetor pouco estudada: a limpeza do peridomicílio. Apesar de não ter sua eficácia comprovada é uma prática recomendada pela Organização Mundial da Saúde. Essa forma de combate é de baixo custo e consiste em práticas já realizadas pela população ao redor de suas residências. Contudo, não podemos excluir do contexto o papel que os animais exercem na atração do vetor ao peridomicílio. Por esse motivo consideramos, no presente estudo, a presença de animais domésticos como coadjuvantes ao manejo ambiental no controle vetorial das leishmanioses.

Dentre os animais domésticos, o cão é o mais importante para a epidemiologia da doença, pois é considerado o principal reservatório de *Leishmania infantum chagasi* (WHO, 2010). No entanto, este não é o animal mais abundante ao redor das residências rurais (ALEXANDER ET AL. 2002). Nesse sentido foi considerada a abundância de cada animal no

peridomicílio, com o intuito de entender como diferentes animais influenciam na abundância dos flebotomíneos nos peridomicílios.

Nesse contexto, também não podem ser ignorados fatores inerente à paisagem onde esses peridomicílios estão inseridos. Assim, foi incluída, baseada em trabalhos realizados em peridomicílios urbanos, a distância dos corpos d'água ao peridomicílio e também a cobertura vegetal desses peridomicílios (FERNÁNDEZ et al., 2010; SANTINI et al., 2015,). Assim, a presente dissertação relata dados científicos originais sobre os fatores que influenciam a abundância de flebotomíneos em peridomicílios rurais, permitindo um melhor delineamento de futuras estratégias de combate ao vetor.

O estudo foi realizado em dois municípios que fazem parte do Parque Nacional dos Lençóis Maranhenses. O parque é um polo turístico e recebe milhares de pessoas de várias regiões do mundo todos os anos. Por esse motivo, se torna um importante local de estudo, pois existe um perigo de contágio pela leishmaniose tegumentar e visceral, que são endêmicas da região (SINAN, 2016).

Essa dissertação foi dividida em dois capítulos. O primeiro trata-se do Referencial Teórico que é uma revisão sobre os aspectos bioecológicos dos flebotomíneos, situação epidemiológica das leishmanioses e avanços sobre o controle vetorial dessas importantes doenças. Além disso, vem relatando as lacunas de conhecimento que servem como base para o desenvolvimento da pesquisa científica.

Na segunda parte apresenta-se o manuscrito com os principais resultados dessa dissertação. Este contém dados originais sobre a ecologia de flebotomíneos, que podem servir para determinar formas de controle das leishmanioses no Brasil e em outras partes do mundo. O manuscrito tem o seguinte título: *Factors Associated With Sand Fly (Diptera: Psychodidae) Abundance in Rural Villages in Brazil*. Este foi enviado para publicação na revista Journal of Vector Ecology, cujas normas de submissão encontram-se anexadas no final deste documento.

1.2 REVISÃO BIBLIOGRÁFICA

Os flebotomíneos são pequenos dípteros bastante diversificados, sendo catalogadas e consideradas como válidas 988 espécies e subespécies, com 27 espécies fósseis, distribuídas em todas as regiões do globo, exceto na Antártida (SECCOMBE et al., 1993; YOUNG; DUNCAN, 1994; BATES et al., 2015).

Esses pequenos dípteros são holometábolos, incluídos na Superordem Endopterygota e possuem seu ciclo de vida dividido em quatro fases: ovo, larva, pupa e adulto (FORATTINI, 1973). A biologia de cada espécie é complexa, não havendo como resumir todos os aspectos da reprodução, alimentação, dispersão e outras atividades (WHO, 2010). Além disso, há dificuldade de se encontrar as três primeiras fases em ambientes naturais (ALEXANDER; MAROLI, 2003). Mas sabe-se que o período na fase de ovo varia de dois a treze dias, em condições de laboratório (BELEN; ALTEN, 2006; WHO, 2010). Na natureza esse tempo pode ser maior, tendo em vista que espécies de regiões quentes e úmidas podem entrar em diapausa (WHO, 2010).

Os ovos são depositados em vários microhabitats como: vários tipos de solos, cupinzeiros, ninhos de aves, tocas de animais, abrigo de animais, serapilheira, ocos e base de árvores e sob rochas (HANSON, 1961; FERRO et al., 1997; FELICIANGELI, 2004; WHO, 2010; CASANOVA et al., 2013). Nos ambientes modificados pelo o homem, podem ser depositados embaixo de armazenagem de água, perto de pocilgas, plantações, rachaduras do solo e de paredes, abrigos de animais domésticos e solo de galpão (FERRO et al., 1997; FELICIANGELI, 2004; CASANOVA et al., 2013).

Quando estão na fase larval, utilizam como principal alimento a matéria orgânica em decomposição presente nos locais de ovipostura (FERRO et al., 1997; CASANOVA, 2001). Em laboratório, essa fase dura pelo menos 25 dias (BELEN; ALTEN, 2006). No entanto, algumas espécies podem hibernar no quarto insta da fase larvar (FORATTINI, 1973) podendo chegar até 81 dias (BELEN; ALTEN, 2006; WHO, 2010).

Os adultos têm uma vida curta, cerca de 30 dias (em laboratório) (BRAZIL; BRAZIL, 2003; BELEN; ALTEN, 2006). Nessa fase, se alimentam de açucares das plantas, provindos de frutas, néctar ou extraído direto de tecidos (seiva) (HAMILTON; NAIEM, 2000; MÜLLER; SCHLEIN, 2005; JUNNILA; MÜLLER; SCHLEIN, 2011). No entanto, as fêmeas

necessitam também de sangue de vertebrados para a maturação dos ovos (WARBURG; FAIMAN, 2011). Neste ato, 93 espécies podem transmitir tripanosomatídeos do gênero *Leishmania*, causadoras de patologias (leishmanioses) em animais e aos seres humanos (WHO, 2010).

Na hematofagia, os flebotomíneos se alimentam de sangue de répteis, aves e mamíferos (QUINNELL; DYE; SHAW, 1992; MORRISON et al., 1993). Destes, se destacam dois grupos importantes: a) animais silvestres reservatórios naturais de *Leishmania*, que abrange roedores, marsupiais, preguiças e carnívoros (WHO, 2010); b) animais que atraem flebotomíneos para próximo das residências humanas – os sinantrópicos e os domésticos (WARBURG; FAIMAN, 2011).

Para o sucesso do repasto sanguíneo, os flebotomíneos têm uma gama de receptores que auxiliam na detecção do animal. Estes são: visuais (olhos compostos) (FORATTINI, 1973), detector de calor (BRAZIL; BRAZIL, 2003), olfativos (HAMILTON; RAMSOONDAR, 1994) e químicos que capturam dióxido de carbono e cairomônios liberados pelos vertebrados (SCHLEIN; JACOBSON, 2000; 2008). Esses receptores levam tanto o macho, quanto a fêmea para a fonte alimentar sanguínea. Esses receptores têm grandes influencias na dispersão dos flebotomíneos.

O raio de dispersão é um fator fundamental para a transmissão de parasitos. Trabalhos realizados no Velho Mundo encontraram um raio de dispersão variando de 200 a 2200 metros (QUATE, 1964; FOSTER, 1972; DOHA et al., 1991). No Novo Mundo os flebotomíneos raramente voam mais que 300 metros (CASANOVA; COSTA; NATAL, 2005; SILVA et al., 2013; DE OLIVEIRA et al., 2013). No entanto, a espécie *Lutzomyia longipalpis*, espécie incriminada como vetor de leishmaniose visceral na América do sul, parece ter um raio de dispersão maior, chegando a quase um quilometro (DYE; DAVIES; LAINSON, 1991; MORRISON et al., 1993). Essas informações podem ser utilizadas para avaliar a taxa de disseminação da *Leishmania* pelo vetor (CASANOVA; COSTA; NATAL, 2005).

O ciclo da leishmaniose é dividido em dois: o silvestre e o rural/urbano. O primeiro ocorre nos ambientes naturais que vêm sendo modificados pela interferência humana (VALDERRAMA; TAVERES; ANDRADE FILHO, 2011; RAMOS et al., 2014; TRUPPEL et al., 2014). Com isso, nasce segundo ciclo, que é favorecido pelas características das

comunidades humanas. Nesse contexto, as características da paisagem, principalmente a presença de arvores frutífera e a cobertura vegetal pode está relacionada com a abundância do vetor (FERNÁNDEZ et al., 2010). É o que acontece com *L. longipalpis* em ambientes urbanos na América do sul (FERNÁNDEZ et al., 2010; 2013; OLIVEIRA et al., 2012; ANDRADE et al., 2014). Esses fatores contribuem com uma manutenção da umidade local, alimentos, abrigos e locais de oviposturas para as fêmeas (FERNÁNDEZ et al., 2013; ANDRADE et al., 2014). No entanto, a espécie *L. whitmani* (responsável pela propagação da leishmaniose tegumentar) não teve a mesma relação (ZEILHOFER et al., 2008). Isso mostra que cada espécie tem características únicas. Além disso, esse fator foi estudado exclusivamente em ambientes urbanos, faltando ainda estudos em ambientes naturais e rurais.

