FARAH DE CASTRO GAMA

COMPORTAMENTO REPRODUTIVO E SELEÇÃO DO HOSPEDEIRO MEDIADA POR SEMIOQUÍMICOS EM *Neoleucinodes elegantalis* (LEPIDOPTERA: CRAMBIDAE)

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

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A minha mãe, meu pai (*in* memoriam), meus irmãos e irmãs, esposo e filhos...

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ÍNDICE

Páginas

LISTA DE FIGURAS

INTRODUÇÃO GERAL

CAPÍTULO I

CAPÍTULO II

CAPÍTULO III

RESUMO

GAMA, Farah de Castro. D. Sc., Universidade Federal de Viçosa, julho de 2011. **Comportamento reprodutivo e seleção de hospedeiro mediada por semioquímico em *Neoleucinodes elegantalis* (Lepidoptera: Crambidae).** Orientador: Eraldo Rodrigues de Lima. Co-orientadores: Og Francisco Fonseca de Souza e Ângelo Pallini Filho.

O comportamento de procura em insetos é importante para a aquisição de alimento, encontro de parceiro, localização de sítios de oviposição e de refúgio contra predadores. Assim, a maioria dos insetos herbívoros seleciona seu hospedeiro e, em particular a parte da planta, em que se alimentarão e depositarão seus ovos, baseando-se, entre outras ferramentas, nos semioquímicos da planta hospedeira. Este trabalho teve como objetivos: (i) determinar o papel dos cairomônios da planta hospedeira nos comportamentos de chamamento e acasalamento de *N. elegantalis*, (ii) verificar se há efeito da densidade de larvas nos parâmetros de sobrevivência e desenvolvimento larval, (iii) Investigar o papel das ferramentas químicas no comportamento geral de oviposição de *N. elegantalis*, (iv) identificar se ferramentas químicas estão envolvidas na discriminação entre diferentes partes da planta hospedeira por fêmeas de *N. elegantalis*, (v) investigar se extratos hexânico e etanólico de frutos de tomate elicitam diferentes respostas de oviposição e (vi) avaliar as respostas comportamentais de adultos de *N. elegantalis* aos compostos emitidos por plantas de tomate induzidas após herbivoria. Alguns parâmetros do comportamento reprodutivo foram alterados na presença dos cairomônios da planta hospedeira. O tempo em que as fêmeas passaram chamando, assim como o tempo em que os casais permaneceram em cópula, foi menor na presença dos odores da planta hospedeira. Com relação ao efeito da densidade larval nos parâmetros de sobrevivência e desenvolvimento, nas maiores densidades foram registradas pupas de menor peso, assim como, maior taxa de mortalidade de pupas e adultos. O estudo do comportamento de oviposição mediado por ferramentas químicas mostrou que quando voláteis de flor, folha e fruto da planta hospedeira são oferecidos não há preferência de oviposição, entretanto na presença de compostos químicos de superfície, fêmeas preferem ovipositar em extrato de frutos quando comparado com extratos de folhas e extrato de flores de tomate. Além disso, fêmeas reconhecem, através

de pistas químicas, frutos sadios e frutos infestados por larvas de coespecíficos e, preferem ovipositar nos frutos infestados.

ABSTRACT

GAMA, Farah de Castro. D. Sc., Universidade Federal de Viçosa, july, 2011. **Reproductive behaviours and host plant selection mediated by semiochemicals in *Neoleucinodes elegantalis* (Lepidoptera: Crambidae).** Adviser: Eraldo Rodrigues de Lima. Co-Advisers: Og Francisco Fonseca de Souza e Ângelo Pallini Filho.

The searching behaviour in insects is important to acquire food, meeting mates, oviposition sites location and protect the insects against predators. Thus, the majority of herbivorous insects select their host plant and, in particular, the plant´s part, which it will feed and oviposit on, using, among other tools, host plants semiochemicals. This paper aims: (i) verify the role of host plant kairomones in calling and mating behaviours of *N. elegantalis*, (ii) investigate larval density effect on survivorship and larval development, (iii) investigate the role of chemical cues in general oviposition behaviour of *N. elegantalis*, (iv) Identify whether chemical cues are involved in discrimination by *N. elegantalis* females among different plant parts, (v) investigate whether chemicals extracted in hexane and ethanol solvents elicit different female oviposition responses, (vi) evaluate the behavioral response of *N. elegantalis* females to compounds emitted by tomato plants induced after herbivory. Some parameters of reproductive behavior have changed in the presence of kairomones of the host plant. The time spent calling as well as the mating duration, was lower in the presence of host-plant odors. Regarding the effect of larval density on the parameters of survival and development, pupae of lower weight were recorded in higher densities, as well as higher mortality rate of pupae and adults. The study of oviposition behavior mediated by chemical tools showed that when host plant volatile from flower, leaf and fruit are offered there is none oviposition preference, however in the presence of surface chemical compounds, females prefer to lay eggs on fruit extract compared with extracts of leaves and flowers of tomato. In addition, females recognize, through chemical cues, healthy fruits and fruit infested by larvae of conspecifics, and prefer to lay eggs on infested fruits.

INTRODUÇÃO GERAL

Neoleucinodes elegantalis (A broca-pequena)

A broca-pequena-do-tomateiro (*Neoleucinodes elegantalis* Guenée) (Figura 1) é uma praga oligófaga (que se alimentam de um número de plantas, geralmente em diferentes gêneros dentro de uma mesma familia) e apresenta, além do tomate, outras espécies de solanáceas como hospedeiros, tais como a berinjela, jiló e pimentão (Hambleton, 1935; Toledo, 1948; Biezanko, 1949; Robbs, 1962; Silva *et al*., 1968; Zucchi *et al*., 1993; Gallo *et al*., 2002). Essa espécie também apresenta como potenciais hospedeiros os frutos de algumas ervas daninhas pertencentes à Solanaceae, como o joá grande (*Solanum ovigerum*), o joá pequeno (*S. reflexus*), o joá vermelho (*S. ciliatum*), o joá doce (*S. sisymbrifolim*), assim como a jurubeba (*S. robustum*) (Benvenga, 2009).

N. elegantalis apresenta ampla distribuição na região Neotropical, ocorrendo do México à Argentina (Leiderman & Sauer, 1953; Zucchi *et al*., 1993). No Brasil, a primeira constatação da existência desse inseto foi feita por Costa Lima em 1922 no Nordeste (Leiderman & Sauer, 1953; Toledo, 1948), e atualmente é considerada uma das pragas principais do tomate em várias regiões produtoras da cultura, em função do ataque às partes reprodutivas da planta (Jordão & Nakano, 2002; Gravena & Bevenga, 2003). Além dos danos diretos causados aos frutos, sabe-se da ocorrência de patógenos que se beneficiam dos orifícios de entrada feitos pelas larvas no momento da penetração do fruto (Gravena & Bevenga, 2003) e, ainda, do baixo poder de germinação das sementes provenientes de furos infestados (Reis *et al*., 1989).

A infestação da broca-pequena em campo pode ser determinada pelas condições climáticas e regionais. Segundo Monte (1933), a partir de observações feitas em Minas Gerais, suas posturas podem ser encontradas facilmente no período de agosto a setembro e, as pupas no inicio de outubro. Os adultos de *N. elegantalis* apresentam em média 25 mm de envergadura. *N. elegantalis* apresenta dimorfismo sexual, sendo as fêmeas de maior peso e comprimento do corpo em relação aos machos (Jaffé *et al*., 2007). As mariposas apresentam coloração geral branca, asas transparentes, trazendo nas anteriores, uma mancha cor de tijolo, e nas posteriores, pequenas manchas marrons esparsas (Muñoz *et al*., 1991; Gallo *et al*., 2002). Outra diferença com relação a fêmeas e machos é que a fêmea possui abdome volumoso, com a parte final truncada, enquanto que o macho apresenta o abdome delgado, com a parte final aguda e recoberta por um tufo de escamas em forma de pincel (Muñoz *et al*., 1991; Carneiro *et al*., 1998). A razão sexual nessa espécie é de 1:1 e a reprodução é sexuada, não havendo, partenogênese (Muñoz *et al*., 1991; Salas, 1992; Carneiro *et al*., 1998; Jaffé *et al*., 2007)

Uma fêmea pode ovipositar até mais de 160 ovos durante sua vida (Marcano, 1991). Os ovos apresentam coloração que varia de branco leitoso, quando recém depositados, até avermelhada próximo da eclosão e são postos, durante a noite, isolados ou em massas de dois a três, diretamente em flores, no pecíolo, cálice ou superfície de frutos (1-3 cm de diâmetro) (Toledo, 1948; Carneiro *et al*., 1998; Blackmer *et al*., 2001; Muñoz *et al*., 1991; Souza, 2001; Gallo *et al*., 2002). De formato elíptico os ovos apresentam largura e comprimento médio de 0,46 e 0,69 milímetros, respectivamente (Muñoz *et al*., 1991).

As larvas de *N. elegantalis* eclodem em cinco a sete dias (Marcano, 1991) e o desenvolvimento larval apresenta-se em cinco ínstares (Muñoz *et al*., 1991). A eclosão ocorre nas duas primeiras horas da fotofase e as lagartas recém eclodidas possuem coloração amarelada (Muñoz *et al*., 1991) enquanto que no último ínstar apresentam coloração rosada uniforme com tamanho de 11 a 13 milímetros de comprimento (Carneiro *et al*., 1998; Gallo *et al*., 2002). Nesta fase passam cerca de 50 minutos caminhando na superfície do fruto (Eiras & Blackmer, 2003), em seguida penetram nos frutos onde se alimentam e permanecem até a pupação (Toledo, 1948). Em condições de moderadas a altas infestações há registros de até 14 larvas/ fruto (Muñoz, *et al*., 1991), mas é necessário apenas uma larva por fruto para torná-lo inapropriado (Toledo, 1949; Salas, 1992). Após o período de alimentação, 15 a 32 dias (Leiderman & Sauer, 1953) as lagartas saem dos frutos através de orifícios de saída, deslocando-se para o solo e neste, tecem um casulo em detritos próximos à planta (Souza, 2001), onde permanecem nas fases de pré-pupa e pupa (Carneiro *et al*., 1998; Gallo *et al*., 2002).

Na fase de pré-pupa, apresenta tamanho reduzido, e possui coloração esbranquiçada. Nessa fase torna-se pouco móvel e inicia a confecção da câmara pupal. A pupa possui coloração variável de amarelo claro à marrom escuro, de acordo com o período de duração. É possível verificar dimorfismo sexual das fêmeas que apresentam abertura genital no início do oitavo segmento abdominal em relação aos machos, que aparece na parte mediana do nono segmento abdominal. O comprimento médio das pupas das fêmeas e dos machos é de 11,05 e 10,33 mm, respectivamente segundo Muñoz *et al*. (1991).

O ciclo biológico de *N. elegantalis* (ovo a adulto) sofre variações em função da temperatura e umidade e, em frutos de tomate dura em média 43,7 dias e a longevidade dos adultos é de 7,2 dias na temperatura de 20° C e 93% de umidade, já na temperatura de 25° C e a 65,6% de umidade, o ciclo de vida completou-se em 30,1 dias e a longevidade foi de 4,6 dias (Marcano, 1991a).

A emergência dos adultos de *N. elegantalis* obedece a ritmos controlados (circadianos) sendo este comportamento bastante comum em Lepidoptera (Matthews & Matthews, 1988; Horodyski, 1996) e ocorre entre a primeira e sétima hora após o inicio da escotofase. Segundo Marcano (1991a), em condições laboratoriais, os adultos permanecem imóveis durante todo o dia (fotofase), com as asas estendidas lateralmente e o abdome recurvado. A maior atividade de cópula foi registrada entre a sexta e sétima hora da escotofase (Marcano, 1991a) e entre a quinta e sexta hora da escotofase (Jaffé *et al*., 2007). A oviposição ocorre entre primeira e décima terceira hora da escotofase, neste período a fêmea varre a superfície do fruto com o abdome recurvado, distendendo-o em seguida para a oviposição.

N. elegantalis é uma mariposa de hábito noturno e são poucos os registros sobre o comportamento de acasalamento e oviposição das fêmeas. Sabe-se que em campo as fêmeas virgens atraem os machos (Miras *et al*., 1997) e, segundo Eiras (2000), o período de chamamento inicia-se durante a escotofase com acasalamento de *N. elegantalis,* em plantações de tomate, entre a sexta e nona hora e, em condições de laboratório, entre a quarta e décim hora da escotofase.

Em *N. elegantalis* o acasalamento se dá após 48 a 72 horas da emergência, também na escotofase, seguido do inicio da oviposição (Eiras, 2000) o que é comum na maioria dos lepidópteros (Roelofs & Cardé, 1987). Jaffé *et al*. (2007) em estudo mais detalhado sobre o comportamento de cópula dos adultos de *N. elegantalis* registraram que não ocorre copula na data da emergência, sendo o maior número de cópulas (52%) após 24 h de emergência. Entretanto, segundo Marcano (1991a) é possível observar cópula entre adultos recém emergidos. A duração da cópula pode alcançar até quatro horas.

Quanto à distribuição de ovos, esta depende do nível de infestação, sendo que a maior parte é depositada na superfície do fruto ou na face inferior do cálice (Blackmer *et al*., 2001). Em baixas infestações (inferiores a 50% das plantas) a distribuição de ovos ocorre no pecíolo, cálice (face superior e inferior) e fruto, com maior concentração de ovos na porção inferior do cálice. Entretanto, em infestações maiores que 50%, a distribuição de ovos se concentra nos frutos. Há também registros de ovos sobre folhas e ramos caulinares (Millán *et al*., 1999). Ainda com relação à oviposição, há preferência por frutos em estádio de desenvolvimento específico, segundo Rodrigues Filho *et al*. (2003), em estudos de campo, o maior número de posturas foi registrado em frutos com diâmetro médio de 2,5 cm, sendo observada uma variação de 1,0 a 3,9 cm. Segundo Muñoz *et al*. (1991), a preferência de postura sobre frutos pequenos é para garantir o desenvolvimento larval do inseto sincronizando-o com o tempo de maturação dos frutos.

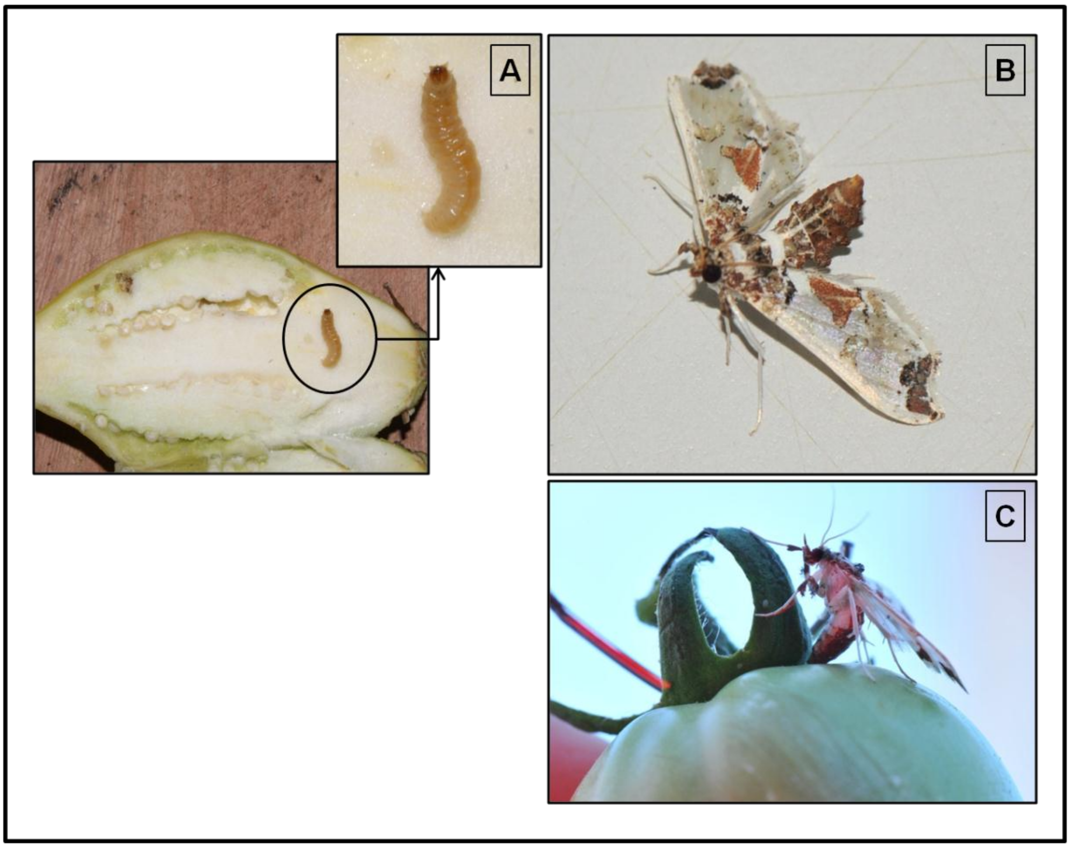

Figura 1: A broca-pequena-do-tomateiro, *Neoleucinodes elegantalis*, (A) larva em fruto de Jiló, (B) adulto (fêmea) e (C) oviposição em fruto de tomate. Fotos de Hernane Dias.

