ECOLOGIA QUÍMICA E REPRODUÇÃO DE *Neoleucinodes elegantalis* GUENÉE (LEPIDOPTERA: CRAMBIDAE)

por

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RESUMO

A reprodução em insetos depende de diversos fatores que afetam diretamente o sucesso reprodutivo, em relação à qualidade e quantidade da progênie: a interação mediada por sinais químicos, físicos e visuais, entre o inseto e seu hospedeiro; taxa de crescimento larval relacionado com o tamanho final do adulto e a frequência de cópulas, como mecanismo de sucesso reprodutivo. Portanto, o estudo da reprodução de insetos é recomendado para se estimar e compreender a dinâmica de populações, tanto para a preservação de espécies em extinção, como para o controle de pragas agrícolas. A broca-pequena-do-tomateiro *Neoleucinodes elegantalis* Guenée (Lepidoptera: Crambidae) é uma das pragas mais importantes das solanáceas no Brasil, o que estimula o desenvolvimento de qualquer estudo voltado para a melhor compreensão de sua biologia e dinâmica populacional. Assim o objetivo deste trabalho é estudar (i) o efeito dos sinais físicos, químicos e visuais que afetam sua oviposição; (ii) as causas do seu dimorfismo sexual e (iii) o efeito da experiência de cópula de machos na sua capacidade de obter novos acasalamentos. Este trabalho ainda propõe uma equação que possa auxiliar em estimar o tamanho inicial de um espermatóforo já fragmentado, baseado nas medidas do fragmento encontrado no trato reprodutivo das fêmeas. Os resultados demonstram que: (i) os sinais físicos e químicos oferecidas estimulam significativamente a oviposição de *N. elegantalis*, bem como revela que

pistas visuais afetam a oviposição; (ii) que a diferença na taxa diária de crescimento larval é responsável pelo dimorfismo sexual nesta espécie e (iii) que machos recém-copulados têm a mesma chance de conseguir uma nova cópula que um macho virgem, num período de 24 horas.

PALAVRAS-CHAVE: Broca pequena do tomateiro, ecologia química, dimorfismo sexual, recópula de machos.

CHEMICAL ECOLOGY AND REPRODUCTION OF *Neoleucinodes elegantalis* GUENÉE (LEPIDOPTERA: CRAMBIDAE)

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ABSTRACT

The reproduction in insects is affected by a wide array of factors that acts directly on the reproductive output, related with offspring quality and quantity: the interaction, by physical, chemical and visual cues, between insects and host plants; the larval growth rate affecting adult size and mating rate, as a mechanism of reproductive sucess. Thus, studies on insect reproduction are recommended to help estimate and to understand population dynamics, for both endangered species and to control crop pests. The tomato fruit borer *Neoleucinodes elegantalis* Guenée (Lepidoptera: Crambidae) is one of the most important pest species on Solanaceae in Brazil, and the aims of this works is to study this species regarding to: (i) the role of physical, chemical and visual cues on oviposition; (ii) the causes of sexual dimorphism and (iii) how male mating history affects his ability to obtain new matings. This work also propose an equation that can help to estimate the initial size of fragmented spermatophores found inside the reproductive tracts of females, based on measures of the fragments. The results showed: (i) that physical and chemical cues increase significantly the oviposition of *N. elegantalis*, as well showed that light intensity also affect oviposition; (ii) that differences in dayly growth rate is the cause of the observed sexual dimorphism in this species, and (iii) that recently mated males are equally able to achieve new matings as virgin ones, within at least 24-h period.

KEY WORDS: Tomato fruit borer, chemical ecology, sexual size dimorphism, male remating.

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CAPÍTULO 1

INTRODUÇÃO

Reprodução geral dos insetos

A reprodução sexuada é o processo através do qual os seres vivos produzem descendentes, tendo sido considerada um dos principais agentes da evolução através da seleção natural, quando sua importância foi salientada pela primeira vez por Charles Darwin, em 1859. Para que a reprodução sexuada se dê, é necessária que haja a sincronia de um conjunto de fatores, como maturação sexual de machos e fêmeas num momento adequado, a presença de ambos os sexos (no caso da reprodução sexuada) ocorrendo em sincronia com condições ambientais favoráveis, o histórico fisiológico decorrente da dieta dos indivíduos quando em fase imatura, entre outros.

O estudo do comportamento reprodutivo de insetos torna-se cada vez mais importante, não apenas para preencher as lacunas existentes sobre o comportamento de espécies que atualmente são consideradas pragas, como aumentar o grau de conhecimento a respeito de novos comportamentos que possam ser utilizados para otimizar os métodos de controle já existentes, e implementar novas formas de manejo. Dentre as áreas de pesquisa que possuem forte relação com o sucesso reprodutivo dos insetos estão os estudos sobre dimorfismo sexual, frequência de cópula e ecologia química.