Outro fator da paisagem, que influencia a abundância e riqueza de flebotomíneos em ambientes urbanos, é a distância dos corpos d'água (SANTINI et al., 2015). Que pode contribuir com umidade, matéria orgânica e temperaturas mais amenas (NAIMAN; DECAMPS, 1997). Fatores fundamentais para o desenvolvimento larval (FERRO et al., 1997; CASANOVA, 2001; FELICIANGELI, 2004; LAINSON; RANGEL, 2005). Além disso, apresentam maior abundância de animais que servem como fonte alimentar sanguínea (MACDONALD et al., 2006). Este fator também não foi estudado em ambientes rurais e naturais.

Outra característica das populações humanas é a criação de animais nos ambientes peridomésticos. Essa prática também contribui para a abundância de flebotomíneos, pois torna os animais fonte fixa de sangue (NEITZKE-ABREU et al., 2012; RAMOS et al., 2014), além de contribuir com locais de ovipostura (RAMOS et al., 2014). Nesse contexto, os cães se destacam, por serem incriminados como reservatórios (WHO, 2010). Como vimos todos esses fatores previamente apresentados têm implicações na ecologia dos flebotomíneos e na epidemiologia das leishmanioses.

Estas são doenças que acometem cerca de dois milhões de pessoa por ano em todo mundo. É endêmica de 98 países, com mais de 350 milhões de pessoas em área de risco (WHO, 2010). Áreas onde a pobreza é mais acentuada têm maiores riscos de contraí-la. As condições sanitárias precárias das habitações e do peridomicílio, aumentam os sítios de reprodução e locais de repouso, bem como o seu acesso dos vetores aos seres humanos (AMÓRA et al., 2009; WHO, 2010). Com tais conhecimentos, a organização Mundial da

Saúde recomenda três formas de controle das leishmanioses: tratamento dos casos humanos utilizando fármacos, controle de animais reservatórios e controle de insetos vetores (WHO, 2010).

Para o tratamento de casos humanos é utilizado alguns fármacos como: Antimoniato de N-metil glucamina, Desoxicolato de anfotericina B e Anforexina B lipossomal (PELISSARI et al., 2011). Em alguns casos, é utilizada a combinação de pelo menos dois ou até três fármacos para a cura definitiva da doença (PELISSARI et al., 2011). O tempo de tratamento varia de acordo com a espécie de *Leishmania*. Além do estado nutricional, idade do paciente e do fármaco utilizado (WHO, 2010). Isso torna essa prática bastante cara e para diminuir o gasto, são utilizadas as outras formas de controle.

O controle de animais reservatório (cães) é realizado com eliminação de cães infectados e cães errantes (vadios). No entanto, tal técnica de abate em massa de cães soropositivos não foi comprovada como método eficaz (DIETZE et al., 1997; ASHFORD et al., 1998). Atualmente, vem sendo sugerido como forma de prevenção a utilização de coleiras impregnadas com inseticidas (piretróides). Essa técnica mostrou ser eficaz na redução de leishmaniose visceral em cães e humanos (KILLICK-KENDRICK et al., 1997; GAVGANI et al., 2002; FERROGLIO; POGGI; TRISCIUOGLIO, 2008). Embora a Organização Mundial de Saúde (WHO) não recomenda tal método, pois nunca foi testado em campanhas em massa e não pode ser aprovado como uma alternativa para interromper a transmissão (WHO, 2010). Além disso, apresenta custo elevado e pode contribuir para aumentar a resistência dos insetos.

Por último, o controle do vetor pode oferecer uma solução mais prática, o que permitiria mais eficácia nas medidas preventivas no controle das leishmanioses (MAROLI; KHOURY, 2004). É uma técnica bastante utilizada em todo mundo, embora seja restrita a fase adulta (WARBURG; FAIMAN, 2011).

A primeira tentativa de controlar flebotomíneos foi com o pesticida Diclorodifeniltricloroetano (DDT), no Peru em 1944, para o controle de *L. verrucarum* (HERTIG; FAIRCHILD, 1948). A eficácia do DDT para controle de flebotomíneos e diminuição da doença é comprovada, principalmente quando foi utilizado para erradicar a malária na década de 60 (DAVIES et al., 1994). O DDT contribuiu para uma drástica queda de infecção por leishmaniose em vários países. No entanto, quando as pulverizações foram

suspensas, os níveis de prevalência da doença voltaram rapidamente (DAVIES et al., 1994; KISHORE et al., 2006; WHO, 2010).

Mazzarri et al., (1997), utilizaram vários inseticidas (DDT, Propoxur, Malation, Fenitrotion, Pirimifos Metílico, Deltametrina, Lambida-cialotrina, Permetrina) na susceptibilidade de *L. Longipalpis*. Como resultados perceberam que as colônias de laboratório e os insetos capturados em campo tinham susceptibilidade a qualquer um dos inseticidas testados. Contudo, alguns trabalhos relatam que o uso de DDT não foi satisfatório (QUINNELL; DYE, 1994 a,b; LAINSON; RANGEL, 2005), principalmente quando a pulverização se dá no interior das habitações humanas.

A utilização de DDT foi proibida na agricultura e programas de controle de vetores em vários países, por sua alta toxicidade e contaminação ambiental (D'AMATO; TORRES; HALM, 2002). Com isso, outros compostos químicos foram utilizados na substituição do DDT, como o grupo sintético dos piretróides, compostos químicos derivado da piretrina (VALENTINE, 1990).

A piretrina é um composto natural encontrada nas plantas do gênero *Chrysanthemum*. Ela apresenta baixa toxicidade para mamíferos, com propriedades tóxicas para insetos e organismos aquáticos (SHAROM; SOLOMON, 1981). A permanência dos piretróides depende da estabilidade da molécula, da intensidade da radiação solar, podendo sua meia-vida variar de acordo com o composto (HOLMSTEAD; CASIDA; RUZO, 1978; TOTH; SPARKS, 1990). Por esses motivos, é utilizada principalmente no interior das residências. No entanto, essa prática diminui apenas os flebotomíneos no interior dos domicílios, mesmo quando os efeitos residuais duram um ano (FALCÃO et al., 1991).

Passerat de Silans et al., (1998), utilizaram a Cipermetrina em paredes no interior das residências na América do sul. Eles perceberam que existem efeitos nos flebotomíneos (*L. longipalpis*) que adentram no intradomicílio. Este efeito durou até dois meses após a aplicação, mas não teve efeito nos flebotomíneos do peridomicílio. Concluindo, que o comportamento extradomiciliar dos adultos dificulta o controle através de pulverização de inseticidas residuais.

Com essas considerações, outras medidas de controle têm surgido como o uso de mosquiteiros e cortinas impregnados com inseticidas (ELNAIEM, 2011). Essa técnica mostrou-se eficaz na proteção de pessoas e em alguns casos reduziu a incidência de leishmaniose (COURTENAY et al., 2007; PICADO et al., 2009; KASILI et al. 2010; MONDAL et al., 2010; 2013). Contudo, deve-se levar em consideração a segurança para os seres humanos e o meio ambiente, além do custo do inseticida (WHO, 2010).

Outro método de controle estudado foi a impregnação de piretróides em telas de janelas. Esse método pode reduzir a abundância de flebotomíneos dentro das residências (BASIMIKE; MUTINGA 1995). Com isso, Das et al., (2014), comparam vários tamanhos de malhas e perceberam que o menor tamanho da malha e a adição de piretróides diminuiu substancialmente a quantidade de flebotomíneos no interior das residências. No entanto, a utilização de inseticidas no controle de flebotomíneos tem que ser bastante estudada em cada local de aplicação. Pois doses altas podem resultar em efeitos excito-repelentes e doses baixas podem selecionar indivíduos resistentes aos inseticidas escolhidos (MILLER; GIBSON, 1994).

A resistência de flebotomíneos já foi comprovada para todos os grupos de inseticidas modernos como os organoclorados, organofosforados, carbamatos e piretróides (KAUL et al., 1978; JOSHI et al., 1979; HEMINGWAY; RANSON, 2000; SURENDRAN et al., 2005; ALEXANDER et al., 2009). A utilização dos inseticidas embora inicialmente atrativos, não é permanente. Assim, a interrupção ou utilização prematura em períodos inadequados, dificulta o controle de flebotomíneos e apresenta risco ambiental (TEODORO et al., 2007). Além disso, nas áreas rurais, onde as habitações humanas são dispersas, e, ao redor do peridomicílio existem reservatórios, a utilização de inseticidas nas casas pode ser ineficaz (ALEXANDER; MAROLI, 2003; MAROLI; KHOURY, 2004; AMÓRA et al., 2009).

A estratégia de controle do vetor baseado na pulverização residual é criticada por ser cara, não é facilmente aceita, é um perigo para organismos não alvo (como os polinizadores) e não é sustentável em longo prazo (ALEXANDER; MAROLI, 2003; MARCONDES, 2011; DAS et al., 2014). Além disso, é recomendável a utilização de medidas de proteção individual como mosquiteiros e repelentes. Porém essas práticas muitas vezes não são disponíveis para as populações em risco (ALTEN et al., 2003).