Cairomônios (voláteis de plantas)

Semioquímicos ("sinais químicos") ou infoquímicos são substâncias químicas envolvidas na comunicação entre os organismos (Vilela & Della Lucia, 2001). Se estas substâncias são transmitidas entre indivíduos de espécies diferentes elas são denominadas aleloquímicos e são classificados como cairomônios, alomônios e sinomônios (Figura 2) (Gullan & Cranston, 1994; Vilela & Della Lucia, 2001). Dentro do contexto estudado nesse trabalho, cairomônios são substâncias que as plantas produzem e que são percebidas e usadas pelos insetos para localização de sítios de alimentaçãoe/ou oviposição.

Diversas substâncias químicas podem ser sintetizadas por uma ampla gama de gêneros ou famílias de plantas e os cairomônios são aquelas capazes de regular a seleção do hospedeiro por insetos herbívoros os quais podem ser monófagos, oligófagos ou polífagos (Metcalf & Metcalf, 1992).

Insetos monófagos são aqueles que se alimentam de uma única espécie de planta podendo se extender àqueles que se alimentam de várias espécies dentro de um mesmo gênero. O termo Oligófago refere-se aos insetos que se alimentam de um número de plantas, geralmente em diferentes gêneros dentro de uma mesma familia. Polífagos são aqueles insetos que se alimentam em um número relativamente amplo de plantas de diferentes famílias (Bernays & Chapman, 1994).

Os voláteis que as plantas emitem são principalmente metabólitos secundários, liberados, tanto por plantas intactas como por tecidos de plantas danificados, e podem ser classificados como gerais, "voláteis verdes" ou específicos, aqueles restritos a certos grupos de plantas (Schoonhoven *et al*., 1998). A mistura dos compostos liberados pelas plantas, a partir da abertura dos estômatos, pode variar entre espécies e raças, ou ainda em função de fatores abióticos e bióticos (Paré & Tumlinson, 1999). Além disso, as quantidades de compostos liberados podem ser específicas de uma determinada espécie de planta e os insetos podem perceber essas variações usando-as para discriminar espécies hospedeiras daquelas não hospedeiras (Schoonhoven *et al*., 1998).

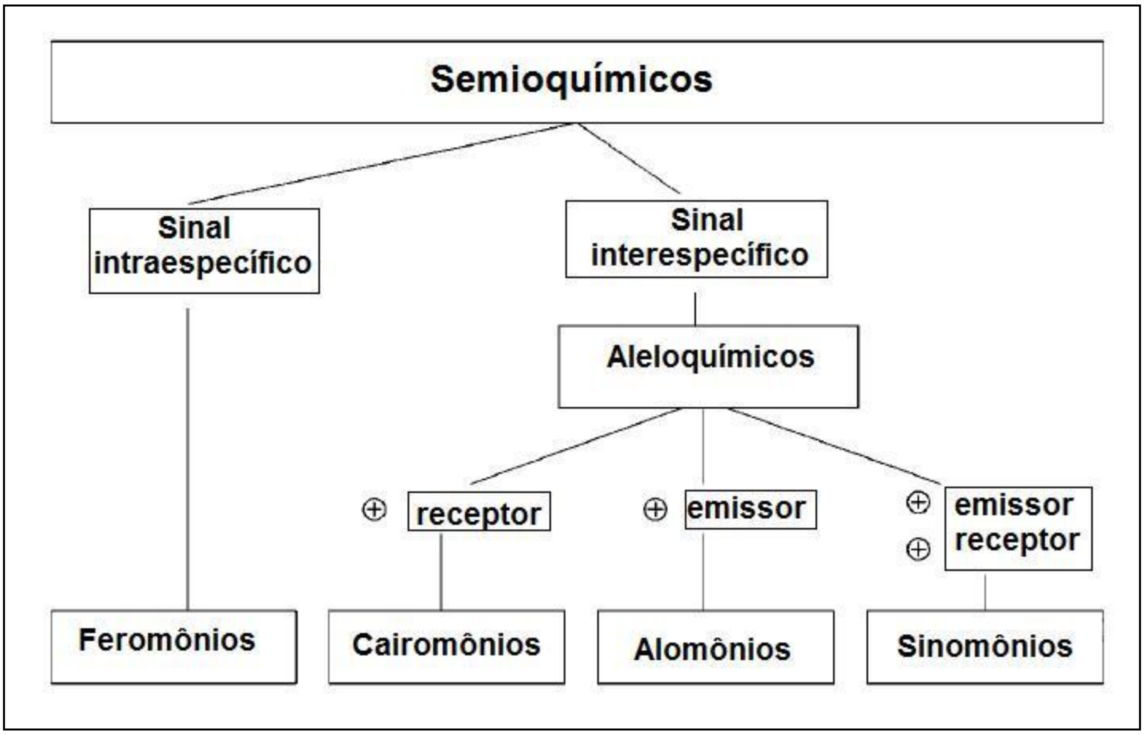

Figura 2: Classificação dos compostos químicos (semioquímicos) mediadores das interações entre os organismos, de acordo com o efeito sobre os organismos envolvidos. ⊕ = benefício. Adaptado de Ansebo (2004).

Compostos (Voláteis e não-voláteis) de atração e oviposição

Os compostos emitidos pelas plantas podem atuar como repelentes, atraentes, deterrentes, assim como estimulantes de oviposição e alimentação (Bernays & Chapman, 1994). Segundo Renwick e Chew (1994) a atração aos voláteis das plantas nem sempre está relacionada ao encontro de um local de oviposição, podendo o inseto estar em busca de fontes de néctar, como ocorre em *Pectinophora gossypiella*, em resposta aos voláteis das flores do algodão, *Lobesia botrana*, em flores de *Tanacetum vulgare* e *Agrotis ipsilon*, aos voláteis de flores de diferentes plantas.

Os compostos responsáveis pela atração dos insetos são altamente voláteis e são percebidos por quimioreceptores olfatórios (sensila olfatória). No entanto, os compostos envolvidos com o processo de oviposição são detectados tanto por quimioreceptores olfatórios, como de contato (Renwick & Chew, 1994). Exemplos de quimiorecepção de contato ocorrem em *Pieris brassicae* e *Plutella maculipennis* em que fêmeas depositam seus ovos em qualquer superfície provida de substâncias secundárias de plantas, sinigrina e alil-isotiocianato, respectivamente (Schoonhoven, 1967).

Há muitos exemplos de insetos atraídos por voláteis de plantas hospedeiras, sejam esses monófagos (*Psila rosae*, *Acrolepiopsis assectella*, *Agraulis vanillae*) ou polífagos, *Heliothis virescens* e *Tricoplusia ni* (Bernays & Chapman, 1994). E, ainda alguns exemplos quanto ao estimulo à oviposição mediada por voláteis de plantas, como *Papilio demoleus* na presença dos voláteis de citrus, *Papilio polyxenes* em plantas artificiais tratadas com voláteis de cenoura (Renwick e Chew, 1994).

O papel dos voláteis no comportamento e reprodução do inseto

Metcalf & Metcalf (1992) sugeriram que o sistema olfatório dos insetos atuais originou-se a 500 milhões de anos atrás, na era Paleozóica, e que plantas e insetos têm co-evoluído desde então. Para a maioria das espécies de insetos e, particularmente para mariposas, as ferramentas envolvidas no reconhecimento de odores fornecem informações biologicamente importantes. Os semioquímicos emitidos por uma diversidade de grupos de plantas estão envolvidos diretamente com o processo de seleção de hospedeiro pelos insetos herbívoros. Alguns autores afirmam que a localização da planta hospedeira por um inseto fitófago é crucial para a aquisição de alimento, assim como, encontrar locais disponíveis para oviposição (Schoonhoven *et al*., 1998; Paré & Tumlinson, 1999; Bruce *et al.*, 2005).

A atmosfera é formada por uma complexa mistura de milhões de componentes voláteis (Ansebo, 2004) e sabe-se que os insetos, apesar de possuirem um número pequeno de receptores olfatórios (50), desenvolveram uma sensibilidade extrema a certos grupos de odores (Keller & Vosshall, 2003). Muitas espécies de insetos apresentam ferramentas especializadas na procura por hospedeiros, seja para alimentação, localização de parceiros e, ou de sítios de oviposição (Ansebo, 2004). Dentro desse contexto, os voláteis emitidos pelas plantas servem de pista no reconhecimento eficiente das plantas hospedeiras pelos insetos herbívoros (Bernays & Chapman, 1994; Schoonhoven, 1998; Bengtsson *et al*, 2006). Entretanto, dentre os compostos que são detectados, apenas poucos são responsáveis por elicitar respostas comportamentais do inseto (Thiéry & Visser, 1986; Cossé & Baker, 1999; Rojas, 1999; Zhang *et al.*, 1999). Ainda, faz necessário conhecer quais os compostos emitidos pelas plantas intactas são necessários e suficientes para desencadear os comportamentos de vôo e oviposição.

O processo de seleção do hospedeiro compreende duas etapas, primeiro o inseto escolhe o seu hospedeiro, à distância, utilizando-se de recursos olfatórios e visuais e, por último, reconhece o hospedeiro, somente após o contato, quando recursos gustativos são empregados. Após a combinação dessas ferramentas sensoriais, a planta é reconhecida como hospedeira e a atração ocorre (Bernays & Chapman, 1994; Schoonhoven, 1998; Bruce *et al*., 2005).

A escolha do hospedeiro pelas fêmeas grávidas é um importante passo já que as larvas são de pouca mobilidade, não podendo migrar a longas distâncias e dependem, em muitos casos, da escolha feita pela fêmea adulta para o seu desenvolvimento e sobrevivência. É provável que uma vez grávidas, as fêmeas procurem um hospedeiro disponível para oviposição e este comportamento é mediado por alterações hormonais no inseto.

A atração de fêmeas aos odores da planta hospedeira pôde ser constatada em várias espécies de lepidópteros, dentre essas, as mariposas *Lobesia botrana*, aos voláteis emitidos por *Vitis vinifera* (Tasin *et al*., 2006; Masante-Roca *et al*., 2007) e, *Cydia pomonella* e *Argyresthia conjugela*, aos voláteis dos frutos de maçã (Yan *et al*., 1999; Ansebo *et al*., 2004).

Respostas positivas foram registradas em ensaios de laboratório com fêmeas de *Heliothis virescens*, mediante os voláteis extraídos de algodão, tabaco e *Desmodium tortuosum* (erva daninha). Com exceção de *D. tortuosum*, a resposta aumentou positivamente com a dose e teve seu máximo com extratos de botões florais de algodão. Quase todas as mariposas que pousaram no liberador do extrato, também ovipositaram após a execução dos comportamentos de procura do hospedeiro, como vôo orientado para a fonte de odor e exame com as antenas (Mitchell *et al*. , 1991).

A localização do hospedeiro em mariposas é amplamente orientada pelos voláteis fitoquímicos (Ansebo *et al*., 2004), principalmente mono e sesquiterpenoides (Bernays & Chapman, 1994; Schoonhoven *et al*., 1998). Insetos fitófagos encontram parceiros e acasalam na planta hospedeira e seu comportamento sexual pode ocorrer principalmente ou exclusivamente nessas plantas (Landolt & Phillips, 1997) e ainda, os voláteis da planta podem estar envolvidos na produção e liberação, ou a percepção de feromônio sexual pelos insetos (McNeil & Delisle, 1989; Landolt & Phillips, 1997).

As interações entre semioquímicos e feromônio sexual manifestam-se como efeito da planta hospedeira na fisiologia e comportamento, refletindo diferentes estratégias dos insetos para aperfeiçoar alimentação, acasalamento e reprodução (Reddy & Guerrero, 2004). A habilidade que as fêmeas apresentam em parar a produção de feromônio ou a liberação até que o hospedeiro seja localizado coordena seu comportamento reprodutivo com a disponibilidade de alimento para seus descendentes (Raina *et al*. 1992). Dentro desse contexto, vários estudos têm relatado a influência da planta hospedeira no comportamento sexual de insetos herbívoros (Herrebout & Van de Water, 1982; Hendrikse & Vos-Bunnemeyer, 1987; McNeil & Delisle, 1989). Riddiford & Williams (1967) foram os primeiros a sugerir que o comportamento de chamamento de uma fêmea de Lepidoptera pode ser alterado pelos voláteis da planta hospedeira. Em contraste, Groot & Visser (2001) concluíram que os voláteis da planta hospedeira não estão diretamente envolvidos na produção de feromônio em *Lygocoris pabulinus*. Cardé & Taschenberg (1984) também não encontraram evidências de que os voláteis da planta hospedeira influenciaram no comportamento de chamamento de *Antheraea polyphemus*.

Disponibilidade de alimento e desenvolvimento larval.

Nos insetos holometábolos, a morfologia do adulto e seu desempenho são influenciados pela disponibilidade e alocação de recursos provenientes da alimentação nas fases de larva e adulto (Boggs & Freeman, 2005). Assim, os nutrientes absorvidos pelos adultos e aqueles armazenados na fase larval são então usados para dar suporte às funções de reprodução, sobrevivência e dispersão (Boggs & Freeman, 2005). Estudos revelam que uma redução na qualidade ou quantidade de alimento larval geralmente resulta em adultos com reduzido tamanho corporal e, conseqüentemente, isso tem efeitos negativos sobre e fecundidade (Scriber & Slansky, 1981; Ernsting *et al*., 1992; Fischer & Fiedler, 2001; Awmack & Leather, 2002).

Uma vez que a dieta da maioria dos adultos lepidópteros consiste principalmente de carboidratos e água e, como os constituintes reprodutivos contêm grandes proporções de nitrogênio e lipídios, além dos carboidratos e água (Marshall,1982), então a reprodução de machos e fêmeas torna-se dependente, em um elevado grau, dos recursos adquiridos durante o estágio larval (Karlsson & Wickman, 1990). Estudos com insetos fitófagos revelam que o desempenho dos indivíduos varia em função do hospedeiro (Tabashnik *et al*., 1981; Lance, 1983; Singer *et al*., 1991), assim como, na ocorrência de competição por recurso. A competição é um fator que afeta o desenvolvimeto geralmente porque variações na densidade afetam a disponibilidade de recurso alimentar para as larvas (Putman, 1977; Applebaum & Heifetz, 1999).

Dois tipos de competição por alimento pode ser descritos. No primeiro, ocorre divisão dos recursos igualmente entre os indivíduos que, conseqüentemente sofrem igualmente com a redução na disponibilidade de recurso e, o segundo, em que alguns indivíduos são mais aptos a explorar os recursos mais eficientemente que os demais (Nicholson, 1954). A competição larval, assim, pode afetar tanto na evolução do comportamento dos insetos como na história de vida (Smith & Lessells, 1985). A competição intraespecífica por recurso alimentar pode ser um importante fator que afeta o desenvolvimento e as características de organismos adultos. Além disso, uma redução na disponibilidade de alimento devido à competição durante o desenvolvimento pode afetar tanto o comportamento quanto o desempenho dos adultos (Marshal, 1990; Mueller, 1997; Hirschberger, 1999).