Reconhecimento da planta hospedeira

Encontrar uma planta hospedeira é um processo fundamental para a reprodução de insetos fitófagos, uma vez que a oviposição num substrato adequado para a sobrevivência e desenvolvimento da progênie é crucial para espécies onde a fase imatura é imóvel e incapaz de se deslocar para hospedeiros adequados (Renwick & Chew 1994). O reconhecimento da planta como um substrato adequado para oviposição pode se dar por fatores tanto físicos, como textura e cores, como químicos, através da detecção de substâncias voláteis, ou avaliação de substâncias químicas na superfície do sítio de oviposição. Tanto a visão como os voláteis são utilizados principalmente para a identificação de potenciais hospedeiros à longa distância, enquanto que logo após o pouso no hospedeiro, a textura e as substâncias da superfície da planta são pistas sensoriais importantes na aceitação ou rejeição do hospedeiro (Ramaswamy 1988, Renwick & Chew 1994). A preferência por superfícies rugosas (Ramaswamy 1988, Rojas *et al.* 2003, Nava *et al.* 2005) ou lisas (Foster *et al.* 1997, Calatayud *et al.* 2008) varia de acordo com as características intrínsecas de cada espécie.

Além disto, a presença de compostos químicos na supefície da planta é crucial na aceitação do hospedeiro. A capacidade do inseto de identificar os sinais presentes no hospedeiro provém tanto da especificidade e variedade dos receptores como da capacidade do sistema nervoso central (SNC) de avaliar a intensidade dos sinais e interpretá-los (Dethier 1982). O SNC responde a presença de substâncias químicas do hospedeiro, identificadas como deterrentes e tóxicas ou atraentes, antes de aceitar ou rejeitar o hospedeiro (Schoonhoven *et al.* 2005).

A maioria das espécies está preparada para reconhecer os compostos presentes na superfície foliar das plantas. Os órgãos sensoriais onde esses quimioreceptores encontram-se são os que mais facilmente podem entrar em contato com a superfície do hospedeiro, como antenas, tarsos, probóscide e ovipositor (Renwick & Chew 1994). Diversos estudos mostram que esses compostos, majoritariamente constituídos por substâncias lipossolúveis (Bernays & Chapman 1994), desempenham um papel decisivo na aceitação do hospedeiro, uma vez que os insetos geralmente costumam ovipositar em qualquer substrato no qual tenham sido aplicados extratos obtidos da superfície de sua planta hospedeira (Foster & Howard 1998, Hora & Roessingh 1999, Heinz & Feeny 2005, Heinz 2008).

Dimorfismo sexual

Em insetos, o dimorfismo sexual é um fenômeno muito comum (Blanckenhorn *et al.* 2007, Gotthard 2008), onde geralmente as fêmeas são maiores que os machos. Essa diferença deve-se principalmente ao fato de que o sucesso reprodutivo das fêmeas tem uma forte relação com o tamanho, onde fêmeas maiores tendem a ovipositar mais (Honek 1993). Essa relação é especialmente importante levando-se em consideração se a espécie é proovigênica, quando a produção de ovos é inteiramente dependente do consumo nutricional obtido na fase de larva (Honek 1993). Outros fatores que podem afetar a ocorrência no dimorfismo sexual são a frequência de cópula e a seleção sexual agindo sobre os machos.

Podem-se enumerar duas causas para gerar o dimorfismo sexual em Lepidoptera: diferenças na taxa de crescimento ou diferenças no tempo de desenvolvimento larval. Se ambos os sexos crescem com a mesma rapidez, não existe razão para esperar que haja dimorfimo. Contudo, fatores como a seleção sexual agem neste ponto, alterando o período de eclosão dos adultos. Em espécies monândricas ou monogâmicas, as fêmeas copulam uma vez. A pressão de seleção age para que os machos consigam eclodir o mais rápido possível, a fim de diminuir a competição com outros machos pelas fêmeas virgens. Machos que emergem mais cedo teriam maiores chances de conseguir copular com fêmeas no momento de emergência (Singer 1982). Se ambos os sexos possuem a mesma taxa de crescimento diário, os machos cujo período de eclosão é antecipado tendem a ter menor tamanho, decorrente do consumo de menor quantidade de nutrientes durante a fase larval mais curta. Portanto, existe uma relação entre a protandria (quando machos emergem antes das fêmeas), a frequência de cópula e o dimorfismo sexual (Wiklund & Forsberg 1991).