Em suma, a eficácia da pulverização utilizando inseticidas residuais depende do quanto os flebotomíneos estão adaptados ao ambiente modificado. Também ao grau de susceptibilidade ao inseticida escolhido, além da eficiência dos programas de pulverização (ALEXANDER; MAROLI, 2003; CHOWDHURY et al., 2011). Atrelado a isso, existe diferença na ecologia de cada espécie, às características de transmissão em cada região, a gama de reservatórios, considerando que em alguns lugares existem mais de uma espécie vetor e mais de uma espécie de *Leishmania*, levam a crer que nenhuma forma de combate acima pode ser generalizada.

Outro método de controle vem sendo sugerido que é a reorganização e limpeza ao redor das habitações humanas (WHO, 2010) que poucos autores abordam o tema. O primeiro trabalho realizado foi de Teodoro et al., (1999), que coletou flebotomíneos antes e depois da limpeza e organização do peridomicílio. A limpeza consistiu em instalação de fossas sépticas, relocaram o abrigo de animais doméstico para uma distância de 100m em relação à residência, retirada de algumas arvores, remoção da matéria orgânica do peridomicílio. Com essas práticas, percebeu-se que a população de flebotomíneo diminuiu.

Outro trabalho publicado pelos mesmos autores e nas mesmas circunstâncias, porém com adição de DDT e Deltametrina nas residências humanas. Foi percebido que houve diminuição na população de flebotomíneos nos dois primeiros anos de coleta e no terceiro ano houve um aumento (TEODORO et al., 2004). Os autores atribuíram esse aumento a negligencia dos moradores da fazenda em não seguir as medidas estabelecidas, além de mudança de moradores para a zona urbana.

Neitzke-Abreu et al., (2012) realizando o manejo ambiental, coletaram seis vezes mais flebotomíneos após a limpeza do peridomicílio, mostrando que as técnicas utilizadas não foram suficientes para reduzir o número de flebotomíneos. Outro autor teve resultados semelhantes, Reinhold-Castro et al., (2013), realizou um estudo no mesmo local, com o mesmo método de limpeza. E adicionou o uso de inseticidas no interior das residências. Percebeu que em alguns locais houve aumento de flebotomíneos quando comparado ao ano anterior (sem manejo). Assim, não houve uma resposta clara sobre a limpeza do peridomicílio.

Contudo, ainda é bastante preliminar qualquer inferência sobre a limpeza do peridomicílio e a sua influência nas populações de flebotomíneos. Muito embora, esse método já vem sendo sugerido pela organização mundial de saúde, ainda há necessidade da realização de estudos no intuito de clarear tal afirmação. Pois, os métodos de controles para a diminuição das leishmanioses utilizados atualmente não estão sendo eficazes no controle da doença.

Partindo desse pressuposto, é necessária uma combinação de estratégias eficazes que devem ser adaptadas a cada contexto, pois o principal fracasso no controle de leishmaniose é a falta de programas integrados (AMÓRA et al., 2009). Mas para integrar as diferentes formas de controle, deve-se comprovar a eficácia de cada método e compilar com fatores inerentes de cada local.

1.3 Objetivo Geral

Estudar a influência do manejo ambiental, da presença de animais domésticos e das características ambientais próximos as residências rurais como estratégia de controle vetorial das leishmanioses.

1.4 Objetivos Específicos

Estudar a composição da fauna de flebotomíneos em peridomicílios rurais;

Analisar se a limpeza do peridomicílio contribui para a redução de flebotomíneos machos e fêmeas;

Saber se a presença de animais no peridomicílio contribui para o aumento de flebotomíneos no peridomicílio;

Saber quais animais domésticos contribui para abundancia de machos e fêmeas de flebotomíneos;

Analisar se a abundancia de flebotomíneos é influenciada pela distancia dos corpos d'água;

Analisar se a maior cobertura vegetal dos peridomicílios rurais contribui para o aumento de machos e fêmeas de flebotomíneos.

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2.1 ARTIGO

Factors Associated With Sandfly (Diptera: Psychodidae) Abundance in Rural Villages in Brazil

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ABSTRACT. Leishmaniasis is a significant public health threat in many countries. The reducing proliferation of the causative agent (*Leishmania* spp.) requires a combination of management practices. This study analyzes how landscape features, animal husbandry, and environmental management practices influence the abundance of sandflies (the biological vectors of leishmaniasis). Sandflies were collected in 112 rural, peridomestic areas, which were grouped into four categories: with or without environmental management (cleaning of peridomestic), and with or without animals. We also quantified the number of domestic animals, distance to the nearest water body (streams and rivers), and vegetation cover in a 50 m zone around each trap. The results indicate that management of peridomestic areas did not reduce sandfly populations, and that the presence of domestic animals, particularly chickens, was the factor that most influenced sandfly abundances. We also found a positive relationship between sandfly abundance and proximity of traps to water bodies, and a negative relationship between vegetation cover and sandfly abundance in peridomestic areas.

KEY WORDS: Phlebotomine, environmental management, vegetation cover, peridomestic, restinga and savannah vegetation.

Sandflies are broadly distributed around the world, with an estimation 988 described species (Bates et al. 2015). From those, about 90 species can transmit parasites of the genus *Leishmania* (Knetoplastida: Trypanosomatidae), which cause diseases such as cutaneous and visceral leishmaniasis in humans and other animals (WHO 2010). There are two types of leishmaniasis that occur in Brazil, which are caused by at least eight species of the genus *Leishmania* (Grimaldi Jr et al. 1989, Cupolillo et al. 1995). These parasites are transmitted by various sandfly species (Grimaldi Jr and Tesh 1993), of which two have particularly high epidemiological importance: *Lutzomyia longipalpis* (Lutz and Neiva 1912), a vector of visceral leishmaniasis, and *Lutzomyia whitmani* (Antunes and Coutinho 1939), a vector of cutaneous leishmaniasis (Young and Duncan 1994, Costa et al. 2007).

The transmission of leishmaniasis is associated with an increase in vector-host contact, which can be due to several factors. Ecosystem alterations due to the destruction or reduction of natural habitat and endemic sandfly breeding areas can favor vector movement into peridomestic areas (Valderrama et al. 2011, Ramos et al. 2014). The presence of domestic animals in the vicinity of human dwellings may also attract vectors, because these animals can serve as blood sources, and accumulated organic matter associated with animals may provide suitable breeding sites (Neitzke-Abreu et al. 2012, Ramos et al. 2014). Sandfly abundance in urban environments may also be influenced by landscape features such as vegetation cover (Fernández et al. 2013, Santini et al. 2015) and the distance between peridomestic areas and water bodies (Santini et al. 2015). These factors must be considered in order to better control leishmaniasis, since current efforts tend to focus on the clinical treatment of human cases rather than management programs for animal reservoirs and insect vectors.

Control of sandfly populations is typically accomplished through use of residual insecticides (Stockdale and Newton 2013). However, the effectiveness of these insecticides is controversial (see Falcão et al. 1991, Passerat of Silans et al. 1998, Barata et al. 2011, Faraj et al. 2013), and their use negatively impacts other insect species (Warburg and Faiman 2011). The cost of this strategy can also be quite high (Alexander and Maroli 2003) and sandflies may become resistant after long-term application (Surendran et al. 2005). Other control methods are thus needed, and some regions have already adopted new strategies. In the old world, for example, control programs have incorporated destruction of rodent burrows using a deep plow (WHO 2010). However, this method would not be practical in the Americas because domestic dogs are the main vertebrate reservoirs of *Leishmania* near human dwellings. Management programs in these areas have instead introduced environmental cleaning; a cost-effective management method with no known negative environmental impacts (Reinhold-Castro et al. 2013). Some studies have evaluated the efficiency of this method in sandfly control (Teodoro et al. 1999, 2004, Legriffon et al. 2012, Neitzke-Abreu et al. 2012, Reinhold-Castro et al. 2013), but a consensus has not yet been reached.

The current study investigates relationships between landscape factors (vegetation cover, distance to streams and rivers) and sandfly abundances, and evaluates whether environmental

management practices in rural environments (i.e., cleaning/clearing of peridomestic areas) can reduce phlebotomine abundances. Finally, we evaluate whether the presence or abundance of domestic animals in peridomestic areas contributes to sandfly abundances.

Materials and Methods

Study Site. The study was carried out in the municipalities of Barreirinhas and Santo Amaro do Maranhão in the State of Maranhão, Brazil. Barreirinhas (2°45' S, 42°5' W) has a total area of 3,111 km^2 and a population of about 56,000 inhabitants, most of them residing in rural areas (59.9%) (IBGE 2011). Santo Amaro do Maranhão (2°30' S, 43°15' W) has a total area of 1,601 km^2 and a population of about 14,000, most of them residing in rural areas (73.7%) (IBGE 2011).

The regional climate is sub-humid megathermic, with an average annual temperature of 26.1 ºC. There are two well-defined seasons: a dry season between July and December, and a rainy season between January and June. The mean annual relative humidity is 85%, and mean annual precipitation is 1,800 mm (GEPLAN 2002). The IBGE vegetation classification map (2011) indicates that the region is characterized by vegetation influenced by marine systems (restinga) and savannah (cerrado), and areas of transition between savanna and seasonal forest (ecotone). The collection points were in the restinga and ecotone regions (Figure 1), both endemic regions of leishmaniasis, with at least 542 cases of cutaneous leishmaniasis and 22 cases of visceral leishmaniasis reported from 2007 to 2015 (SINAN 2016). The relatively high numbers of cases are due to leishmaniasis endemism and the presence of competent and efficient sandfly vectors in the region, in addition to high contact rates with humans. The latter have been exacerbated due to the municipalities being situated along an access route to the Lençóis Maranhenses National Park, which receives thousands of visitors annually.