O aumento na densidade de insetos reduz a disponibilidade de recursos por indivíduo, o que se reflete em aumento na mortalidade juvenil (Livdahl, 1982; Simmons, 1987; Agnew *et al*., 2002). Nos insetos o tamanho maior está geralmente correlacionado com alta fecundidade (Honék, 1993), enquanto que o tempo de desenvolvimento menor possibilita maior probabilidade de sobrevivência na fase adulta (Sibly & Calow, 1986). Sabe-se que uma redução na disponibilidade dos recursos pode se refletir em um prolongado período de desenvolvimento e na redução no tamanho do adulto (Stearns & Koella, 1986; Roff, 1992; Stearns, 1992; Abrams *et al*., 1996; Nylin & Gotthard, 1998).

Interação entre compostos de plantas induzidos por herbivoria e insetos herbívoros.

Em resposta aos danos por herbivoria plantas sintetizam e liberam misturas de compostos voláteis que atuam como defesa indireta contra os insetos responsáveis pelos danos (Vet & Dicke, 1992; De Moraes *et al*., 1998; Kessler & Baldwin, 2001; de Boer *et al*., 2004; Turlings & Wackers, 2004).

Esses compostos desempenham importantes papéis como mediadores de comportamento de procura e aceitação da planta hospedeira nos insetos fitófagos, além de atrair parasitóides ou predadores de insetos praga e, também direcionam a oviposição assim como influenciam nas interações entre plantas (Visser, 1986; Anderson & Alborn, 1999; Dicke & Van Loon, 2000; Wink, 2003; Dudareva *et al*., 2004; Konstantopoulou *et al*., 2004; Steinbauer *et al*., 2004; Rassmann *et al*., 2005; Ruther & Kleier, 2005; Carroll *et al*., 2006; Raguso, 2008).

Sabe-se que plantas de tomate (*Lycopersicon esculentum*) são conhecidas por liberar compostos, emitidos após danos mecânicos e por herbivoria, que atuam como defesa indireta da planta (Takabayashi & Dicke, 1993). Danos causados por herbívoros, incluindo em plantas de tomate, podem causar aumento nos níveis de ácido jasmonico (JA) e ácido salicílico (AS) (Doares *et al*., 1995). Ambos compostos são components chave das respostas das plantas ao ataque de herbívoros e importantes na resistência de plantas aos insetos (Felton & Korth, 2000).

Nos insetos herbívoros, a presença de coespecíficos na planta hospedeira pode influenciar nas decisões da fêmea com relação à seleção do local para oviposição (Nufio & Papaj, 2001; Prokopy & Roitberg, 2001). Embora pareça obvio que a fêmea adulta deva selecionar a planta ou a parte da planta na qual o desenvolvimento de suas larvas seja o melhor, em algumas espécies isso ocorre, de modo que fêmeas ovipositam mais em plantas ocupadas por larvas de coespecíficos (Rausher, 1979; Rausher & Papaj, 1983; Quiring & McNeill, 1987; Damman & Feeny, 1988; Preszler & Price, 1988; Craig, Itami & Price, 1989; Minkenberg & Fredrix, 1989, Baur *et al*., 1996; Shiojiri & Takabayashi, 2003), ao passo que em outras espécies as fêmeas preferem ovipositar em plantas livres de coespecíficos (Prokopy, 1972; Mitchell, 1975; Rausher, 1979; Mackay, 1982, 1985; Karban & Courtney, 1987; Futuyma & Moreno 1988; Auerbach & Simberloff, 1989, Sato *et al*., 1999, de Moraes *et al*., 2001).

As respostas podem variar dependendo do contexto em que as espécies estão inseridas (Nufio & Papaj, 2001; Prokopy & Roitberg, 2001). Desse modo, dentro de uma determinada condição ecológica e/ou fisológica, uma fêmea pode ovipositar ou não em um hopedeiro já ocupado por outro da mesma espécie (Papaj *et al*., 1992; Papaj e Messing 1996). Além disso, as respostas também dependerão da extensão alimentar desses insetos. Assim, especialistas, por exemplo, podem responder positivamente àquelas mudanças sofridas pela planta, ao passo que generalistas podem ser negativamente afetados (Karban & Baldwin, 1997).

Respostas positivas de insetos aos compostos emitidos após herbivoria podem ser explicadas pelo fato de que voláteis de plantas induzidos por herbivoria são ferramentas altamente detectáveis que revelam a identidade dos herbívoros e a intensidade do dano. Além disso, as plantas danificadas são muito mais aparentes que plantas não danificadas, as quais apresentam menores taxas de emissão daqueles compostos (Schoonhoven *et al.*, 1998).

Exemplos de respostas positivas e negativas podem ser observados em diferentes espécies. Fêmea de *Cydia pomonella* (Lepidoptera: Tortricidae) foram mais atraídas por maçãs infestadas por larvas de coespecíficos do que por frutos não infestadoss (Hern & Dorn, 2002). Do mesmo modo, fêmeas de *Leptinotarsa decemlineata* (Coleoptera: Crhysomelidae), espécie também especialista, foram mais atraídas por compostos produzidos pelas plantas de batata em resposta à herbivoria por coespecíficos (Landolt *et al*., 1999). *Delia radicum* (Diptera: Anthomyiidae) mostrou preferência por plantas cujas raízes haviam sido danificadas por larvas da mesma espécie quando comparadas com plantas sadias (Baur *et al*., 1996) e diferentes estudos com *Plutella xylostella* (Lepidoptera: Plutellidae), mostraram que as fêmeas preferiram plantas danificadas do que plantas não danificadas (Uematsu & Sakanoshita,1993; Shiojiri & Takabayashi, 2003). Em contraste, alguns exemplos de respostas negativas aos danos causados por herbivoria podem ser vistas em *Pieris rapae* (Lepidoptera: Pieridae) (Sato *et al*., 1999), *Pieris brassicae* (Lepidoptera: Pieridae) (Schoonhoven, 1990), *Heliothis virescens* (Lepidoptera: Noctuidae) (de Moraes *et al*., 2001) e *Phtorimaea operculella* (Lepidoptera: Gelechiidae) (Arab *et al*., 2007). Compostos de plantas induzidos por herbivoria podem tornar as plantas mais visíveis entre plantas vizinhas não danificadas e, como resultados, aquelas plantas atraem mais herbívoros (Dicke & Vet, 1999). Uma vez que as plantas danificadas mecanicamente emitem *blends* diferenciados daquelas danificadas por herbívoros, então, é disponibilizada para os insetos herbívoros uma informação específica sobre o nível de competição existente naquele hospedeiro (Dicke & Vet, 1999).

Diferentes respostas podem ser encontradas para uma mesma espécie e isso dependerá de aspectos relacionados com tamanho, idade da planta e época de coleta dos compostos. Por exemplo, *Spodoptera littoralis* (Lepidoptera: Noctuidae) preferiram ovipositar em plantas pequenas danificadas por larvas do que em plantas sadias.

Entretanto, quando foram utilizadas plantas maiores a resposta foi inversa (Anderson & Alborn, 1999). *Trichoplusia ni* (Lepidoptera: Noctuidae) foram mais atraídas para plantas de algodão infestadas e danificadas artificialmente quando comparadas com plantas sadias. No entanto, as respostas foram diferentes quando a espécie de planta foi trocada, ou seja ao oferecer plantas de repolho as fêmeas preferiram aquelas que não haviam sido danificadas, com as plantas sadias recebendo maior número de ovos (Landolt, 1993).

Objetivos

Este trabalho tem como objetivo geral determinar o papel dos semioquímicos do tomateiro nos comportamentos reprodutivo e de seleção de hospedeiro em *N. elegantalis*, tendo como objetivos específicos:

1. Determinar o papel dos cairomônios da planta hospedeira nos comportamentos de chamamento e acasalamento de *N. elegantalis*.
2. Investigar o papel das ferramentas químicas no comportamento geral de oviposição de *N. elegantalis.*
3. Identificar se ferramentas químicas estão envolvidas na discriminação entre diferentes partes da planta hospedeira por fêmeas de *N.* elegantalis, além de investigar se extratos hexanico e etanolico de frutos de tomate elicitam diferentes respostas de oviposição.
4. Verificar se há efeito da densidade de larvas nos parâmetros de sobrevivência e desenvolvimento larval
5. Avaliar as respostas comportamentais de adultos de *N. elegantalis* aos compostos emitidos por plantas de tomate induzidas após herbivoria.

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CAPÍTULO 1

Host plant chemical cues and reproductive behavior of *Neoleucinodes elegantalis*.

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Abstract- Conflicting results exist concerning host plant effects on pheromone production and sexual behavior in moths. Some experiments confirm the effect of plant volatiles on sexual behavior, while others show that plant volatiles are not directly involved in pheromone production. This divergence has been explained as a consequence of insect host plant range; therefore, the insect-plant interaction would be most likely to occur in species that are monophagous or oligophagous. The insect studied here was the tomato fruit borer, *Neoleucinodes elegantalis*, a specialist of solanaceous plants and an important economic pest of tomato fruits. We tested whether there are significant differences in the calling and mating behavior of *N. elegantalis* in the presence or absence of the host plant volatiles. Most females called for the first time the first day. Onset calling was not influenced by the presence or absence of host plant volatiles. Calling activity started at the sixth hour of the scotophase and was consistent regardless of treatments or calling age. However, the time spent calling was significantly less in the presence of tomato fruit extract. In both treatments, first-time mating occurred during the first night after emergence and did not differ between treatments. Onset of mating did not differ between treatments and began after the onset of the first scotophase. However, duration of the first mating was shorter in the presence of plant volatiles. In conclusion, volatiles released by tomato fruits had some significant behavioral alteration on tomato fruit borer reproduction.

Introduction

Host plant kairomones are commonly associated with the production, release or perception of sex pheromones in some phytophagous insects (McNeil & Delisle, 1989; Landolt & Phillips, 1997). Furthermore, they play an important role in the selection of oviposition sites by adult Lepidoptera (Feeny *et al*, 1983).

Herbivorous insects meet and mate on plants (Landolt & Phillips, 1997), so they oviposit on or near plants that provide food for their larvae (Schoonhoven *et al.*, 2005). These plants may release chemical signals that stimulate insect mating to synchronize oviposition and larval food availability (Raina *et al.*, 1992).

To attract conspecific males, mating in most moth species is mediated by female production and release of sex pheromones (Raina *et al.*, 1989). Production, release and perception of sex pheromone in moths can be mediated by host plant volatiles (Hendrikse & Vos-bunnemeyer, 1987; McNeil & Delisle, 1989; Landolt & Phillips, 1997; Groot & Visser, 2001; Reddy & Guerrero, 2004). However, contradictory studies have shown that the host plant is not directly involved in pheromone production (Gomez & Rojas, 2006; Groot & Visser, 2001; Cardé, 1984).

The tomato fruit borer, *Neoleucinodes elegantalis* (Lepidoptera: Crambidae), is an important pest of solanaceous plants (Toledo, 1948; Leiderman & Sauer, 1953). After eclosion, the larvae dig and subsequently feed inside the green tomato fruits, where they will develop until they leave to pupate in the soil (Blackmer *et al.*, 2001; Jaffé *et al.*, 2007). When females were provided with oviposition sites treated with or without surface chemical extracts from tomato fruits, considerably more eggs were deposited in the presence of tomato fruit surface extracts (Pontes *et al*. 2010). Subsequently, it was shown that females are more stimulated to oviposit in presence of surface extracts than in volatiles one (Gama *et al*., submitted). Because visual and chemical cues from host plant play a major role in site selection for the oviposition of Lepidoptera (Feeny *et al*., 1983) and given the importance of surface chemical extracts from tomato fruits for oviposition, we hypothesized that these cues might also play a role in the female calling and mating behaviors in *N. elegantalis*.

Moths are present on many different plants at differing times of day; therefore, pheromones are necessary to synchronize mating. In the case of the sunflower moth, *Homeossoma electellum*, sex pheromone production is retarded in the presence of host-plant pollen, which affects the synchronization of mating and oviposition (McNeil & Delisle, 1989). Larvae of the black army cutworm, *Actebia fennica*, feed primarily on a specific plant species that have been burned; their reproductive behavior is mediated by smoke volatiles, indicating the presence of its larval host food and low population densities of natural enemies (Everaerts *et al.*, 2000)

In tomato plants, such as *Solanum lycopersicum* Mill, or wild tomato, *Solanum granulosoleprosum* Dunal, single plants may contain both flowers and fruits at differing degrees of development (Cáceres & Moura, 2003). Thus, with the permanent availability of resources for larval development we can predict that *N. elegantalis* does not need to anticipate mating to identify a suitable oviposition site that contains the appropriate nutrients for offspring development. We examined the role of host plant odors on the sexual behavior of females by observing whether the mating and calling behavior of *N. elegantalis* was altered by the presence of host plant volatiles.

Methods

Insects and Experimental conditions

Neoleucinodes elegantalis were collected from a commercial tomato crop in Minas Gerais State, Brazil, and reared in the laboratory for 2 years. Larvae were reared on *Solanum gilo* until pupation. Pupae were sexed and separated in cages (50 x 50 x 50 cm) until emergence. After emergence, they were held separately in plastic containers so that females were isolated from males. Pupae and adults were housed at 25 ± 1 ºC and 71 ± 10 % RH with the light regime set to 12L:12D. Newly emerged virgin males and females were supplied with a 10 % honey-water solution.

Plant material and chemical extraction

Fruits *Solanum lycopersicum* Mill. were collected from greenhouse plants, grown from commercial seed (cv. Santa Clara) for collection of surface chemicals to be used in subsequent bioassays. Plants were watered daily and an N. P. K fertilizer treatment was applied at 15 days intervals. No fungicide nor insecticides treatments were performed on plants. Green tomato fruits were removed from plants to allow collection of surface chemicals. Surface chemicals were collected using the immersion technique (Breeden *et al.*, 1996). Green tomato fruits (3 cm in diameter) were placed in a glass container with hexane (1 mL for each fruit) and gently shaken in a brief extraction for 30 seconds to remove surface chemicals, resulting in a concentration of 1 equivalent fruit/mL of solvent. This solution was stored at -19 ºC until use.

Observations of calling behavior

Experiments started during the first complete scotophase after emergence. The observation was performed in a dark room under laboratory conditions mentioned above. Newly emerged females were separated in numbered plastic cages containing filter paper supplied with (i) 10 µL of a hexanoic tomato extract (1 equivalent fruit) or (ii) 10 µL of pure hexane as a control. Treatments were replaced every day before the beginning of the scotophase. Moths were supplied daily with a 10% solution of natural honey to ensure that females had food *ad libitum*. Two groups of fifty-five females were maintained separately in two rooms, under identical conditions, to avoid interference of odors. A flashlight covered by six layers of red cellophane assisted observations made during periods of darkness.

All emerged females were observed during the scotophase following eclosion. The day on which a female first displayed calling behavior was designated as calling day 1, and the subsequent days were designated as calling day 2, 3, …, 6. To determine the pattern of calling behavior, 110 females were observed every 10 min throughout scotophase for four days or until female death. Moths were considered to be calling if the ovipositor was extended. A calling bout was each event in which the ovipositor was extended. If a female called in only one observation, the calling bout was considered to have lasted 10 min. If a female was calling during two consecutive observations, it was considered as a calling bout of 20 min, and so on. We used this information to obtain the mean onset time of calling (MOTC, expressed as min. after the onset of the scotophase), the mean total time spent calling (MTSC), the number of calling bouts (NCB) and the duration of calling bout (DCB).

Mating experiments

Newly emerged male and female virgin moths were used for mating observations. Eighty pairs of moths were divided into two groups, randomly selected, and placed in cages (50 x 50 x 50 cm) at the end of the first photophase. These two groups were maintained separately in two rooms, as described above. The moth pairs were either given, on a piece of filter affixed to the cage ceiling by a piece of a wire, (i) 30 µL of hexanic tomato extract (1 equivalent fruit) or (ii) 30 µL of hexane as a control. Only moths emerging on the same day were paired; thus, the male and female in each pair were approximately the same age. Experimental conditions and the routine of changing tomato extract were executed as mentioned above. Moth pairs were subjected to observation starting from the first scotophase after emergence until the first mating was completed. The pairs were observed every 10 min throughout scotophase. The pairs were removed from the cage and maintained together in plastic containers until the end of mating. We used this information to obtain the number of mating moths, onset-time of the first mating and mating duration.

Statistical analysis

Reproductive behavior was observed using two treatments (tomato fruits extract and control). A total of 110 females were observed for changes in calling behavior, and two groups of 40 male-female pairs were observed for mating experiments.

All statistical analyses were performed using generalized linear modeling (Crawley, 2007) in the R statistical system (R Development Core Team, 2006). For calling behavior experiments, the number of females that initiated calling were analyzed using Poisson errors with a log link (Crawley, 2007), followed by residual analyses to verify error distribution and suitability of the models employed, including checks for over-dispersion. The other parameters evaluated were the age at which females started calling, mean time onset of calling, mean time spent calling, number of calling bouts and the duration of calling bouts. These parameters were analyzed using a generalized linear modeling followed by an ANOVA with test F.

Matings were analyzed with Poisson errors using log link (Crawley, 2007), followed by residual analyses to verify error distribution and suitability of the models employed, including checks for over-dispersion. Differences in the onset of first-time mating and mating duration in mating bioassays were tested with analysis of variance (ANOVA), followed by a test F.

Results

Calling behavior

The number of females that initiated calling varied considerably with the age of females in both the treatment and control groups ($F = 34.2465$; $P<0.01$). 72% of females called for the first time on the first day in the presence of extracts compared to the control group, which called 82% of the time. The age at which females started calling did not differ significantly with the presence of host plant chemicals ($F = 0.1643$ $P = 0.6862$) (Figure 1). Females provided with hexanoic extract ($n = 47$) or hexanes ($n = 47$) called 1.27 ± 0.45 and 1.23 ± 0.56 days following emergence, respectively. Because not all females initiated calling at the same chronological age, the calling age was used to compare calling behavior (Turgeon & McNeil, 1982). Thus, calling patterns were compared among individuals that started calling on the same day; similar comparisons were made for the subsequent calling days.

Calling activity was initiated at the sixth hour of scotophase for females in the presence or absence of tomato volatiles. The mean onset time of calling did not differ significantly between treatments (F = 0.0123, P = 0.91169) or with calling age (F = 0.4139; P = 0.5208) (Figure 2a). Nevertheless, the time spent calling showed significant differences between treatments and age. Females, in the presence of tomato fruit extract, spent significantly less time calling (F = 5.2906; P < 0.05) than did the control group. The mean time spent calling differed with female age (F = 7.2614; P < 0.01) and was the highest on the second day and the first day in the extract-treated and control group, respectively (83.33 ± 12.92 min and 115.95 ± 17.78 min, respectively) (Figure 2b). The number of calling bouts did not differ between treatments (F= 0.7062, P = 0.40183) or with female age (F= 2.9484; P = 0.0877) (Figure 2c). The duration of calling bouts did differ between treatments (F= 4.1698; P<0.05) but not among ages (F=2.3282; P = 0.1288) (Figure 2d). In the presence of tomato fruit extract, females called for shorter periods than the control group (26.64 ± 0.96 min and 35.54 ± 3.01 min, respectively) (Figure 3).

Mating behavior

The average age at which first-mating occurred in the presence of tomato volatiles was 1.23 ± 0.14 days compared to 1.23 ± 0.11 days for control moths. The occurrence of mating varied considerably with age of pairs ($X_{1,3}^2 = 2.153$ P<0.01) but not between treatments ($X_{1,4}^2 = 36.492$ P = 1.0). The highest occurrence of mating in both the presence and absence of host plant chemicals occurred on the first night after emergence (Figure 4).

The onset of mating did not differ between treatments (F = 0.475 P = 0.495); the pairs started mating at 258.23 ± 24.45 and 280.00 ± 23.71 min after the onset of the first scotophase in presence and absence of plant volatiles, respectively. The duration of the first-mating was significantly different in treatments (F= 4.423; P<0.05) with 139.28 ± 12.60 and 185.38 ± 20.86 min in the presence and absence of host plant volatiles, respectively (Figure 5).

Discussion

The onset of calling and mating in the presence of tomato fruit volatiles was not significantly different from that observed in the absence of tomato volatiles. However, the fact that females spent less time calling in the presence of tomato plant volatiles than in their absence suggests an interaction between females and tomato plants. This interaction does not reveal a relationship in synchronizing oviposition with host plant availability to achieve resources for larval development and survival.

It was demonstrated that the host plant odor affected the time that females spent calling and mating. The results also revealed that age influenced the number of females calling for the first time, with the largest number of females calling on their first day. This finding suggests that *N. elegantalis* females are reproductively mature upon adult emergence.

The influence of plants on calling behavior is mediated by host plant availability (Landolt & Phillips, 1997). This availability can stimulate insect behaviors that optimize feeding, mating and reproduction (Gomez & Rojas, 2006). Some moths, such as *Zamagiria dixolophella* (Gomez & Rojas, 2006) and *Antheraea polyphemus* (Cardé, 1984), do not require a host plant for pheromone production and release. However, several studies have shown that a host plant or its volatile chemicals stimulate the production, release and perception of the sex pheromone in some moth species (Hendrikse & Vos-bunnemeyer, 1987; Raina, 1988; McNeil & Delisle, 1989; Raina *et al*., 1992, 1997; Pittendrigh & Pivnick, 1993; Sadek & Anderson, 2007).

Some reproductive behavior was modulated by the presence of host plant volatiles. Compared to control conditions, the presence of host chemicals had effects on the duration of both calling and mating. This response seems to be, most likely, intended to find mates and avoid natural enemies. Nonetheless, it did not affect the other reproductive parameters evaluated. The reduction of time spent calling in the presence of host chemicals may reflect the time spent to find a mate, to mate and to successfully exploit resources once the host plant is identified as a suitable site to meet, mate and feed (Visser, 1986; Schoonhoven *et al.*, 2005; Bruce *et al.*, 2005).

The peak of first-time mating in paired moths was similar to that reached at the peak of first-calling in individually housed females (first scotophase). This result demonstrates the ability of males to perceive female pheromones and mate. Mating in moths usually occurs during a discrete period of a cycle and is nocturnal in most cases.

Temporal control of pheromone release is often critical to synchronize reproductive behavior (Raina, 1993). One of the characteristics of insects that have a short life span is that mating occurs shortly after emergence (Matthews & Matthews, 1988). This is in agreement with the fact that in non-migratory species, males respond to the female sex pheromone directly after hatching (Anderson *et al*., 2003). Furthermore, mating in insects is generally ruled by female readiness, manifested in calling, and the responsiveness of males (Sadek & Anderson, 2007).

The response of males to female pheromones may also be directly influenced by stimuli from host plants (Landolt & Phillips, 1997). Attraction to sex pheromone was enhanced by host plant volatiles in some Lepidoptera species, such as *Plutella xylostella* L. (Reddy & Guerrero, 2000), *Heliothis virescens* (Dickens *et al.*, 1993), *Heliothis zea* (Light *et al.*, 1993)and *Helicoverpa armigera* (Fang & Zhang, 2002).

Reduced time of mating were in accordance with the shorter time spent calling, exhibited by individually housed females in the presence of host plant chemical cues. Enhancement of the mating duration may increase the predation risk for both sexes (Rowe, 1994; Bissoondath & Wiklund, 1996). Thus, the lowest mating duration in the presence of the host plant could be explained by the fact that plant volatiles may be especially important in guiding predators to their prey’s habitat (Vet & Dicke, 1992; Turlings *et al*., 1991; Whitman, 1988; Price, 1986; Price *et al*., 1980).

Thus, by these work results we can infer that the host plant chemicals may act as (i) an indicator that there are nearby host plants where, consequently, the females can find a mate and (ii) an important cue to the insect evaluating the risk of predation when mating. We argue that plant semiochemicals are not directly involved in pheromone production, release (calling behavior) and perception (mating behavior) in *N. elegantalis* as suggested by Eiras (2000). Therefore, the role of host plant chemicals on insect pheromone production may not be a general rule in Lepidoptera and depends on the insect species studied.

Acknowledgments

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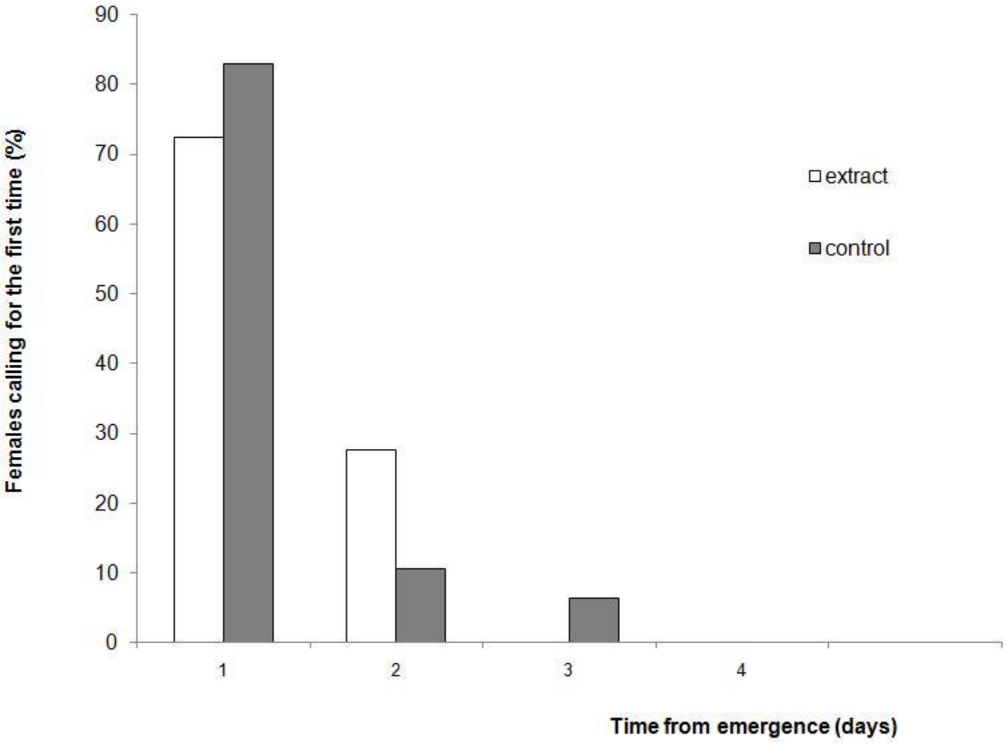

Figure 1: The age at which virgin females of *Neoleucinodes elegantalis* initiate calling behaviour for the first time following emergence when held in presence of hexanic extract of tomato fruit (1 eq. fruit) or only hexan.

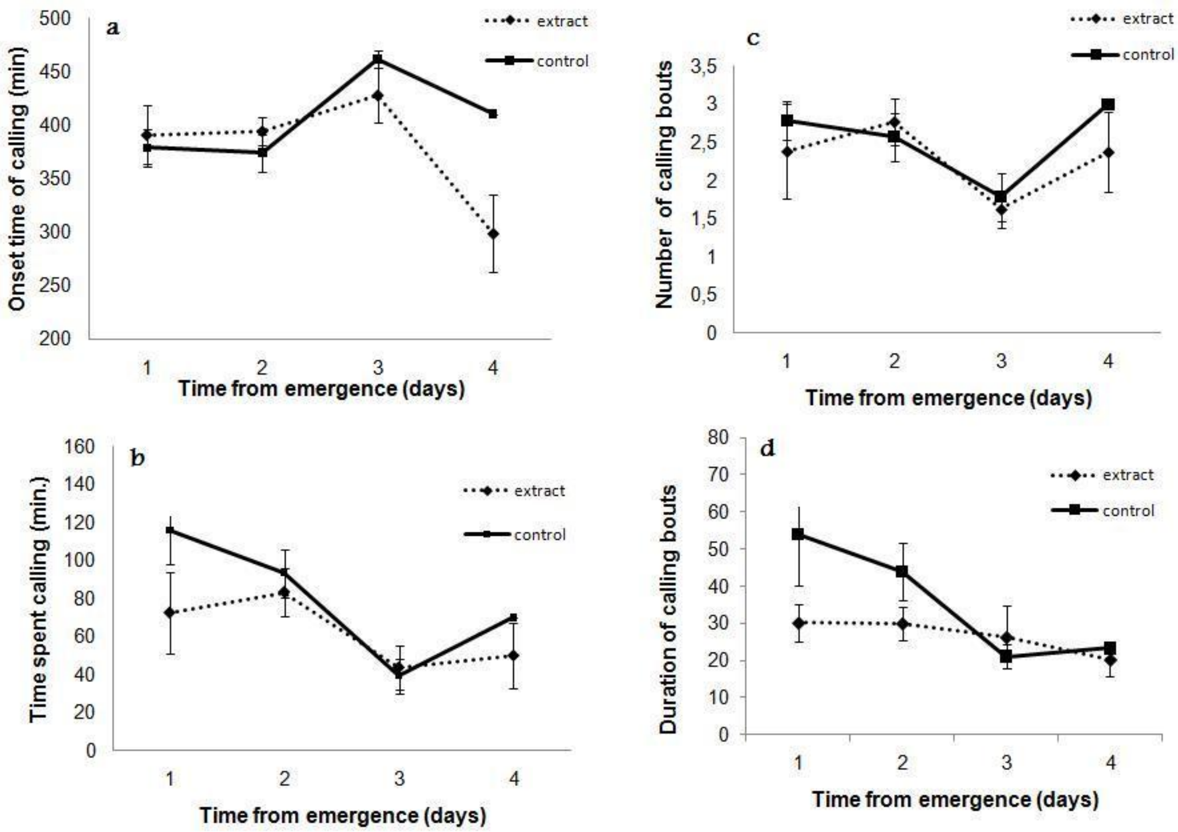

Figure 2: a) The mean onset time of calling (min. after lights off) (± S.E.), b) the mean time spent calling (min.) (± S.E.), c) the mean number of calling bouts (± S.E.), and d) the duration of calling bout (± S.E.), during the first four days of calling when virgin females of the *Neoleucinodes elegantalis* where held in presence of hexanic extract of tomato fruit (1 eq. fruit) or only hexane (control).

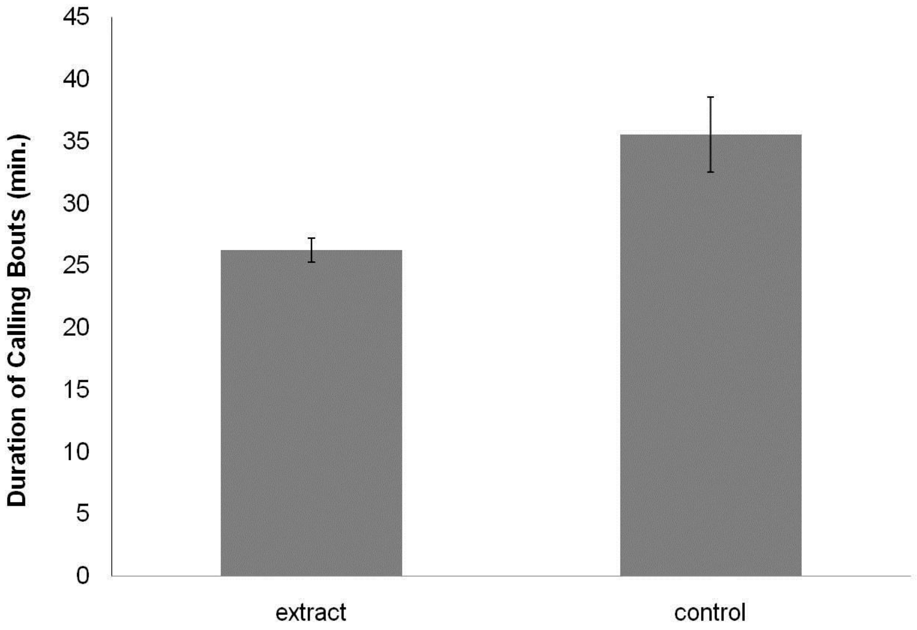

Figure 3: The mean duration of calling bout (± S.E.) for the four days of calling when virgin females of *Neoleucinodes elegantalis* where held in presence of hexanic extract of tomato fruit (1 eq. fruit) or only hexane (control).