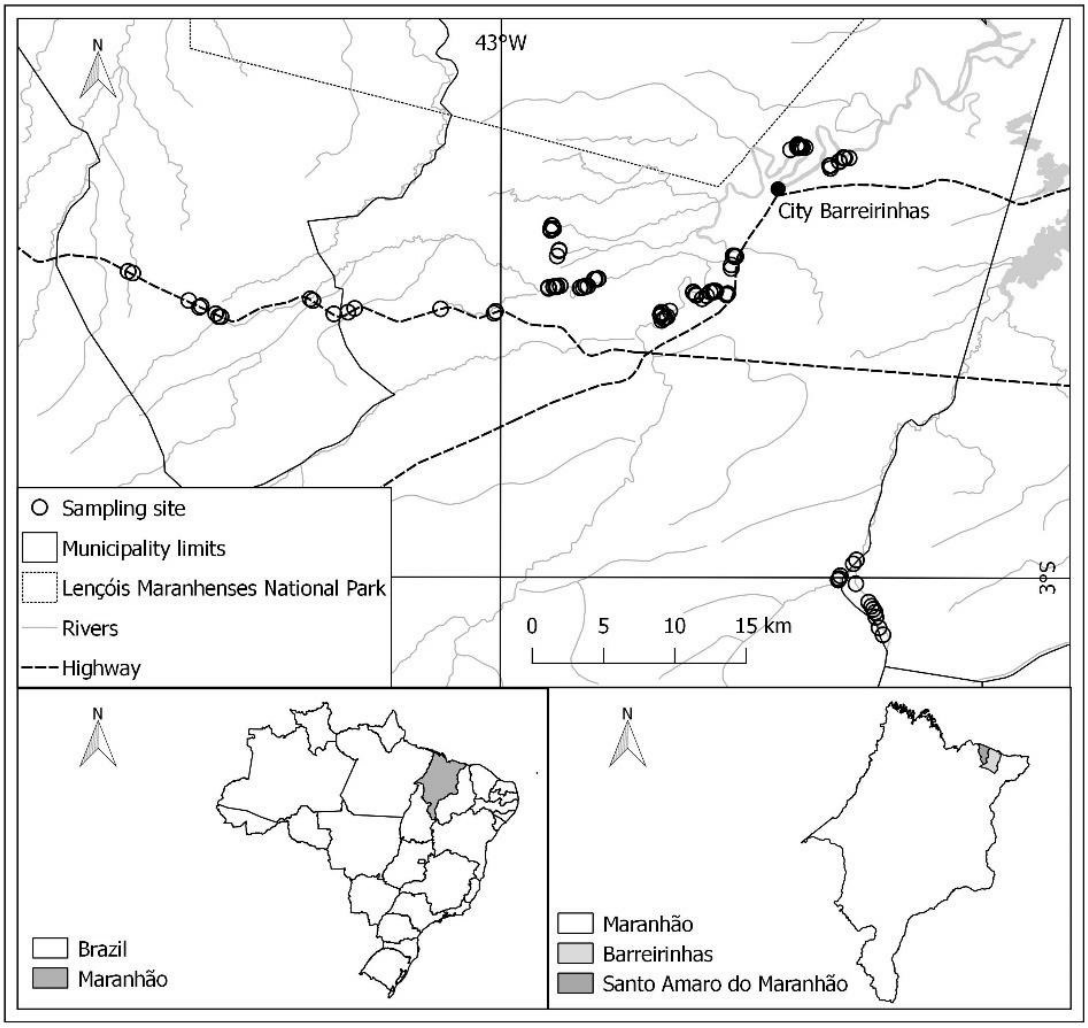

Fig. 1. The Barreirinhas and Santo Amaro do Maranhão municipalities in Maranhão, Brazil. Circles indicate the residences (peridomestic) where sandflies were collected.

Experimental design. The environmental management practices employed in the current study included cleaning areas around homes, following Teodoro et al. (1999, 2004), Neitzke-Abreu et al. (2012) and Reinhold-Castro et al. (2013) with some modifications. A 'managed' peridomestic area was considered one in which topsoil organic matter, organic and inorganic waste, and objects that may serve as substrate such as wood, bricks, or tiles were removed, in addition to ruderal plants such as shrubs and grasses. Areas without management had some combination of these characteristics.

We selected 112 houses spaced at least 50 meters apart and classified them according to the presence (P) or absence (A) of cleaning (C) and animals (An). The four resulting categories were thus as follows: PC + PAn, PC + AAn, AC + PAn, and AC + AAn, with a total of 28 residences per category. These residence characteristics were pre-existing, thus collection areas were not manipulated in any way.

Sandfly collection. HP-type CDC light traps (Pugedo et al. 2005) were used, however incandescent light bulbs were replaced by white, LEDs (Fernández et al. 2015). Traps were

installed at each collection point at 1.5 m height. Traps were run continuously for 12 hours per site (6:00 pm to 6:00 am). If the residence had domestic animals, the trap was installed near animal shelters. Traps were installed on consecutive days from the new moon, from April 18-19 and May 16-18 of 2015 (period 1) and August 18-20 of 2015 (period 2). Sandflies were captured from each of the four types of peridomestic area in both periods, totaling 52 areas in period 1 (capture effort = 624 hours), and 60 areas in period 2 (capture effort = 720 hours).
Collected specimens were immediately frozen, then clarified and mounted according to Forattini (1973). Specimens were then identified using dichotomous keys by Young and Duncan (1994) and Shimabukuro et al. (2011).

Descriptive variables. Descriptive variables were classified into three groups: A) landscape variables: trap distance to water bodies (Dist.water) and vegetation cover in the 50 m radii zone around trap (Veg.50); B) managed (cleaning) or not managed peridomiciles; and C) presence/absence, quantity, and types of domestic animals in the areas. Quantum GIS version 2.14 was used to measure the shortest distance between each trap and the nearest water body (streams and rivers), and the coordinates of each trap were plotted in a raster with high resolution images. To calculate the percentage of vegetation cover around each trap (50 m), we used a raster available on the Global Forest Change 2000-2014 website, using a green band to measure vegetation cover (see Hansen et al. 2013). The explanatory variables were standardized (plant cover, distance to the water body, abundance of captive birds, horses, goats, cattle, canines, and pigs), where mean values were transformed to zero and the variation was between -1 and + 1.

Statistical analysis. We used generalized linear mixed models (GLMM) with negative binomial distribution to model sandfly abundance to analyze 15 possible combinations for the first analysis and 63 combinations for the second analysis. We used the Akaike Information Criteria (AIC) to select the models that best described patterns of male and female sandfly abundances. We used collection period (1 or 2) as the random variable. All analyses were done in R version 3.2 (R Development Core Team 2010) using the glmm ADMB (Bolker et al. 2012) and vegan (Oksanen et al. 2013) packages.

Results

Explanatory variables. The distances between traps and the nearest body of water ranged from 34 to 776 meters, with an average of 197 meters. Percent vegetation cover within a 50 m radius around the traps ranged from 5.5 to 70.9%, with an average of 33.9%. Data on environmental variables are shown in Table 1. There were 672 animals found in the 56 peridomestic areas that contained animals. Chickens were the most commonly encountered animal (present in 55 of 56 areas), with numbers of individuals per area ranging from two to 60 hens. Dogs were the second most common, with 53 animals in total present in 28 peridomestic areas. Data describing the presence, abundance, and types of other animals are shown in Table 1

Phlebotomine sampling. We collected a total of 3,169 specimens, of which 11 species were identified. *Lutzomyia longipalpis* (Lutz and Neiva 1912) and *Lutzomyia whitmani* (Antunes and Coutinho 1939) together represented 88.3% of the total sample (Table 2). Males were more common than females, representing 77.8% of the sample.

Generalized linear mixed models (GLMM). For male sandfly abundances, the model with the lowest AIC value included the presence of domestic animals, cleaning of peridomestic area, distance between trap and water bodies, and vegetation cover as explanatory variables (Figure 2). Chickens and pig abundances also had a significant effect on the abundance of male sandflies (AIC = 703.7) (Figure 3). The descriptive variables that best predicted female sandfly abundance was the distance between traps and water bodies, percent vegetation cover (AIC=513.9) (Figure 2), and the presence of domestic animals, specifically chickens and horses (AIC=547) (Figure 3).

Discussion

In the present study, management of the peridomicile did not significant reduce sandfly abundances. The presence of domestic animals was the factor most associated with the presence of sandflies in rural peridomestic areas, which generally agrees with results from other studies (Neitzke-Abreu et al. 2012, Reinhold-Castro et al. 2013). Although management practices did not affect female sandfly abundances, which are responsible for the spread of parasites, they did favor infestation by males. Cleaning of the peridomicile is a strategy that cannot be considered in isolation, because it is difficult or impossible to partition effects of other factors that cannot be controlled on sandfly abundances in these areas. For example, among the kairomones released by vertebrates, CO^2 is highly attractive and can be detected from long distances (Takken 1991, Kline 2006, Muller et al. 2015), and male sandflies have higher capacity for detection (Pinto et al. 2001). This may partially explain the greater abundance of males in managed peridomestic areas with animals. Further, higher wind speeds and physical barriers such as trees and shrubs in the environment are known to dilute or divert odor plumes (Murlis et al. 2000, Cardé and Willis 2008) and influence CO^2 dissemination; peridomestic areas with fewer obstacles may in turn favor reception of odor plumes by the phlebotomines. Finally, open peridomestic spaces may optimize light trap visibility and consequently, may increase sandfly abundances. Similarly, lightbulbs used in and around homes in rural areas may further increase the abundance of sandflies in associated peridomestic areas.