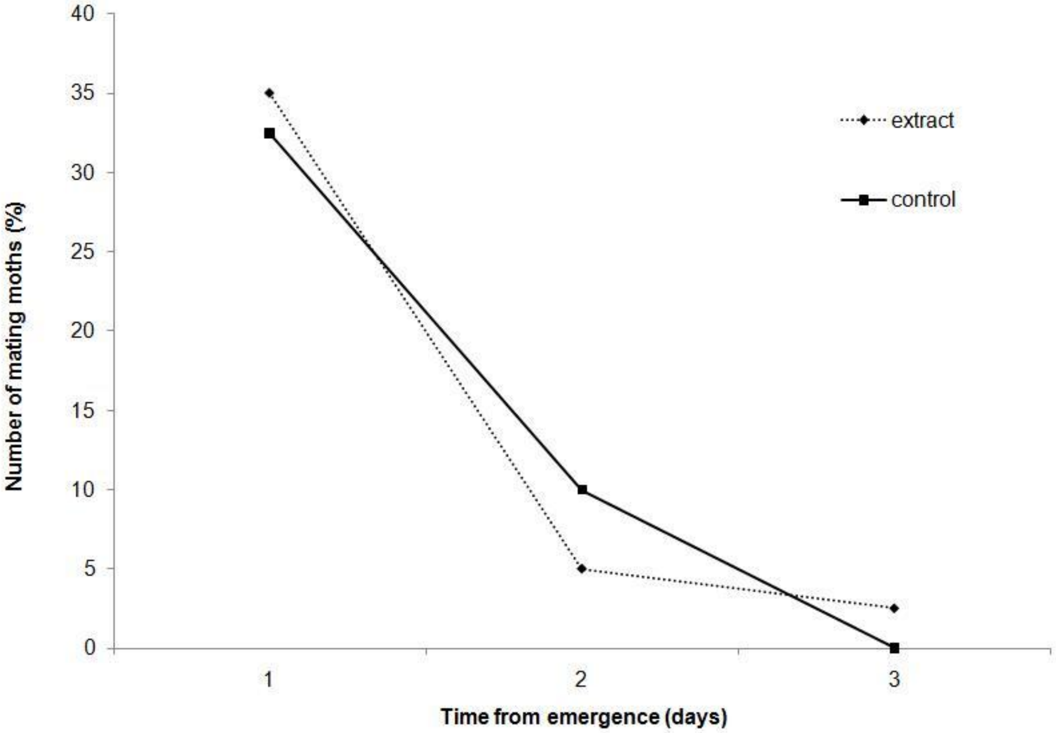

Figure 4: Temporal distribution of first mating in *Neoleucinodes elegantalis* when they were held in presence of hexanic extract of tomato fruit (n= 40 pairs) or control (n= 40 pairs). The two distributions are not significantly different.

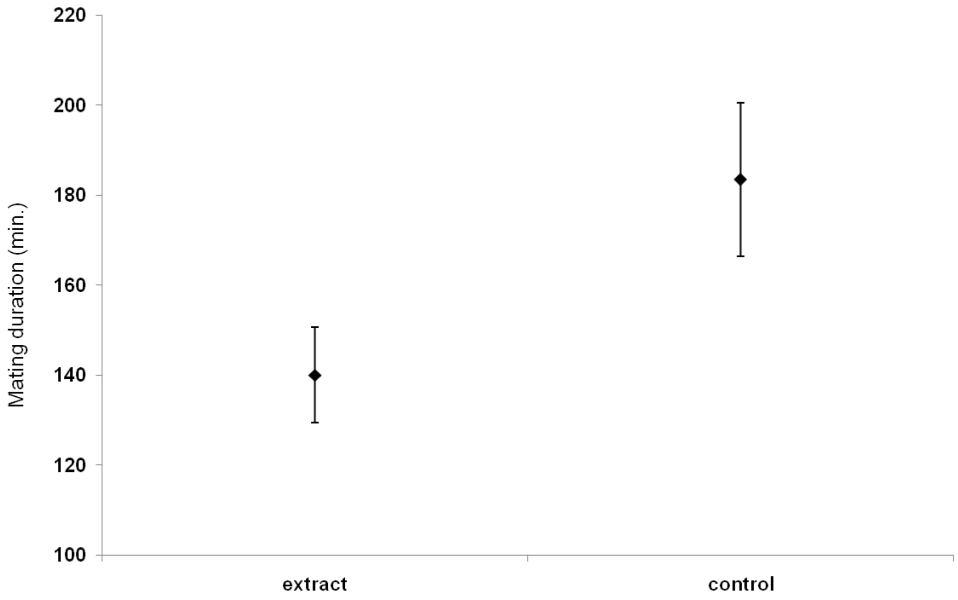

Figure 5: Mating duration in minutes (± S.E.) in *Neoleucinodes elegantalis* when they were held in presence of hexanic extract of tomato fruit (n= 40 pairs) or control (n= 40 pairs). The two distributions are significantly different.

CAPÍTULO 2

Effect of larval density on development and survival of *Neoleucinodes elegantalis* (Guenée) (Lepidoptera: Crambidae)

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Abstract - Many insects has its fitness reduced by intraspecific competition. This occurs mainly because most holometabolous insects depend of nutrients accumulated during larval fase. Intraspecific competition depends on larval density, which in turn affects food availability in larval stage. Laboratory studies of larval densities of the tomato fruit borer *Neoleucinodes elegantalis* confirmed that intraspecific competition affected development and survival. *N. elegantalis* pupae were lighter in weight. Pupae were lighter in high densities. Larval development time did not changed with increasing density; however, survivorship was significantly lower. Once larval density affected insect size and this trait is generally correlated with high fecundity, the intraspecific competition during the larval stage would appear to carry a large cost for female´s fitness. This may confer a disadvantage since *N. elegantalis* is monandrous and depends on their larval acquired resources to reproduce as they have no opportunity to receive additional resources through nuptial gifts at mating. This work suggests that shortages of larval food could affect fecundity directly. For males, it is possible that the small size will affect the opportunity of mating when in competition with larger ones.

Keywords: Larval competition, fitness, food availability.

Introduction

Adult fitness in holometabolous insects is under influence of the availability and allocation of resources to larval and adult feeding. When larvae are feeding, at some stage, the growth stops and nutrients accumulated are allocated during metamorphosis into the adult for somatic maintenance. Some insects may feed when adults and the combination of incoming of adult nutrients and stored larval nutrients are then used to support the functions of reproduction, survival and dispersal (Boggs & Freeman 2005). Thus, variation in food availability may affect the reproductive output of adults.

Populational variation affects the amount of food available to larvae (Dethier 1959, Putman 1977). A reduced availability of food due to crowding during development can affect adult behaviour, morphology, physiology, fitness and larval development and survival (Marshall 1990, Denno & Roderick 1992, Denno *et al* 1995, Mueller 1997, Hirschberger 1999, Mercer 1999, Tammaru *et al* 2000b, Rhainds *et al* 2002, Gibbs *et al* 2004, Bauerfeind & Fischer 2005, Fantinou *et al* 2008).

Larval development time may increase when in competition (Gibbs *et al* 2004). *Pararge aergeria* (Linnée) (Lepidoptera: Nymphalidae) and *Epirrita autumnata* (Linné) (Lepidoptera: Geometridae) intraspecific competition resulted in increased larval development time (Gibbs *et al* 2004). Increased periods of larval development generally are disadvantageous in two ways: (i) the insect will spent more time to reach mating age (Gotthard *et al* 1994, Fischer & Fiedler 2000) and (ii) the longer time exposured to predators and parasitoids (Klok & Chown 1999). On the other hand, longer development time may be advantageous if it results in an increased body mass (Wiklund & Forsberg 1991) because the insect may compensate the lower quantity of food by longer period of food consumption (Rahman 1969).

At reproductive stage, monogamous females rely primarily on reserves accumulated during larval stage, since adults have little opportunity to accumulate additional resources. Feeding rate, utilization and allocation of food may be constrained by shortage of food during larval stage. Hence, competition for food among larvae that develop at different densities affects pupal size and fecundity (Jones *et al* 1982, Honek 1993, Tammaru *et al* 2000a).

This study was undertaken to see the effects of larval densities in life history traits of the tomato fruit borer *Neoleucinodes elegantalis*. We aimed investigate the effect of larval density on larval development and survival. So, we expect that larval mortality and development time increases, whereas size decreases with increasing density. We choose this specie, since females are monandrous (Jaffé *et al* 2007) and it does not receive any gift from males as nutritious ejaculate.

Material and Methods

Rearing conditions

N. elegantalis used in our experiment were reared in the laboratory for some generations at the Federal University of Viçosa, Minas Gerais, Brazil. Larvae were reared on *Solanum gilo* until pupation. Pupae and adults were kept at 25 ±1º C and 71,2 ± 10 % RH and light regime set to 12L:12D. Newly emerged virgin males and females were remained together, in cages (50 x 50 x 50cm), with artificial substrates to oviposition, made with polystyrene ball covered with a thin layer of green paraffin and hanging inside the cage. The adults were supplied with a 10 % honey-water solution dispensed on cotton wool hung on the top inside the cages. The eggs on the artificial substrates were maintained on cages (20 x 15 x 10 cm) until larvae hatched. Larvae newly hatched were used in larval bioassays. We assessed larval development and survival using only healthy, active larvae (i.e. with no visible morphological or behavioral defects).

Larval density assay

Newly hatched larvae were randomly assigned to fruits of *S. gilo* at densities of 1, 6, 15 and 30 larvae per fruit. *S. gilo* was choossen because these fruit are more resistant and so it allows the larval development in laboratory conditions. Previously to assays, the fruits were selected with similar weight and size, to avoid experimental error. Older larvae have been show to have a significant competitive advantage (Krebs & Barker, 1995; Briggs *et al*., 2000) and so, all of the larvae assigned to each fruit shared the same hatching date. The treatments were replicated 100, 30, 11 and 11 times to 1, 6, 15 and 30 densities, respectively. A total of 775 larvae were distributed over 152 fruits. Fruits were similar in size and weight. Fruits were placed into separate 250 ml plastic containers and maintained in the same rearing conditions (above) until the end of the experiment. The fruit were checked daily and any fruit rotten was discarded.

Pupation dates, pupal sex and masses were recorded for each individual. At pupation, individuals were removed from the container, weighted, sexed and separate in 20 ml transparent plastic containers until emergence. Emergence dates were recorded for each individual.

Statistical analysis

All statistical analysis was done using R (version 2.8.0; R Development Core Team, www.R-project.org) following the method of Crawley (2007).

The effects of density on development time, pupal mass and larval/pupal surviving were investigated by comparing treatments 1 to 4 (1, 6, 15 and 30 larval densities). The experimental unit was a container. For larval time, pupal mass and pupal time, the mean values were calculated separately for both sexes. However, when analyzing survival, an individual larva was used as an independent unit, which allowed the highest order interactions to be estimated.

The effect of larval density on pupal mass and development time (larval and pupal) were analyzed with generalized linear models fitted with the function GLM and using normal distribution followed by F test. Treatment (densities) and sex were set as independent variables.

The survival probability of an individual larva was analyzed in relation to densities. The dichotomic response of each larva (alive/dead) was analyzed with generalized linear model with treatments (densities) as independent variables. The models were fitted with the function GLM, and all possible interactions between the independent variables were included in the models. As the response variable was dichotomic, binomial distribution with logistic link function was utilized.

Results

Larval development time

Larval density (F=1.3459; d.f.=1,53; p=0.2514) and sex (F= 0.0478, d.f.=1,52, P=0.8278) did not affected the larval developmental time (Fig 1).

Pupal masses and **pupal period**

Pupal mass was significantly affected by larval density (F= 16.40, d.f.= 1,53, P<0,001) and sex (F= 36.09, d.f= 1,52, P<0,001) but not by the interaction density:sex (F= 0.03; d.f= 1,51; p = 0.865) (Fig 2). Females and males differed and females weighted (53,8±1,77 mg) significantly more than males (41,20 ± 1,03 mg) (Fig 3).

Pupal period was not affected by larval density (F=1.17, d.f.=3,40, P=0.3341) or sex (F= 0.0072, d.f.=1,39, P=0.9329) (Fig 4).

Survivorship

The influence of larval density was seen on the total developmental mortality (to both larval and pupal stages). Mortality in the larval and pupal stages considered separately also changed as the density increased.

Density had a significant effect on survivorship at pupation (d.f=1,150, P<0,001). The number of larvae that reached pupal stage declined with larval density (Fig 5). Pupal survival affected differently males and females. Female survivorship was more affected than males.

Survival to adulthood was also affected as comparing different larval density (1, 6, 15 and 30 larvae/fruit) (d.f=1.15, P<0,001) (Fig 6). Males and females were affected differently by larval density. In both cases, survivorship (pupae and adults) was high in individuals reared alone (P<0,001) (Figs 5 and 6). In both cases (pupae and adult), survivorship was highest when individuals were reared alone.

Discussion

In this work density did not affect the duration of larval development in both sexes of *N. elegantalis*. This response could be related to the increased larval mortality rates at the highest density (Figs 5 and 6) due to the lower probability of survival under conditions of low food availability. This is in agreement with the results of Kivelä & Välimäki (2008) found in *Pieris napi*. However, our data are not in agreement with previous studies in which larval density affects larval development by increasing (Gibbs *et al* 2004; Fischer & Fiedler, 2001) or decreasing their time (Bauerfeind & Fischer, 2005; Tammaru *et al* 2000b).

Under food shortage, many larvae of Lepidoptera increase their developmental time and consequently their feeding period in order to reach the critical size for metamorphosis (Bauerfeind & Fischer 2005; Fischer & Fiedler 2001). Larval density appeared to affect the life history traits of individual larvae apparently through changes in food availability or quantity. The increasing in density decreases the availability of resources, wich results in competition.

The shorter larval development period of males compared to females was observed when compared 1, 6, 15 and 30 larval densities (Fig 1). Theory predicts that rapid growth of males could be attributed to selection for protandry in males to maximize the number of matings and to reduce the pre-reproductive period of females (Fagerstrom & Wiklund 1982). The patterns of growth in females may explain why the stronger selection for males produces shorter developmental time and it may be an adaptation to the short adult lifespan of *N. elegantalis*. In laboratory conditions Gama et al. (unpublished) and Eiras (2000) found that mating in this moth started at the first scotophase following emergence and Jaffé *et al* (2007) suggest that this species is monandrous, mated females did not mate again whereas a few males mated more than once (Pontes *et al*. 2010).

Changes in developmental time may be seen under different perspectives: Increasing larval development time may be disadvantageous, byreducing the opportunities in reproducing and by exposing larvae to predation and parazitation actions (Gotthard *et al* 1994, Klok & Chown 1999, Fischer & Fiedler 2000), but can also be advantageous because enlarged larval development results in increased body mass since the major time dispended to consume more food over a longer period resulting in larger adults (Rahman, 1969, Wiklund & Forsberg 1991). The expected effects of low resources availability are both prolonged development time and reduced adult size,as consequence of lower pupal masses.

Density affected pupal mass and there was a sex dependent effect on size. Pupal mass, for both sexes, decreased with increasing of larval densities and females were heavier than males (sexual dimorphism). In insects, large size is generally correlated with high fecundity (Honek 1993). It is expected that small size will be more costly for females than males.

Size dimorphism occurs in *N. elegantalis* (Jaffé *et al* 2007). The investment of males is in lipid reserves that enables them to longer mating flights or in terrestrial disputes, whereas females investments are in nitrogen reserves, which are allocated for reproduction (Sibly *et al* 1997). *N. elegantalis* responded to intraspecific competition by producing small pupae at the same development time. Apparently females and males had the same growth rate once the response of both was similar when subjected to different populational densities.