We found that the presence of domestic animals in general in the peridomestic area increases sandfly abundance, however the correlations were stronger for chickens, swine, and horses. This may be explained by the higher output of kairomones, mainly CO^2, in these animals (Pinto et al. 2001, Alexander et al. 2002, Müller et al. 2015). However, the presence of cattle was not associated with sandfly abundances, which contrasts studies of *Phlebotomus orientalis* in Ethiopia (Gebresilassie et al. 2015) and *Phlebotomus argentipes* in India (Dinesh et al. 2001). Morrison et al. (1993) also analyzed the blood meals of *L. longipalpis* in

Colombia and found that the species fed mainly on cattle. The preferred vertebrate blood source for sandflies seems to differ regionally, thus knowledge of the animals are most attractive for vectors in specific regions is extremely relevant for control of leishmaniasis. In the current study there were few residences with cattle in the peridomicile, mainly due to extensive livestock farming in the study areas, in which animals sleep in the pasture.

No correlation was found between abundance of sandflies and the abundance of dogs in the peridomestic area, despite the fact that the dog is the most important animal for propagation of *Leishmania* in rural and urban environments (Ashford et al. 1998). This may be partially explained by the fact that all peridomestic areas containing dogs also contained chickens. Poultry (chickens, turkeys, and ducks) are considered zooprophylactic barriers (Schlein et al. 1982, Alexander et al. 2002) due to having a body temperature of 41° C, which makes them resistant to leishmania infection (Zilberstein and Shapira 1994). However, poultry should ideally be kept at least 100 meters from human residences (Legriffon et al. 2012), while chickens at our study sites were rarely more than 10 meters from homes. Thus, instead of forming a barrier, chickens may have instead attracted sandflies to areas immediately surrounding human dwellings, and to associated dogs (the common reservoir).

Sandflies frequently acquire blood meals from several domestic and synanthropic animals (Dias et al., 2003), and it is possible that after feeding on chickens, they may also feed on a mammal infected with leishmaniasis. Chicken blood does not inhibit the development of leishmania in the insect midgut (Sant'Anna et al. 2010) and may increase the number of eggs sandflies can produce (Noguera et al. 2006), thus indirectly impacting the spread of the disease in rural settings. In addition, chickens in peridomestic areas may provide suitable sites for sandfly oviposition (Casanova et al. 2013), placing the vector life cycle even closer to humans.

Equines contributed to the increase of female sandflies in the peridomicile. These animals are commonly found with the cutaneous lesions associated with *Leishmania* (Viannia) *braziliensis* in South America (Oliveira-Neto et al. 1988, Brandão-Filho et al. 2003, Soares et al. 2013, Truppel et al. 2014), and for this reason, some authors have suggested that they are actively involved in the transmission of American cutaneous leishmaniasis (Aguilar et al. 1984, Menezes et al. 2002, Vedovello Filho et al. 2008). Our results reinforce this idea, since equines seemed to strongly attract female sandflies.

Swine were also commonly found near residences in our study areas, which on the island of São Luís, Maranhão, have frequently been found infected with amastigote forms of *Leishmania* (Brazil et al. 1987). Pigs were also found to increase abundances of male sandflies, which have a habit of visiting vertebrate blood sources in search of females for mating (Kelly and Dye 1996).

One variable that is not always considered a factor in the epidemiology of leishmaniasis is the presence of natural water bodies (e.g., streams and rivers). The distance from traps to the nearest body of water was found to be a good predictor of sandfly abundance and richness, with a positive correlation found between abundance and richness and water body proximity to traps. Although sandflies do not reproduce in water, they do require moist environments with abundant organic matter for larval development (Lainson and Rangel 2005), a condition

that can be found year-round in riparian forests (Naiman and Decamps 1997). In months with less rainfall (July to December), riparian forests can act as refuges for sandfly reproduction and maintenance by providing blood meal sources such as mammals and birds (Macdonald et al. 2006).

The negative correlation between sandfly abundance and percent vegetation cover was unexpected, as this result contrasts with other studies of urban environments in other biogeographic areas (Oliveira et al. 2012, Santini et al. 2012, 2015, Fernandez et al. 2013, Andrade et al. 2014). The explanation, however, is likely related to the physiognomy of these vegetation ecosystems, which includes tree and shrub species that are spread over grassy substrate without forming a continuous canopy (Ribeiro and Walter 1998). In addition, because of the intense degradation of these forests due to farming by local communities (Pereira Filho et al. 2015), perhaps the captured species are simply better adapted to low vegetation cover, especially the most abundant species (*Lutzomyia*. *longipalpis* and *Lutzomyia whitmani*).

We conclude that environmental management of the peridomicile was not effective for controlling sandflies in rural environments, because it does not appear to reduce sandfly abundance in these areas. The presence of domestic animals and distance from traps to water bodies were the most important factors for predicting sandfly occurrence, with chickens apparently attracting higher abundances. Furthermore, rural, peridomestic areas with lower vegetation cover had increased abundance of sandflies.

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Table 1. Descriptive variables that were present in peridomestic rural environments in Barreirinhas and Santo Amaro do Maranhão, Maranhão, Brazil

Descriptive variables	Minimum	1st Quartile	Median	Mean	3rd Quartile	Maximum	Presence Peridomestic
Dist.water (m)	34	90.75	146	197.1	276	776	112
Veg.50(%)	5.5	24.8	33.5	33.9	42.7	70.9	112
Chickens	0	10.75	19	22.46	30.5	60	55
Canids	0	0	0.5	0.9464	1.25	5	28
Pigs	0	0	0	0.3214	0	8	13
Equines	0	0	0	0.7321	0	8	7
Cattle	0	0	0	0.2143	0	6	5
Goats	0	0	0	0.5357	0	15	2

Dist.water= Distance to water bodies, Veg.50= vegetation type in 50m radii zones around the trap.

Table 2. Sandflies collected in peridomestic rural environments in Barreirinhas and Santo Amaro do Maranhão, Maranhão, Brazil

Species	PC + PAn		AC + Pan		PC + AAn		AC + AAn		Abundance		Total	Relative abundance
	F	M	F	M	F	M	F	M	F	M		
Lutzomyia whitmani (Antunes & Coutinho,1939)	171	1190	145	674	11	66	2	5	329	1935	2264	71,44
Lutzomyia longipalpis (Lutz & Neiva, 1912)	64	153	102	157	15	23	10	9	191	342	533	16,62
Lutzomyia lenti (Mangabeira, 1938)	21	41	43	47	5	6	0	2	69	96	165	5,21
Lutzomyia evandroi (Costa Lima & Antunes, 1936)	33	25	34	44	7	7	1	2	75	78	153	4,83
Lutzomyia trinidadensis (Newstead, 1922)	11	0	8	2	1	0	0	0	20	2	22	0,69
Lutzomyia migonei (França, 1920)	5	8	2	2	0	0	0	0	7	10	17	0,54
Lutzomyia flaviscutelata (Mangabeira Fo, 1942)	2	0	1	2	0	0	0	0	3	2	5	0,16
Lutzomyia sordellii (Shannon & Del Ponte, 1927)	1	1	2	0	0	0	0	0	3	1	4	0,13
Lutzomyia termitophila (Martins, Falcão & Silva, 1964)	0	0	1	1	2	0	0	0	3	1	4	0,13
Lutzomyia barretoi (Mangabeira, 1942)	1	0	0	0	0	0	0	0	1	0	1	0,03
Lutzomyia shannoni (Dyar, 1929)	0	0	1	0	0	0	0	0	1	0	1	0,03
Total	309	1418	339	929	41	102	13	18	702	2467	3169	100
Relative abundance	17,89	82,11	26,74	73,3	28,67	71,33	41,94	58,06	22,15	77,85	100	

F=Females, M= Males, P= presence, A= absence, C = cleaning of the peridomestic, An= animals.