There was not a sex: time density interactions because females and males gained similar weight as densities increased. *N. elegantalis* female does not receive nuptial gifts and there is no opportunities for adult females acquire additional resources for reproduction through mate. Once female fecundity is largely dependent on the resources accumulated during the larval stage (Wiklund *et al* 2001, Bergström *et al* 2002), so its may be negatively affected by food shortages during larval development (Gibbs *et al* 2004).

Female fecundity in insects generally shows a strong positive relationship to adult body mass (Nylin & Gotthard 1998). This study revealed that the individuals spent the same developmental time under different larval densities, which should have contributed to the lower weight at pupae. It was observed that females are heavier than males and we predicted that females should reach large size more than males because females reproductive success, unlike that of males, is largely dependent of body size due to absence of male nutrient provisioning in the adult stage (Leimar *et al* 1994, Wiklund & Kaitala 1995, Bissoondath & Wiklund 1997).

We cannot determine whether there would be adverse effects of density on reproduction traits because the number of adult surviving within the treatments were too low to evaluate the fecundity, fertility and other reproductive traits. So, further work is needed to investigate the responses in fitness of both sexes, as well as to access the relationship among larval density, adult size and longevity, mating and reproductive success.

Intraspecific larval competition ha some effects in larval and pupal survival of *N. elegantalis* as described for other insects (Barros-Bellanda & Zucoloto 2002, Gibbs *et al* 2004, Kivelä & Välimäki 2008). Pupation and adulthood surviorship were significantly higher for larvae that were reared alone. This result suggests that *N. elegantalis* females could enhance the fitness of their larvae by avoiding to lay eggs on host plants that are already occupied by conspecific larvae. Investigations would be valuable to determine whether the oviposition behavior of *N. elegantalis* is directed in order to avoid intraspecific competition and if this behavior affects the survival of its offspring.

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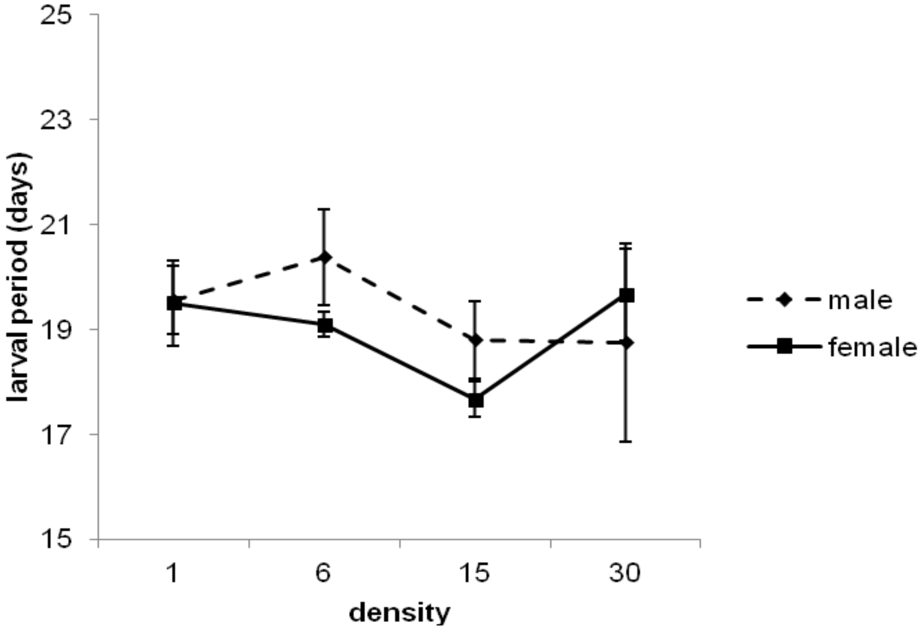

Fig 1 Larval development (days) in *Neoleucinodes elegantalis* reared in *S. gilo* and submitted to different populational densities and sexes. Mean number of days (± S.E.).

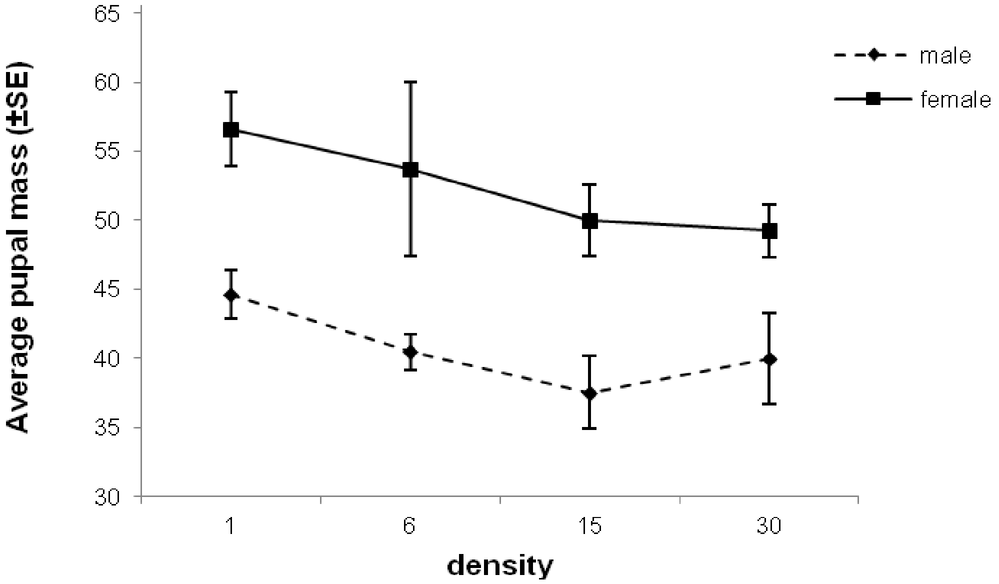

Fig 2 Pupal mass (mg) in *Neoleucinodes elegantalis* reared in *S. gilo* and submitted to different populational densities and sexes. Mean pupal mass (± S.E.).

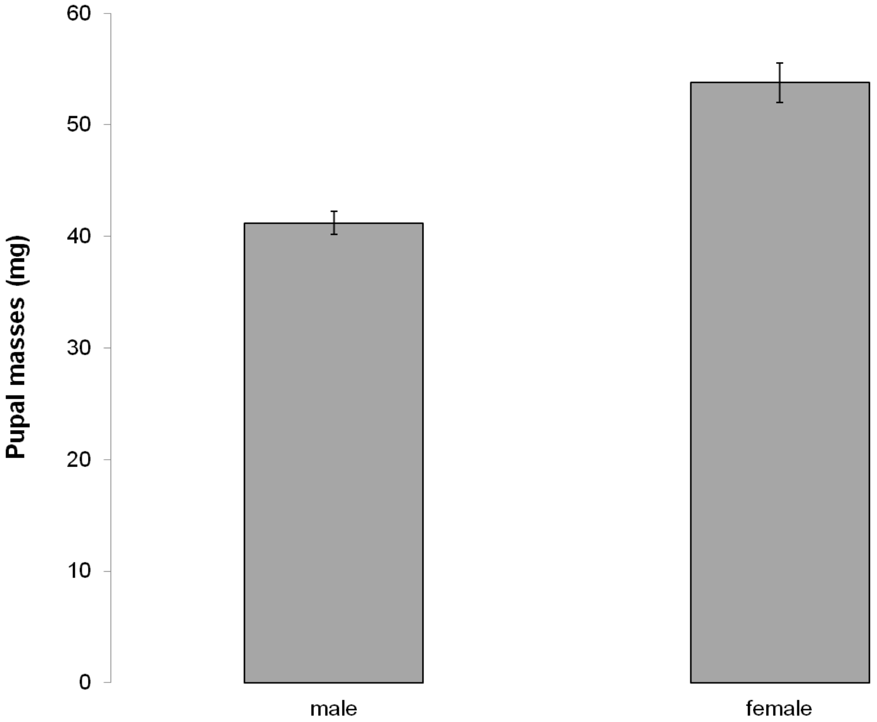

Fig 3 Pupal mass (mg) in *Neoleucinodes elegantalis* reared in *S. gilo* and submitted to different populational densities and sexes. Mean pupal mass (± S.E.).

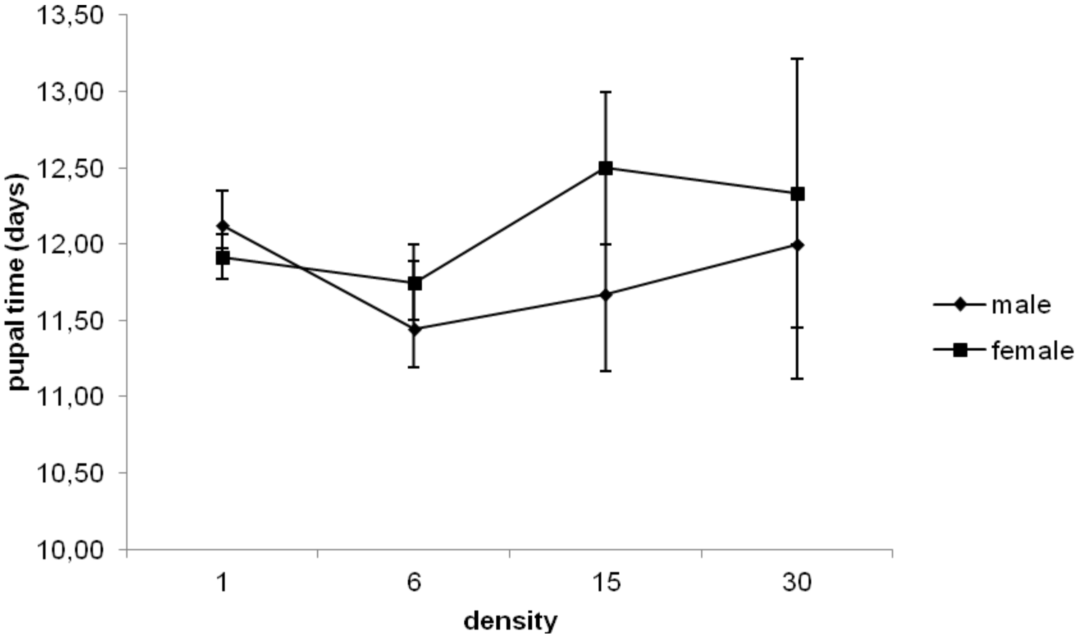

Fig 4 Pupal time (days) in *Neoleucinodes elegantalis* reared in *S. gilo* and submitted to different populational density and sexes. Mean number of days (± S.E.).

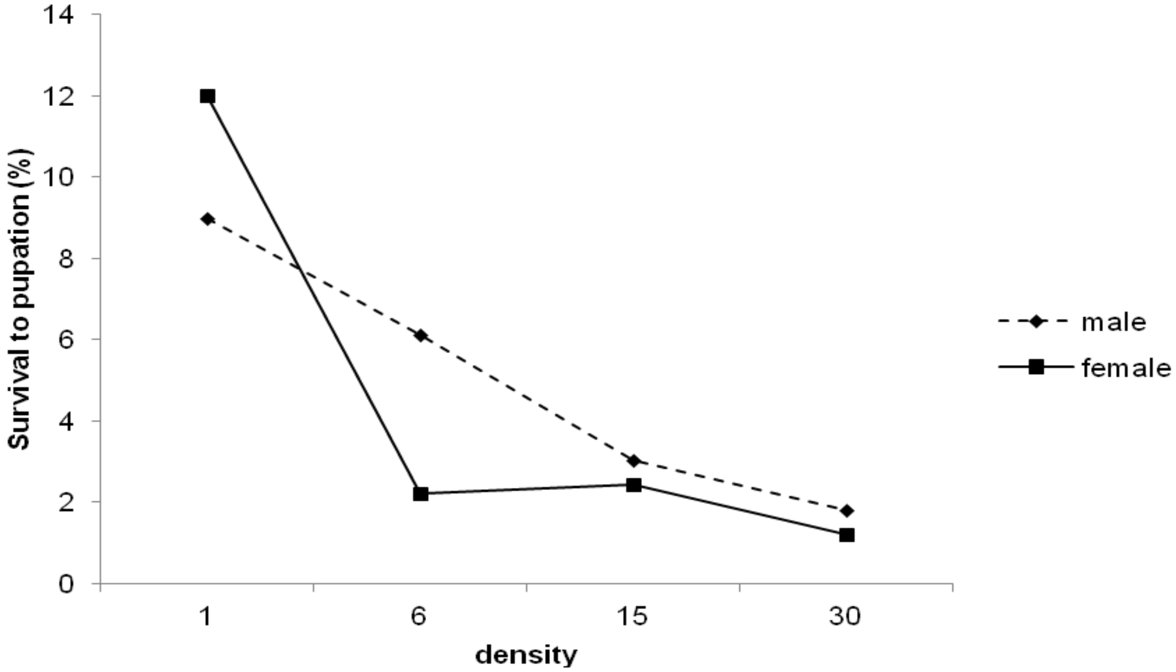

Fig 5 Pupae survival probabilities of *Neoleucinodes elegantalis* in relation to populational density and sexes. Percentage of larvae surviving to pupae.

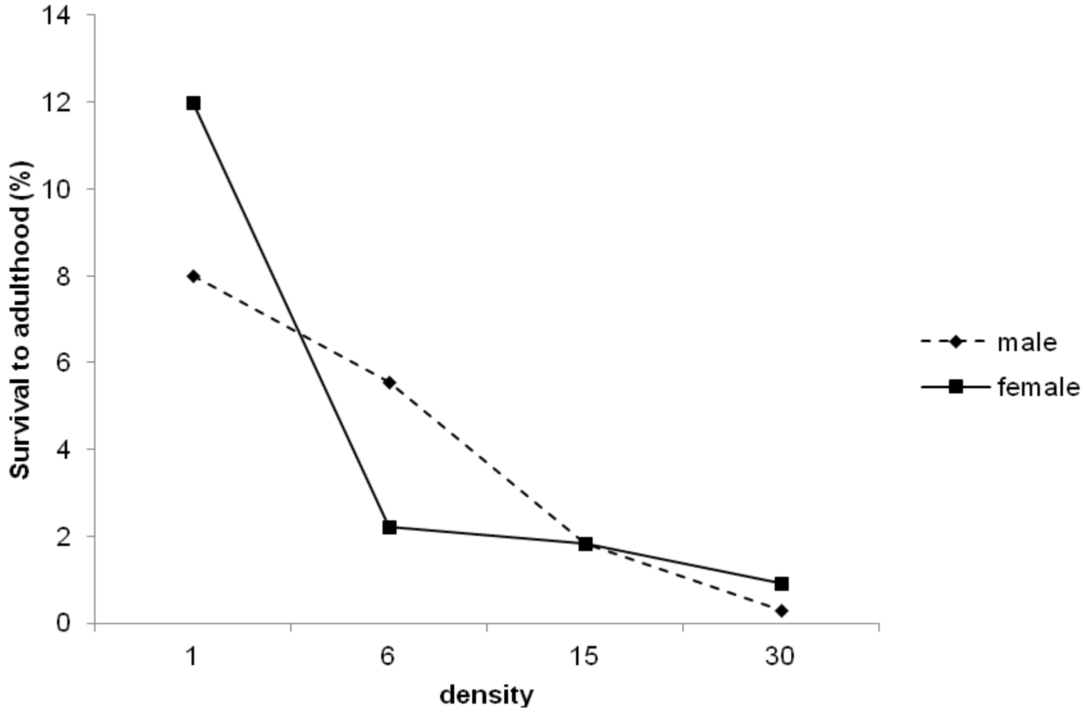

Fig 6 Adult survival probabilities of *Neoleucinodes elegantalis* in relation to density and sexes. Percentage of pupae surviving to adulthood.

CAPÍTULO 3

Selection of oviposition sites by *Neoleucinodes elegantalis* (Lepidoptera: Crambidae) mediated by semiochemicals.