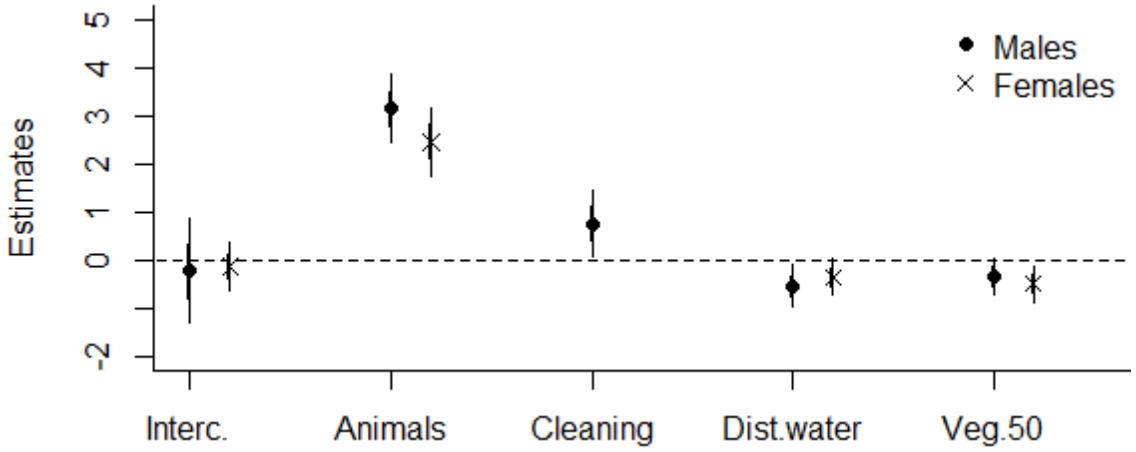

Fig. 2. GLMM referring to the abundance of male and female sandflies of rural environments peridomestic of Barreirinhas and Santo Amaro do Maranhão, Maranhão, Brazil.

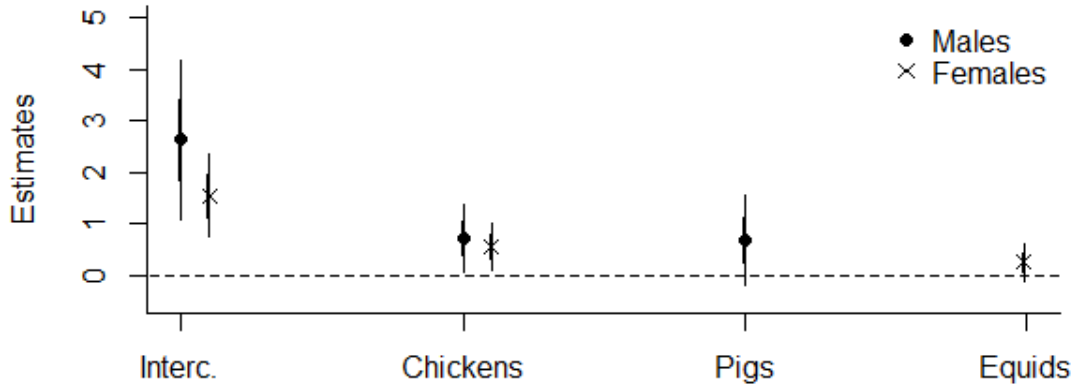

Fig. 3. GLMM referring to animals that influence the abundance of sandflies males and females in peridomestic rural environments of Barreirinhas and Santo Amaro do Maranhão, Maranhão, Brazil.

APÊNDICE

Tabela dos Modelos (GLMM) e o valor AIC

Supp. Table S1. Generalized linear mixed model (GLMM) and the Akaike value (AIC) for each model. Subtitle: A. females: Abundance of females, A. males: Abundance of males, Animals: peridomiciliary with animals presence, Cleaning: peridomiciliary cleaning presence, Dist.water: Distance to water bodies, Veg.50: vegetation type in 50 m radii zones around the trap, Ch: Abundance of chickens, Cat: Abundance of cattle, Go: Abundance of goats, Can: Abundance of canids, Eq: Abundance of equines, Pi: Abundance of pigs, *: smaller value AIC.

Models	Abundance females	Abundance males
Animals + Cleaning + Dist.water + Veg.50	515.1	653.2*
Animals + Cleaning + Dist.water	517.7	654
Animals + Cleaning + Veg.50	516.1	656.8
Animals + Dist.water + Veg.50	513.9*	656.1
Cleaning + Dist.water + Veg.50	548.9	702
Animals + Cleaning	517	655.8
Animals + Dist.water	517.4	657.7
Animals + Veg.50	514.7	659.2
Cleaning + Dist.water	548.5	701
Cleaning + Veg.50	556.4	712.6
Veg.50 + Dist.water	547	701.6
Animals	516.3	658.7
Cleaning	554.8	710.8
Veg.50	554.5	710.9
Dist.water	546.9	701.3
Ch+Cat+Go+Can+Eq+Pi	554.2	714.2
Ch+Cat+Go+Can+Eq	552.5	715.3
Ch+Cat+Go+Can+Pi	554.8	712.7
Ch+Cat+Can+Eq+Pi	552.5	712.2
Ch+Cat+Go+Eq+Pi	552.3	712.3
Ch+Go+Can+Eq+Pi	552.4	712.7
Cat+Go+Can+Eq+Pi	557.2	717.1
Ch+Cat+Go+Can	552.9	713.5
Ch+Cat+Go+Eq	550.5	713.3
Ch+Cat+Go+Pi	553.1	710.7
Ch+Cat+Can+Eq	550.8	713.3
Ch+Cat+Can+Pi	552.8	710.7
Ch+Cat+Eq+Pi	550.5	710.3
Ch+Go+Can+Eq	550.6	713.6
Ch+Go+Can+Pi	553.1	711.2
Ch+Go+Eq+Pi	550.5	710.8
Ch+Can+Eq+Pi	550.6	710.7

Models	Abundance females	Abundance males
Cat+Go+Can+Eq	555.3	715.7
Cat+Go+Can+Pi	556.6	715.3
Cat+Go+Eq+Pi	557.6	716.2
Cat+Can+Eq+Pi	555.3	715.1
Go+Can+Eq+Pi	555.6	715.6
Ch+Cat+Go	551.3	711.5
Ch+Cat+Can	551	711.5
Ch+Cat+Eq	548.8	711.3
Ch+Cat+Pi	551.2	708.7
Ch+Go+Can	551.2	711.9
Ch+Go+Eq	548.7	711.7
Ch+Go+Pi	551.3	709.3
Ch+Can+Eq	548.9	711.7
Ch+Can+Pi	551.2	709.3
Ch+Eq+Pi	548.7	708.9
Cat+Go+Can	554.6	713.8
Cat+Go+Eq	555.7	714.7
Cat+Go+Pi	558.7	714.6
Cat+Can+Eq	553.4	713.7
Cat+Can+Pi	554.6	713.3
Cat+Eq+Pi	555.6	714.2
Go+Can+Eq	553.6	714.2
Go+Can+Pi	555.1	713.7
Go+Eq+Pi	555.6	714.4
Can+Eq+Pi	553.7	713.6
Ch+Cat	549.4	709.5
Ch+Go	549.5	709.9
Ch+Can	549.3	709.9
Ch+Eq	547*	709.7
Ch+Pi	549.4	707.3*
Cat+Go	556.8	713
Cat+Can	552.6	711.8
Cat+Eq	553.7	712.7
Cat+Pi	556.7	712.6
Go+Can	553.1	712.2
Go+Eq	553.7	712.9
Go+Pi	556.7	712.8
Can+Eq	551.7	712.2
Can+Pi	553.1	711.7
Eq+Pi	553.6	712.4
Ch	547.6	707.9

Models	Abundance females	Abundance males
Cat	554.8	711.1
Go	554.8	711.2
Can	551.1	710.2
Eq	551.8	710.9
Pi	554.7	710.9

ANEXO

Normas de submissão da revista Jounal of Vector Ecology

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Abbreviations
Sentences should not start with an abbreviation or new acronym. For example: use: "*Aedes aegypti* is a mosquito." Don't use: "*Ae. aegypti* is a mosquito." For example: use "Plaque forming units…." and not "PFU…." ESA accepted acronyms such as DNA or PCR can be used but should be discouraged.

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When presenting results of probit/logit analysis, these columns should be included in tables (in this order, left to right); n, slope + SE, LD (or LC) (95% CL), and chi-square. When a ratio of one LD versus another is given, it should be given with its 95% CI.

Statistical tests to show what model best fits data intended to estimate the 99.9986% level of effectiveness should be presented to justify use of any model, including the probit model. Thus, we do not recommend use of the Probit 9 without tests to show that the probit model fits the data.

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When presenting the results of analysis of variance or a *t*-test, specify F (or t) values, degrees of freedom, and P values. This information may be placed in parentheses in the text. Example: ($F = 9.26$; df = 4, 26; $P < 0.001$). If readability of the text is affected by the presence of repeated parenthetical statistical statements, place them in a table.

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Equations
Consult *Mathematics into Type* for correct formatting of equations and mathematical variables. Italicize all mathematical variables. Center more complex equations on a separate line.

$$R = A \text{ barrtype} + B\log 10 (f) \quad (2)$$

Validation or the Testing of Model Results
Authors must state why the model did not require testing (e.g., theoretical study), why it cannot be tested (e.g., lack of data), or how it was tested. Data used for testing must be independent of data used to build or calibrate the model. Describe the data and procedures in Materials and Methods. Authors should be aware that the testing of models is an important step that should be a part of most studies.

Structure of Computer Code
For models solved or simulated by computers, mention the programming language and computer used. Describe the important numerical methods used in calculating the model (e.g., integration and random number generation). Mention how the program's logic and algorithms were tested and verified. When published software is computed, provide a reference and state which procedures were used. Discuss in any section of the manuscript the limitations of the published software. Original computer programs should be made available at the request of reviewers and readers.

Gene Sequencing
Inclusion of a GenBank/EMBL accession number for primary nucleotide and amino acid sequence data is a criterion for the acceptance of a manuscript for publication. Sequences from new species and new genes must indicate the proportion of the gene sequenced and should include data from both strands. The accession number may be included in the original manuscript or the sequence may be provided for review and an accession number provided when the manuscript is revised. A manuscript will not be accepted for publication until the accession number is provided.