Abstract - Plants produce volatile and nonvolatile compounds that signal both their phenological and physiological state, and may play a role as attractants and/or stimulant to herbivorous insects. Phytophagous insects use these signals to identify and select suitable food plants that may improve larval development and survival. The current study investigates the role of tomato fruits chemical cues on the oviposition preferences and larval orientation in *N. elegantalis*, an important pest of tomatoes. This study aimed also verify: (i) whether females show some preference in relation to chemicals from different plant parts (leaves, flowers or fruits) and (ii) whether chemical compounds from uninfested and infested fruits of tomatoes *Solanum lycopersicum* Mill. were engaged in oviposition preference and larval orientation in this moth. Compounds for bioassays were collected by headspace or immersion. Adult females of *N. elegantalis* showed dependence on semiochemicals for acceptance of its host plants. Oviposition assays with tomato plant parts revealed that when only volatiles from headspace are offered there is no oviposition preference, however when surface chemical compounds are available the females deposited most eggs on the surface of fruits, according to the moth behavior in the field. Furthermore, females recognized chemicals from uninfested and infested fruits and preferred oviposit on the last.

Introduction

The selection of oviposition site by herbivorous insects is crucial for their offspring survival, mainly because neonate larvae are relatively immobile and subject to desiccation (Feeny *et al.*, 1983; Renwick, 1989; Renwick & Chew, 1994). So, females should choose the plants, on which they deposit their eggs (Schoonhoven *et al.*, 2005).

It seems obvious that adult female should select individual host plants, or parts of one host-plant in a way that allow the best larval development. However, this is not a general rule, once this occur in some species (Rausher, 1979; Rausher & Papaj, 1983; Quiring & McNeil, 1987; Damman & Feeny, 1988; Preszler & Price, 1988; Craig, T.P. & Price, 1989; Minkenberg & Fredrix, 1989), whereas in others species this do not take place (Rausher, 1979; Mackay, 1982, 1985; Karban & Courtney, 1987; Futuyma & Moreno, 1988; Auerbach & Simberloff, 1989). The lack of oviposition site selection may represent large risk for that species which immature phase completes their total development at the same host where the eggs were deposited.

The host plant selection steps depend on a wide variety of sensory cues as vision, mechanoreception, olfaction (Renwick & Chew, 1994) and contact chemoreception (Ramaswamy, 1988). It is generally assumed that the host selection process in specialist insects is governed primarily by volatile chemical signals, later by visual stimuli, and finally by non-volatile chemical signals (Hern *et al.*, 1996; Hooks & Johnson, 2001).

Volatiles are the first chemicals detected and they are result of lipids metabolism whose releasing is linked to water loss during stomata opening, and their perception by insects arise mainly on the antenna receptors but also on the labial palps of some Lepidoptera (Bernays & Chapman, 1994). In contrast, contact semiochemicals are thought to consist of non-volatile chemicals (Renwick, 1989) derived from plant trichomes or leaf waxes (Stadler, 1986), and in Lepidoptera their detection occur through chemoreceptors present on mouthparts, tarsi and ovipositor (Bernays & Chapman, 1994). Thus, the combination of volatiles and contact chemical cues enable females to determine the identity of a potential host plant (Heinz, 2008).

Plant chemistry may be greatly altered in response to herbivory (Turlings *et al.*, 1995; Karban, 1997) and, many of these inducible defense compounds are non-volatile acting as toxins or reducing plant digestibility (Karban, 1997; Tallamy & Raupp, 1991). In herbivore insects, the presence of conspecific brood on plants can influence the host selection decisions of the ovipositing female (Nufio & Papaj, 2001; Prokopy & Roitberg., 2001).

In some species, the female oviposits more eggs on plants used by conspecific (Baur *et al.*, 1996; Shiojiri & Takabayashi, 2003), whereas in other species, the female lay more eggs on plants free of conspecifics (Prokopy, 1972; Mitchell, 1975; Sato *et al.*, 1999; Moraes *et al.*, 2001). Thus, responses to plants already used by conspecific brood can depend on species context (Nufio & Papaj, 2001; Prokopy & Roitberg, 2001). Thus, taking into account different ecological and/or physiological conditions, a female may refrain from ovipositing on used hosts or may accept occupied hosts (Papaj *et al.*, 1992; Papaj & Messing, 1996).

Ovipositing females of *N. elegantalis*, a specialist in solanaceous plants (Leiderman & Sauer, 1953, Gallo *et al.*, 2002), are chemically stimulated to oviposit on tomato fruits (Pontes *et al* 2010). The question is, should females use these kairomones to select available site to subsequent larval development?

The aim of this work was to determine if the host plant chemical cues from infested fruits could be discriminated from the uninfested ones by *N. elegantalis*. The chemicals cues involved in the discrimination among different host plant parts by *N. elegantalis* have also been evaluated. The specific questions addressed in this paper are: females use chemical cues to select within the host plant the best place to oviposit on? If so, then what chemicals cues are involved in the discrimination among different host plant parts?

Material and Methods

Insects and Experimental conditions

N. elegantalis used were reared in the laboratory for two years at the Federal University of Viçosa, Minas Gerais, Brazil. Larvae were reared on *Solanum gilo* until pupation. Pupae were sexed and separated in cages (50 x 50 x 50cm) until adult emergence. After emergence they were held separately in plastic containers, so females did not have contact with males. Pupae and adults were kept at 25 ±1ºC and 71,2 ± 10 % RH and light regime set to 12L:12D. Newly emerged virgin males and females were supplied with a 10% honey-water solution. In trials to obtain mated females, all females and males that emerged in the previous day were released (sex ratio 1:2) into experimental cages (50 x 50 x 50cm) from the 5th to 10th hours of scotophase. Mating pairs were carefully transferred to plastic containers where they remained until they finished the copula. Females that had been in copula were presumed mated and were submitted to the bioassay at the same day.

For larval bioassays, newly emerged virgin males and females were remained together, in cages, in the conditions described above, with artificial fruits to oviposition (Pontes *et al*., 2010). The eggs, on the artificial fruits, were maintained on cages (20 x 15 x 10 cm) until larvae hatched.

Throughout this article an *artificial fruits* refers to a 3,5-cm-diameter ball used as model sites for studying *N. elegantalis* ovipositional responses. It was adapted from a shape found to be attractive to the *N. elegantalis* female (Pontes *et al*., 2010).

Plant material

Leaves, flowers and fruits of tomatoes *Solanum lycopersicum* Mill. (Solanaceae, cv. Santa Clara), were collected in the greenhouse to proceed the collection of volatiles or surface chemicals to be used in bioassays.

Feeding damage

Experiments were started when green tomatoes fruits had 2-3 cm in diameter. We inoculated 20 *N. elegantalis* first instar larvae on each fruit. To assure that the larvae did not take out the fruit, the fruit was enclosed within cloth sleeves. Another plant remained free of larvae (uninfested fruit) and in separate greenhouse to prevent plant to plant signaling (Bruin & Dicke, 2001; Bruin & Sabelis, 2001; Farmer, 2001). The larvae were allowed to damage the plants for 24 h. For infested and uninfested fruits, six pairs of plants (replicates) were used. After 24 hours, infested fruits were removed from the plant and examined in a stereomicroscope to verify larval entry holes on the fruit. The uninfested fruits were also analyzed to assure that they are undamaged. Immediately after, the collection of surface chemicals (uninfested and infested fruits) was performed.

Preparation and extraction of plant material

Chemical extraction from surface

Surface chemical compounds from leaves, flowers and fruits and from uninfested and infested fruits were collected on April and July 2009, respectively, as proposed by Breeden *et al.* (1996).

Tomato leaves, flowers or fruits were placed in a glass container with n-hexane (non-polar) or ethanol (polar) and gently shaken in a brief extraction for 30 seconds to remove surface chemicals in order to obtain a concentration of 1 equivalent plant material/ml of solvent and stored at -19 ºC until it could be used in bioassays. The extraction of chemicals from uninfested and infested tomato fruits were performed by using only hexane, as solvent.

Volatile collections

Volatiles were collected on August and September 2008 from tomato leaves, flowers and fruits. For the push-pull headspace sampling, the material was placed in a glass chamber. Clean humidified air was forced through the chamber, regulated by a flowmeter at 150 ml/min positioned at the lower part of the chamber. Volatiles were trapped in a glass tube attached to the top of the glass chamber containing 50 mg Super Q adsorbent (80/100 Mesh, Alltech Assoc., IL, USA). The trap was connected to the outlet of the chamber and via another flowmeter to the vacuum compressor. Volatile collection was finalized after 24h in controlled room and the extractor was maintained at 22ºC and luminosity of 0,5 lux, simulating the field conditions. After 24 h of aeration the volatiles were eluted with 350 µL of hexane. All collections were conducted at 25 ±1ºC and 71.2 ± 10 % RH. Each treatment was repeated nine times following the same protocol. The samples were placed in a microcapillar sealed in the two extremities at -19ºC to subsequent use in bioassays.

Bioassays

General design for oviposition choice experiments

The importance of the semiochemicals on the egg-laying behavior of *N. elegantalis* was evaluated by testing the behavioral responses of female moths to chemical cues from: (i) tomatoes leave, flowers or fruits and (ii) uninfested or infested fruits, in different ovipositional bioassays. The experimental cages (50 x 50 x 50cm) were kept in room at 25 ±1ºC and 71,2 ± 10 % RH and light regime set to 12L:12D.

Artificial fruits were hung from ceiling of the cage with a fine wire 5 cm long. They were equally distributed on the top of each cage, between replicates and, on each trial, artificial fruits were distributed at random position. At the beginning of each assay, chemicals were applied directly on a 5 mm-wide strip of filter paper, which was wrapped horizontally around the artificial fruit, like a ring, and glued on the ends. For each treatment, we applied the chemicals using a micropipette, 15 minutes before the releasing of moths into the cage for evaporation of solvent. Females were allowed to lay eggs *ad libitum* for 48 h and supplied with a 10 % honey-water solution. The artificial fruits were removed from the cages after 48 hours and the number of eggs on each was counted. Samples of females used in experiments were dissected for evidence of mating and mature eggs.

Role of plant parts volatiles in oviposition

For the volatiles obtained from each plant part, the oviposition response was examined in a three choice bioassay (Experiment 1). Five mated females were placed in experimental cages with three artificial fruits, each one containing 30 µL aliquots of leaves, flowers or fruits volatiles. Data for each treatment were collected from 5 replications.

Role of plant parts chemical surface in oviposition

Experiment 2 tested the role of *non-polar* surface chemicals in the oviposition preference of *N. elegantalis*. Five mated females were placed in experimental cages with three artificial fruits, each one containing 30 µL aliquots of leaves, flowers or fruits *hexanic* extracts. Data for each treatment were collected from 5 replications.

Experiment 3 tested the role of *polar* surface chemicals in the oviposition preference of *N. elegantalis*. Five mated females were placed in experimental cages with three artificial fruits, each one containing 30 µL aliquots of leaves, flowers or fruits *ethanolic* extracts. Data for each treatment were collected from 5 replications.

Oviposition response to non-polar versus polar surface compounds of tomatoes fruits

In both experiments 2 and 3, females had their ovipositional response significantly affected by surface chemicals of tomatoes and they preferred oviposit on fruits. On the basis of these responses, a subsequent experiment (Experiment 4) was performed to investigate if surface compounds of fruits with different polarity elicit different oviposition response in *N. elegantalis*. We used hexanic and ethanolic extracts from fruits which elicitated high ovipositional response. Five mated females were placed in experimental cages containing two artificial fruits, each one with 30 µL aliquots of hexanic or ethanolic fruits extracts. The assay was conducted five times with a different group (n=5) of moths.

Role of uninfested and infested fruit chemicals extracts in oviposition

The behavioral response of female moths *N. elegantalis* to surface chemicals extracts from uninfested and infested fruits was evaluated in a two choice ovipositional assay (Experiment 5). Five mated females were placed in experimental cages with two artificial fruits, each one containing 20 µL aliquots of uninfested or infested fruits extracts. The trials were replicated nine times and each one was performed with five female moths.

Statistical analysis

To test the effects of chemical cues on oviposition of females, eggs were counted on each artificial fruit in all treatments and the proportion of eggs was calculated and analyzed with generalized linear model with a quasi-binomial error distribution. To build the statistical model, the response variable was the proportion of eggs (y= number of eggs loaded under each chemical treatment/ total number of eggs loaded in all treatments), and the explanatory variables were the chemicals from different plants parts or from uninfested and infested fruits. Full models were built by including all explanatory variables and when possible we simplified it amalgamating explanatory variables that have similar parameter values to reach a minimum adequate model.

All analyzes were performed with R statistical system, version 2.4.1. (R Development Core Team, 2006) (Crawley, 2007).

Results

All experiments demonstrated that females responded positively to tomato chemical cues and a kairomone seems to be implicated in mediating host plant selection in *N. elegantalis*.

Role of plant parts volatiles in oviposition. In experiment 1, there are no difference in the mean number of eggs in leaves, flowers or fruits, indicating no preference for plant parts ($F_{2,12}$= 0.4 P= 0.6543) (Figure 1).

Role of plant parts chemical surface in oviposition. After landing on the artificial oviposition site, the females were more likely to oviposit on sites with fruit hexane extracts than leaves and flowers ones ($F_{2,12}$= 18.307 $P<0.001$) (Figure 2). There was no difference between treatments leaves and flowers, so these categories were grouped in one and contrasted with fruits in order to reach a minimum adequate model.

Similarly, artificial fruits with polar fraction extracts received significantly more eggs than the sites treated with flower or leaves extracts ($F_{2,12}$= 35.644 $P<0.001$) (Figure 3). There was no difference between treatments leaves and flowers, and so as before, these categories were grouped in one and contrasted with fruits in order to reach a minimum adequate model.

Oviposition response to non-polar versus polar surface compounds of tomatoes fruits. When two artificial sites with either hexanic or ethanolic fruit extracts were offered simultaneously, there were a significantly higher mean number of eggs laid on ethanolic than the hexanic extracts (Figure 4). Polar surface chemical compounds elicited higher ovipositional response in females than non-polar surface chemicals ($F_{1,8}$= 67.924 $P<0.001$).

Role of uninfested and infested fruit chemicals extracts in oviposition. The attractiveness of the tomato fruit surface extracts to mated female *N. elegantalis* was affected by the status of the fruit (uninfested or infested by first instar larvae).

Chemicals from infested fruits seems to be more stimulant for oviposition than uninfested ones ($F_{1,16}$= 63.027 P<0.001) (Figure 5). It is clear that extracts of damaged or undamaged fruits elicited orientation of females, once eggs were present on both treatments, but they were significantly more attracted to extracts from damaged fruits.

Discussion

It has been previously established that *N. elegantalis* females are dependent of chemical, physical and visual cues from tomato fruits (Pontes *et al*., 2010). Our results show that females cannot discriminate the preferred patch for oviposition, the fruits, relying only on the volatiles chemicals, as no difference in egg laying was found among the plant parts. However, when surface chemicals of different parts of the plant were offered in choice experiment females oviposited on leaves, flowers and fruits extracts but clearly discriminated between different parts of their host plant. This species is known to oviposit predominantly on green fruits (Blackmer *et al.,* 2001). Plant parts (leaves, flowers and fruits) are also responsible for attraction in another insects as *L. botrana* (Masante-Roca *et al.,* 2007) and *Neoceratitis cyanescens* (Brevault & Quilici, 2010).

When potential oviposition sites are available, female will lay most eggs on her most preferred plant part, fewer eggs on her next preferred, and so on (Thompson & Pellmyr, 1991). So, the plant phenology affects chemically mediated oviposition behavior resembling the response of this moth in the field. This suggests that in *N. elegantalis* the host recognition and acceptance are also based on contact cues such as plant surface compounds. We can visualize a scenario were females use olfactory stimuli associated with appropriate visual and tactile stimuli to locate suitable sites (Pontes *et al*., 2010) and the fruit should be choosen by exploiting chemicals from contact with the plant parts.