GenBank may be contacted at their website at http://www.ncbi.nlm.nih.gov/Genbank/submit.html. The EMBL Data Library may be contacted at their website at http://www.ebi.ac.uk/embl/Submission/index.html.

Reporting Taxonomy
Follow the *International Code of Zoological Nomenclature*, 4th ed., for taxonomic style. Center the heading that indicates the name of the taxon in bold type. Center figure numbers in parentheses under the main heading; do not use bold type. Start all synonomies at the left margin with runovers indented. Include authors and date. References must appear in References Cited section. Use telegraphic style throughout descriptions.

Taxonomy Headings
Use only acceptable 3rd-level subheadings such as:

- Male
- Female
- Material Examined
- Type Material
- Distribution
- Etymology
- Biology
- Discussion

Avoid using Description as a subheading.

Dates
Use Roman numerals I through XII to designate month of collection. Use Arabic numerals 00 through 99 to designate collection years in the 20th century. Do not abbreviate other years, including the 21st century. Express data in this format: day-month (use a Roman numeral)-year. Example: 2-V-97.

Locality Other than Principal Types
Start with the largest area followed by successively smaller areas separated by colons. Capitalize countries. Arrange data for each locality in the following order: count of specimens and sex or stage (as applicable), city or vicinity, date, collector, and depository. Example: MEXICO: Tamaulipas: 1 male, 1 female, Ciudad Mante, 15-III-97, K. Haack; 5 females, Ciudad Victoria, 3-VII-99, C. Hughes, MCZ. Arrange localities alphabetically. Use a semicolon to separate data for different localities. Define depositories in the Materials and Methods.

Type Material
Start description with the principal type in capital letters. Follow this immediately with count and sex of specimens (use male and female symbols if possible), then place additional data in the order of locality, date, additional data, and collector. Separate these items with commas. Example: HOLOTYPE: 1 male, Locust Grove, VA, 22-X-98, on Cercis canadensis, R. H. Foote. PARATYPES: 2 males, same data.

Voucher Specimens
Voucher specimens of arthropods serve as future reference for published names used in scientific publications. Although the deposition of voucher specimens is not required as a condition for publication, authors are encouraged to deposit specimens in an established, permanent collection and to note in the published article that the expected deposition has been made and its location. Authors should contact the curator of a voucher repository before deposition concerning the procedures required for curation to ensure that the collection will accept the voucher materials. The designation and proper labeling of voucher specimens is the author's responsibility. When available, at least three specimens should be deposited. Each specimen should have the following information provided at the time of deposition:

- Standard label data that are required for the specimens collection (i.e., locality, date of collection, collector, host, ecological data, whether the specimen is from a laboratory collection, etc.).
- An identification label that includes the identifier and date of identification.
- A label that designates the specimen as "voucher."

Acknowledgments
Place the acknowledgments after the text. Organize acknowledgments in paragraph form in the following order: persons (omit all professional titles and degrees), groups, granting institutions, grant numbers, and serial publication number.

Human and Animal Use in Research and Testing
For research articles that involved the use of humans or animals, the Entomological Society of America requires that the following types of notification, as applicable, be included in the acknowledgement section of the article.

Humans. All human subjects work should reference approved Internal Review Board protocols or compliance with Health Insurance Portability and Accountability Act information policies for their organization, if the protocols are not available.

Animals. All studies should reference an approved Institutional Animal Care and Use Committee protocol or similar documents from their institutions. For trapping/collecting wild animals/birds, reference to collecting permits at the national or state level should be referenced.

Pathogens. Reference should be made to Biological Use Authorization approved by an institutional Environmental Health and Safety committee or similar body.

Sample notification: The collection and infection of wild birds with encephalitis viruses was done under Protocol 11184 approved by the Institutional Animal Care and Use Committee of the University of California, Davis, California Resident Scientific Collection Permit 801049-02 by the State of California Department of Fish and Game, and Federal Fish and Wildlife Permit No. MB082812-0. Use of arboviruses was approved under Biological Use Authorization #0554 by Environmental Health and Safety of the University of California, Davis, and USDA Permit #47901.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

Potential conflicts of interest include any relationships of a financial or personal nature between an author or coauthor and individuals or organizations within three years of submission which, in theory, could affect or bias an author's scientific judgment, or limit an author's freedom to publish, analyze, discuss, or interpret relevant data. Sources of financial support originating outside the coauthors' home institution(s) for any aspect of a study must be indicated in the Acknowledgments section of the paper. Financial support includes not only funding, but gratis provision of materials, services, or equipment. Any additional potential conflicts of interest, not covered in the acknowledgments of financial support, must be revealed to the editor at submission, and disclosed in a statement immediately following the Acknowledgments. If an author or coauthor has entered into an agreement with any entity outside that authors' home institution, including the home institution of another coauthor, giving that entity veto power over publication of the study or over presentation, analysis, discussion, or interpretation of any results of the study, whether or not such veto power was exercised, this information must be disclosed in a statement immediately following the Acknowledgments. As a suggestion, such a statement could take the following form: "This manuscript is published with the concurrence of [*Institution / Company / Individual / etc. X*]." If no potential conflicts of interest exist, this must be stated in the cover letter to the editor at submission.

REFERENCES CITED

Cite only those articles published or formally accepted for publication (in press). Include all references mentioned in text. Include enough information to allow reader to obtain cited material (e.g., book and proceedings citations must include name and location [city and state or country] of publisher).

Abbreviate journal titles according to the most recent issue of BIOSIS Serial Sources. For non-English titled journals that are cited in the references, the title of the journal should be spelled out, and not abbreviated. Systematics-related articles may specify that all serial titles be spelled out for final publication. Citations and References should not be numbered.

Alphabetical order (chronological for one author or more than two authors, and alphabetical order [by surname of second author] for two authors)

Journal Articles

Evans, M. A. 2000. Article title: subtitle (begin with lowercase after colon or dash unless first word is a proper noun). J. Abbr. 00:000–000.

Evans, M. A. 2001a. Article title. J. Abbr. 00: 000–000.

Evans, M. A. 2001b. Article title. J. Abbr. 00: 000–000.

Evans, M. A., and R. Burns. 2001. Article title. J. Abbr. 00: 000–000.

Evans, M. A., and A. Tyler. 2001. Article title. J. Abbr. 00: 000–000.

Evans, M. A., A. Tyler, and H. H. Munro. 2000. Article title. J. Abbr. 00: 000–000.

Evans, M. A., R. Burns, and A. A. Dunn. 2001. Article title. J. Abbr. 00: 000–000.

In Press

Evans, M. A. 2002. Article title. J. Econ. Entomol. (in press).

Books

Burns, R. 2001. Title (initial cap only): subtitle (no initial cap after colon). Publisher, city, state abbreviation or country.

Evans, M. A. 2001. Colorado potato beetle, 2nd ed. Publisher, city, state abbreviation or country.

Tyler, A. 2001. Western corn rootworm, vol. 2. Publisher, city, state abbreviation or country.

Article/Chapter in Book

Tyler, A. 2001. Article or chapter title, pp. 000–000. *In* T.A.J. Royer and R. B. Burns (eds.), Book title. Publisher, city, state abbreviation or country.

Tyler, A., R.S.T. Smith, and H. Brown. 2001. Onion thrips control, pp. 178–195. *In* R. S. Green and P. W. White (eds.), Book title, vol. 13. Entomological Society of America, Lanham, MD.

No Author Given

(USDA) U.S. Department of Agriculture. 2001. Title. USDA, Beltsville, MD.

(IRRI) International Rice Research Institute. 2001. Title. IRRI, City, State or Country.

Patents

Harred, J. F., A. R. Knight, and J. S. McIntyre, inventors; Dow Chemical Company, assignee. 1972 Apr 4. Epoxidation process. U.S. patent 3,654,317.

Proceedings

Martin, P. D., J. Kuhlman, and S. Moore. 2001. Yield effects of European corn borer (Lepidoptera: Pyralidae) feeding, pp. 345–356. *In* Proceedings, 19th Illinois Cooperative Extension Service Spray School, 24–27 June 1985, Chicago, IL. Publisher, City, State.

Rossignol, P. A. 2001. Parasite modification of mosquito probing behavior, pp. 25–28. *In* T. W. Scott and J. Grumstrup-Scott (eds.), Proceedings, Symposium: the Role of Vector-Host Interactions in Disease Transmission. National Conference of the Entomological Society of America,10 December 1985, Hollywood, FL. Miscellaneous Publication 68. Entomological Society of America, Lanham, MD.

Theses/Dissertations

James, H. 2001. Thesis or dissertation title. M.S. thesis or Ph.D. dissertation, University of Pennsylvania, Philadelphia.

Software

SAS Institute. 2001. PROC user's manual, version 6th ed. SAS Institute, Cary, NC.

Online Citations

Reisen, W. 2001. Title. Complete URL (protocol://host.name/path/file.name) and/or DOI (Digital Object Identifier)

Tables
Place tables after the References Cited section. Double-space and number all tables. Boldface table title. Do not repeat data already presented in text. If a table continues on more than one page, repeat column headings on subsequent page(s).