The importance of contact chemoreception in oviposition behavior was demonstrated in polyphagous *Helicoverpa armigera* (Jallow *et al*., 1999), *Lobesia botrana* (Gabel & Thiéry, 1996), *Ostrinia nubilalis* (Udayagiri & Mason., 1997; Derridj *et al*., 1992), *Helicoverpa zea* (Coates *et al.,* 1988), *Heliothis subflexa* (Mitchell & Heath, 1987) and *Heliothis virescens* (Ramaswamy *et al*., 1987; Jackson *et al*., 1986; 1984), as in monophagous Lepidoptera *Yponomeuta cagnagellus* (Roessingh *et al*., 2000; Hora & Roessingh, 1999), *Acrolepiopsis assectella* (Thibout & Auger, 1996)

Plutella xylostella (Justus & Mitchell, 1996; Spencer, 1996), *Sesamia nonagrioides* (Konstantopoulou *et al.*, 2002) and *Keiferia lycopersicella* (Burton & Schuster, 1981).

Secondary metabolites present on the plant surface may also give specific information of plant organ and its stage of development (Stammitti *et al.*, 1995). *N. elegantalis* females always scan with their ovipositor before egg-laying and this organ seems to be used to evaluate the oviposition site (Personal observation). This is reinforced by the presence of chemo and mechanoreceptors on the ovipositor of *N. elegantalis* (Araújo *et al.*, unpublished). *H. virescens* females drag the ovipositor on the leaf surface prior to egg deposition and this behavior suggests that the moths are using the ovipositor chemosensilla in order to test surface chemicals before acceptance and this behavior is also observed in other species of Lepidoptera (Ramaswamy, 1988). Chemosensory sensila are located on antennae, probosces, tarsi and ovipositors of moths (Chadha & Roome, 1980; Valencia & Rice, 1982; Faucheux, 1988; Fenemore, 1988; Ramaswamy, 1988; Marion-Poll *et al.*, 1992a; 1992b; Qui *et al.*, 1998).

In experiments 2 and 3 females moths were stimulated to oviposit on ethanol and hexane extracts of the tomato parts, suggesting that *N. elegantalis* females are either stimulated to oviposit by different classes of compounds. When ethanol and hexane extracts of fruits were offered, semiochemicals soluble in ethanol were more efficient in eliciting oviposition behavior of these moths. So, polar compounds in the host extract served as ovipositional stimulants for *N. elegantali*, as in other moths as *L. botrana* (Maher & Thiéry, 2004), *Yponomeuta cagnaellus* (Hora & Roessingh, 1999). Electrophysiological and behavioral bioassays are currently underway to identify attractive compounds emitted from active fractions.

Tomato plants are known to release compounds that play a role in indirect defense following herbivory and mechanical damage (Takabayashi & Dicke, 1993). Herbivore damage to plants can increase the endogenous jasmonic acid levels (Mueller *et al.*, 1993; Doares *et al.*, 1995) that are key signal components of plant responses to herbivore attack important in plant resistance (Felton & Korth, 2000).

Insect response to plants depends on host plant range. As pointed out by Karban and Baldwin (1997), specialist herbivores may respond positively to induced chemicals, whereas generalist herbivores may show a negative response. Thus, some herbivores show preference for infested plants, as *Cydia pomonella* (Hern & Dorn, 2002), *Leptinotarsa decemlineata* (Landolt *et al.*, 1999), *Delia radicum* (Baur *et al.*, 1996) and *Plutella xylostella* (Uematsu & Sakanoshita, 1993; Shiojiri & Takabayashi, 2003). In contrast, some examples of negative response to damaged plants by herbivorous insects are described in *Pieris rapae* (Sato *et al*., 1999), *Pieris brassicae* (Schoonhoven, 1990), *Heliothis virescens* (De Moraes *et al*., 2001) and *Phtorimaea operculella* (Arab *et al*., 2007).

Furthermore, divergent responses may occur at the same species. *Spodoptera littoralis* showed preference to oviposit on small plants damaged by larvae over undamaged control plants. However, when using larger plants the preference was reversed (Anderson & Alborn, 1999). *Trichoplusia ni* were more attracted to infested or artificially damaged cotton plants as compared to undamaged plants, in wind tunnel experiments. However, the oviposition response was different and, undamaged plants were preferred in comparison to damaged plants. However, when the host plant was exchanged for cabbage, undamaged plants were more attractive and received more eggs than the damaged ones (Landolt, 1993). Therefore, the influence of induced changes upon conspecific herbivores may affect behaviors at different stages of host plant selection (orientation and acceptance) and appears to be dependent on the system (insect-plant) under study.

Our results demonstrate that tomato fruit borer females can discriminate between tomato plants uninfested and infested by conspecific larvae and so, oviposited more on plants that were previously damaged by *N. elegantalis* larvae than on undamaged plants. So, from an evolutionary perspective, why females would choose infested fruits to oviposit on? One possible explanation is that this behavior is the way to avoid risk of predation, once the search for healthy fruits would require longer time exposed to natural enemies. The searching behavior for suitable food plants by herbivores may be directed by exploitation of plant volatiles (Visser, 1986). However, volatiles are emitted at low rates from uninfested plants. In contrast, herbivore infested plants emit volatiles in much larger amounts (Dicke *et al*., 1990a; Turlings *et al*., 1990; Schoonhoven *et al*., 1998). Thus, herbivore-infested plants are more easily perceived from a distance. These cues may convey more information than only about the presence of the host plant. For instance, those defenses have been induced from releasing plants or, in contrast, that plant defense has been overcome by herbivores, which, in turn, makes it more susceptible to herbivores attack (Dicke & Loon, 2000).

Based on the results we propose the following scenario for interactions among tomato plants and *N. elegantalis*: Mated females locate at a distance its host through volatiles emmited by the plant that, in turn, will be evaluated by females using contact chemoreception, a decisive role during the final steps of egg-laying. Non-volatiles compounds are perceived and, the plants parts, available for larval development and survival, are selected by females that prefer lay eggs on fruits. Additionally, females discriminates damaged fruit from undamaged ones and prefers oviposit on the first one. So, the final decision in host plant selection is guide by female on the time of oviposition. *N. elegantalis* responses to tomato is then modulated by kairomones and these moths might have adapted to associate plant kairomonal signs with the potential presence of suitable host plants.

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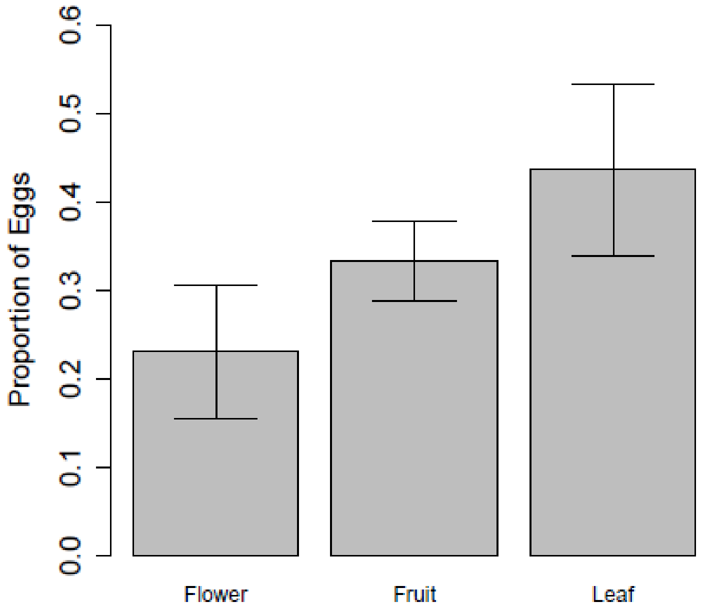

Figure 1: Oviposition response of *Neoleucinodes elegantalis* to volatiles from different phenological stages (leaves, flowers and fruits) of its host plant. Mean number of eggs (± S.E.) laid. Volatiles from plant parts did not affected oviposition preference.

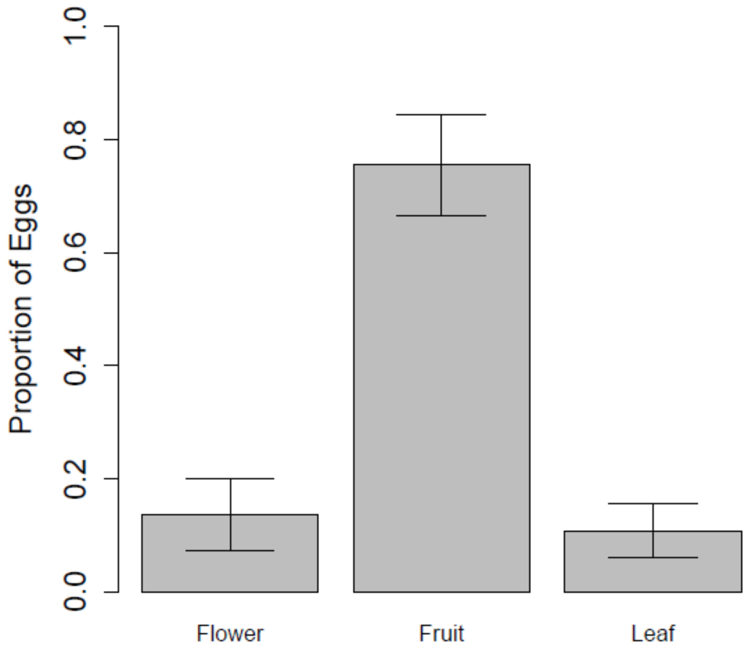

Figure 2: Oviposition response of *Neoleucinodes elegantalis* to hexanic extract from different phenological stages (leaves, flowers and fruits) of its host plant. Mean number of eggs (± S.E.) laid.

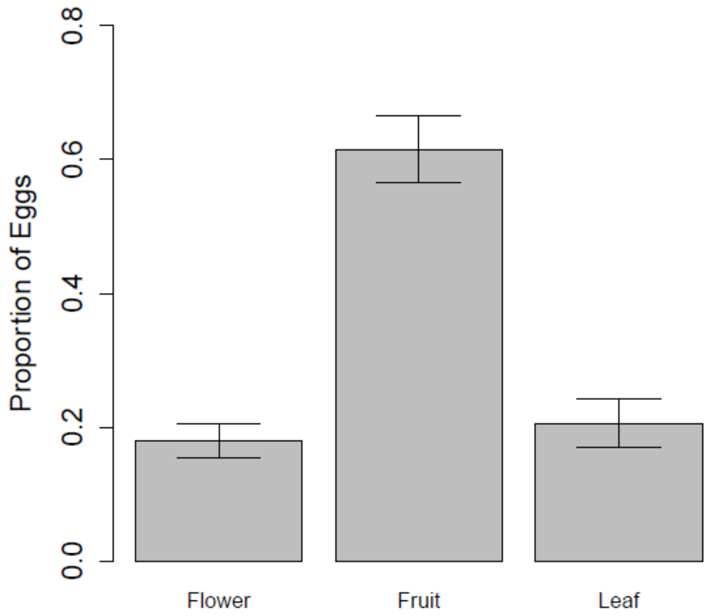

Figure 3: Oviposition response of *Neoleucinodes elegantalis* to ethanolic extract from different phenological stages (leaves, flowers and fruits) of its host plant. Mean number of eggs (± S.E.) laid.

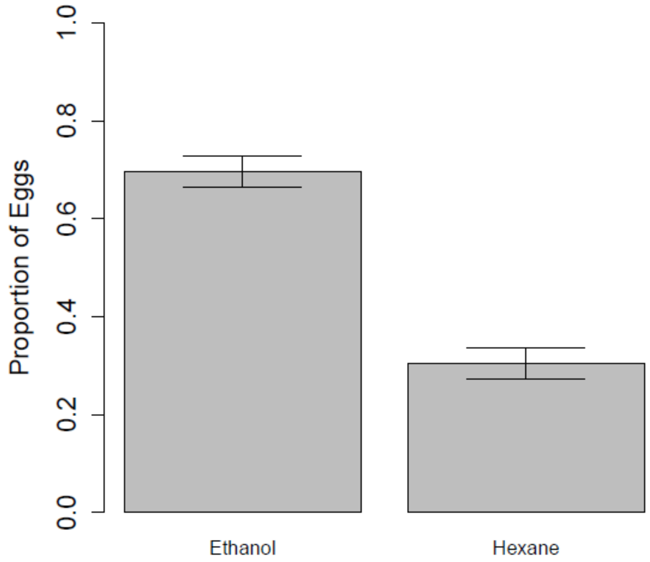

Figure 4: Oviposition response of *Neoleucinodes elegantalis* to non-polar (hexanic) and polar (ethanolic) surface extracts from tomato fruits. Mean number of eggs (± S.E.) laid.

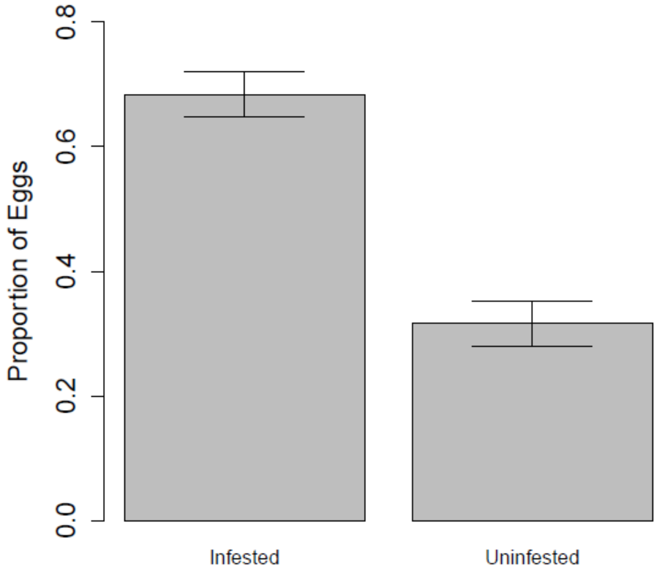

Figure 5: Oviposition response of *Neoleucinodes elegantalis* to hexanic extract from uninfested and infested fruit. Mean number of eggs (± S.E.) laid. Twenty microliter solution of the extracts were applied to filter paper on each artificial fruit. Females were released in the cage and the eggs counted after 48 hours.

CONCLUSÕES GERAIS

1. Fêmeas de *N. elegantalis*, independente da presença da planta hospedeira, iniciam seu comportamento de chamamento na sexta hora da primeira escotofase. O chamamento não é contínuo e ocorre o mesmo padrão de comportamento tanto na presença quanto na ausência da planta hospedeira.
2. O tempo gasto chamando, assim como o tempo gasto no acasalamento é influenciado pela presença da planta hospedeira. A idade interfere no tempo em que cada fêmea passa chamando, de modo que fêmeas mais novas chamam por mais tempo.
3. A densidade larval tem efeito direto sobre alguns aspectos biológicos de *Neoleucinodes elegantalis*, como peso e sobrevivência dos indivíduos. Os efeitos podem interferir indiretamente nos parâmetros de fecundidade e fertilidade dessa mariposa.
4. Diferentes modalidades influenciam fêmeas de *N. elegantalis* no processo de aceitação da planta hospedeira. Com base nos resultados encontrados nós propomos que as fêmeas localizam à distância sua planta hospedeira através dos voláteis emitidos pela planta que, em contrapartida, será avaliada pelas fêmeas através de quimiorrecepção de contato, um passo decisivo durante a etapa final da oviposição.

5. Compostos químicos presentes na superfície da planta são percebidos e, então, as fêmeas selecionam a parte da planta que é mais apropriada para o desenvolvimento e sobrevivência larval, preferindo ovipositar em frutos.
6. As respostas comportamentais de oviposição de *N. elegantalis* são moduladas por cairomônios da planta hospedeira e essas mariposas podem associar os sinais químicos da planta com a potencial presença de um hospedeiro apropriado para o desenvolvimento de seus descendentes.
7. Fêmeas selecionam o sítio de oviposição, entretanto, a seleção não é feita de modo a evitar competição intra-específica, ocorre preferência por frutos já infestados por larvas de co-específicos, de modo que o desenvolvimento larval ocorre nos frutos selecionados pela fêmea no momento da oviposição.