Click here to see a sample table

Title
Title should be short and descriptive. Boldface table number and title only. Include "means + SEM" in title if applicable. Do not footnote title; use the unlettered first footnote to include general information necessary to understand the title (e.g., define terms, abbreviations, and statistical tests).

Lines
Use horizontal lines to separate title from column headings, column headings from data field, and data field from footnotes. Do not use vertical lines to separate columns. All columns must have headings.

Abbreviations
Use approved abbreviations. Use abbreviations already defined in the text and define others in the general footnote. Use the following abbreviations in the body or column headings of tables only: amt (amount), avg (average), concn (concentration), diam (diameter), exp (experiment), ht (height), max (maximum), min. (minimum), no. (number), prepn (preparation), temp (temperature), vs (versus), vol (volume), wt (weight). Use the following abbreviations for months: Jan., Feb., Mar., April, May, June, July, Aug., Sept., Oct., Nov., and Dec.

Operational Signs
Repeat operational signs throughout data field. Insert a space on either side of sign (1.42 ± 1.36).

Spacing
Leave no space between lowercase letters and their preceding values (e.g., 731.2ab).

Footnotes to Tables
Use footnotes to define or clarify column headings or specific datum within the data field. Do not footnote the title; use the unlettered first footnote to include general information necessary to understand the table (e.g., define terms, abbreviations, and statistical tests). The use of asterisks is reserved for statistical significance only.

Example:
Means within a column followed by the same letter are not significantly different ($P < 0.05$; Student *t*-test [Abbott 1925]). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant).

Use lowercase italicized superscripted letters to indicate footnotes. Footnote letters should appear in the table in consecutive order, from left to right across the table then down the page.

Figures
For review purposes, it is acceptable to include figures, whether in black and white or color, as part of the manuscript file, with each figure on a separate page. Figures should be inserted in the manuscript file in one of the following formats:

- Tagged Image File Format (.tif)* (please check settings when exporting to TIFF from the original application).
- Encapsulated PostScript (.eps)*
- Rich Text Format (.rtf)
- Editable Microsoft Word (.doc/.docx) (image files embedded into Word are often not good quality)
- Editable Microsoft PowerPoint (.ppt/.pptx) (image files embedded into PowerPoint are often not good quality).
- Microsoft Excel (.xls/.xlsx)
- Editable Portable Document Format (PDF)
- Postscript (.ps)
- Photoshop (.psd)
- Adobe Illustrator (.ai)
- Graphics Interchange Format (.gif)

- Portable Network Graphics (.png)

GIF formats, such as from websites, are not acceptable and produce poor quality printouts because of low resolution, even for peer review purposes. Charts from Excel and SigmaPlot should not be inserted unless they are in one of the above formats.

Maximum figure sizes are as follows:

- Maximum height: 240 mm (9 inches)
- Maximum width (2-column figure): 171 mm (6 inches)
- Maximum width (1-column figure): 82 mm (3 inches)

When authors are asked to submit revisions, they are also asked to provide all figures as separate, high-quality image files to allow papers to move quickly and efficiently into production upon acceptance.

For more information on preparing figures, see OUP's Author Resource Centre on figures.

Abbreviations and Symbols
Abbreviations and symbols in figures should match those in the text or be defined in legends.

Figure Captions
Type all captions double-spaced on a separate page. All captions should be in paragraph form as shown by the example below.

Fig. 1. Relationship between percentage of defoliation of oak trees and gypsy moth population density. (A) Defoliation and egg mass density. (B) Defoliation of egg density.

Letter locants on figures composed of more than one element should match those in the text (either upper- or lowercase). Do not use equal signs to define abbreviations; use commas (e.g., Ap, barometric pressure).

SUPPLEMENTAL MATERIAL
Supplemental Material may be submitted in the form of one or more (8 maximum) files to accompany the online version of an article. Such material often consists of large tables, data sets, or videos which normally are not possible or convenient to present in print media. Supplemental Material represents substantive information to be posted on the ESA journal website that enhances and enriches the information presented in the main body of a paper. However, the paper must stand on its own without the need for the reader to access the supplemental information to understand and judge the merits of the paper. Any files containing Supplemental Material must be provided at the time of manuscript submission, and will be distributed to reviewers as part of the normal peer-review process. Authors should alert the editor to the presence of Supplementary Material in their cover letter at submission. Once a paper is published, the content of accompanying Supplemental Material files cannot be altered. Although the content of any submitted Supplementary Material is subject to normal peer-review and any changes required by the editor, no copy editing will be performed by the journal's production staff. Therefore, the authors are responsible for suitable format and final appearance of Supplemental Material after acceptance of the paper.

Supplemental Material should be referenced in the body of the main paper (e.g., Supp. Table S1; Supp. Video S1), where a link will take the online reader to the file. Each supplemental file must be labeled with an appropriate title and prefaced by a short (50 words maximum) summary description of the contents. Within each file, any tables, figures, videos, or other material must be accompanied by an appropriate caption. Citations for any literature referenced within a Supplemental Material file should be listed in a References Cited section at the end of the file, even when a citation is duplicated in the main body of the paper. Videos should be brief (< 5 min) and kept to a reasonable size to facilitate downloading by readers.

NOTES ON TERMINOLOGY

Scientific Names

Scientific names and authorities must be spelled out (except for Fabricius and Linnaeus, which are abbreviated as F. and L., respectively) the first time a species is mentioned in the abstract and again in the main body of text.

Common Names

Use only those common names cited in the current *ESA Common Names of Insects & Related Organisms* online database, or those names approved by the ESA Common Names Committee. Do not use any other common name. Do not abbreviate common names (e.g., CPB for Colorado potato beetle).

Give scientific name and authority at first mention of each organism (including plants) in the abstract and again in the text.

Use of "Stadium," "Stage," and "Instar"

Manuscripts received for publication in ESA periodicals refer to arthropods and the periods of time in their development in various ways. These designations should be used consistently.

Stadium (Plural: Stadia): The period of time between two successive molts.

Stage: One of the successive principal divisions in the life cycle of an arthropod (e.g., egg, nymph, larva, prepupa, pupa, subimago, and adult).

Instar: The arthropod itself between two successive molts. For the purposes of the definition, hatching is considered a molt.

Examples of Usage:

Nymphs feed on the underside of leaves during the first stadium.

Larvae of some dermestids go through an indefinite number of stadia (or have an indefinite number of instars).

The nymphs were reared through the fifth stadium. Immature stages (e.g., eggs, larvae, and pupae; eggs and nymphs) are illustrated.

First instar of cerambycids make galleries in wood.

Some 200 first-instar spiderlings were collected. The predators fed readily on early instars of the face fly.

NOTES ON FORMATTING

Capitalization
Do not capitalize the following words in titles or subheadings: a, an, and, as, at, be, by, for, in, of, on, per, to, the.

Abbreviations
Use standard abbreviations as listed in the Council of Science Editors' *Scientific Style and Format, The CBE Manual for Authors, Editors, and Publishers*, 8th ed., or those listed in this guide. Avoid nonstandard abbreviations.

Abbreviations for Time
Use the following abbreviations for time: h (hour), min (minute), s (second), yr (year), mo (month), wk (week), d (day). Do not add "s" to create plurals (e.g., wks).

Fig./Figs.
Use "Fig." if singular and "Figs." if plural (e.g., Fig. 1; Figs. 2 and 3).

Dates
When citing dates in the text (not in tables or taxonomic reports), do not abbreviate month, and use this format: 26 January 1997.

Metric Units
Use metric units. English units may follow within parentheses only if they are of direct practical purpose.

Liter
Do not abbreviate "liter" by itself or when accompanied by a numeral.

% versus percentage
Use "%" only with numerals and in tables and figures. Close up space to numerals (e.g., 50%). Otherwise, use the word percentage (e.g., percentage of defoliation).

Per versus slash
Use "per" rather than a slash unless reporting measurements in unit to unit (e.g., insects per branch, not insects/branch; but g/cm2, not g per cm2.

Numbers
Spell out numbers at the beginning of a sentence. Spell out the numbers one through nine (10 and up are always used as numerals), unless they are used as units of measure (e.g., eight children, three dogs, 8 g, 3 ft, 0600 hours; NOT 8 children, 3 dogs, eight grams, three feet, or six o'clock am). This includes spelling out the ordinals first through ninth, along with twofold, one-way ANOVA, and one-half. Ordinals from 10 and higher are numerals, such as 10th or 51st. In some cases, such as where there is a long list of items (e.g., 8 flies, 6 mosquitoes, 4

butterflies, and 10 bees), exceptions can be made if the editor concurs. The editorial staff will have flexibility in interpreting the rule.

Zeros with P values
All numbers <1 must be preceded by a zero (e.g., $P < 0.05$).

Commas
When a number is >1,000, use a comma to separate hundreds from thousands.

Semicolon
Use a semicolon to separate different types of citations (Fig. 4; Table 2).

Repeating symbols
It is not necessary to repeat symbols or units of measure in a series (e.g., 30, 40, and 60%, respectively).

Footnotes to the Text
Avoid footnotes in the text. Use unnumbered footnotes only for disclaimers and animal use information. Place all footnotes on a separate page after References Cited. Examples of footnotes are:

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation by the USDA for its use.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association of Laboratory Animal Care.

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