Quando a frequência de cópulas é diferente, espera-se que também haja uma diferença no dimorfismo sexual. Em sistemas poliândricos, os machos precisam minimizar as chances da fêmea em recopular e substituir seu esperma (Drummond 1984). Para isso, os machos procuram transferir espermatóforos grandes o suficiente para que as fêmeas permaneçam por mais tempo possível no período entre cópulas, chamado de período refratário, garantindo que seu esperma fecunde o maior número de ovos da fêmea e diminuindo as chances de recópula e por consequência haver precedência espermática por outro macho (Thornhill & Alcock 1983). Existe uma correlação positiva entre o tamanho do macho e o tamanho do espermatóforo que ele produz (Bissoondath & Wiklund 1996) e que em espécies poliândricas, o ejaculado do macho é mais rico em proteínas (Bissoondath & Wiklund 1995), contribuindo com a fertilidade e longevidade das fêmeas (Wiklund & Kaitala 1995). Desta forma, os machos em espécies poliândricas são selecionados para maximizar seu tamanho a fim de transferirem espermatóforos maiores e mais nutritivos para serem utilizados pela fêmea na fertilização dos ovos, aumentando assim o sucesso reprodutivo do macho. Neste caso, os machos permanecem o maior tempo possível na fase larval, consumindo uma maior quantidade de nutrientes para refletir em maior tamanho do adulto e consequentemente maior contribuição pelo ejaculado. Espera-se assim que o dimorfismo sexual não exista, ou seja, relativamente menor em espécies poliândricas do que em espécies monândricas, pressuposto confirmado por Wiklund & Forsberg (1991), quando mostrou uma forte correlação negativa entre o grau de poliandria e o dirmofismo sexual em Lepidoptera.

As diferenças entre poliandria e monandria podem afetar os insetos na sua relação com plantas hospedeiras. Leimar *et al.* (1994) testou a hipótese de que em espécies monândricas, o crescimento das lagartas de ambos os sexos seriam afetados diferentemente pela disponibilidade de recursos na fase imatura. Quando fêmeas e machos são colocados para se alimentar num hospedeiro de baixa qualidade, é esperado que as fêmeas sofram pela falta de alimento adequado, através da redução de seu tamanho, visto que não podem contar com a contribuição do macho via espermatóforo no aumento do sucesso reprodutivo. Nestas condições, o dimorfismo sexual foi reduzido, quando fêmeas cresceram relativamente menos que os machos na espécie monândrica *Pieris napi* (Leimar *et al.*, 1994). Por outro lado, Karlsson *et al.* (1997) previu que se espécies poliândricas fossem colocadas para crescer em hospedeiros de baixa qualidade, seria esperado que houvesse diminuição do dimorfismo sexual, desta vez pela redução do tamanho do macho, que sofreria por crescer num ambiente inadequado, exatamente o oposto do que Leimar *et al.* (1994) havia observado. Em testes com a espécie poliândrica *Pararge aegeria*, Karlsson *et al.* (1997) confirmou sua previsão de que em sistemas poliândricos são os machos que sofrem mais pela permanência num hospedeiro de baixo valor nutricional, diminuindo o dimorfismo sexual nesta condição.

Em alguns casos, existe conciliação entre ausência de protandria e manutenção do dimorfismo sexual (Nylin *et al.* 1993). Em algumas espécies poliândricas, machos podem aumentar sua taxa de crescimento, resultando em semelhança no período de desenvolvimento larval para ambos os sexos, o que implica na falta de protandria, e manutenção do dimorfismo sexual (Nylin *et al.* 1993, Gotthard *et al.* 1994).

Informações sobre o desenvolvimento larval de insetos é util para avaliar sua dinâmica populacional. O fato de se saber se uma espécie é inteiramente dependente do alimento obtido na fase larval para produzir ovos pode afetar o tamanho da população de insetos, baseado na qualidade e distribuição dos hospedeiros disponíveis na natureza.

Avaliação da contribuição do macho pela medida do espermatóforo

O esperma transmitido pelos machos através do estojo quitinoso chamado espermatóforo persiste na bursa copulatrix das fêmeas ao longo de sua vida (Burns 1968, Wedell *et al.* 2002). A presença e contagem desses fragmentos no trato reprodutivo das fêmeas capturadas em campo permitem saber quantas vezes uma fêmea copulou (Arnqvist & Nilsson 2000), visto que cada espermatóforo equivale apenas a uma cópula. Os espermatóforos também são usados para se medir o tamanho da contribuição do macho na cópula, quando além de esperma são transferidas substâncias nutritivas, como lipídios e proteínas, que aumenta a longevidade e a produção de ovos. Diversos métodos são usados para medir o tamanho ou volume do espermatóforo, como pesagens (Oberhauser 1997, Wedell 2006, Wedell & Cook 1999, Marcotte *et al.* 2007) ou associando a forma do espermatóforo com alguma figura geométrica, e estimando a partir daí seu volume (Rutowski 1980, Royer & McNeil 1993, Jimenez-Perez *et al.* 2003). Contudo, apenas o número de cópulas pode ser avaliado em campo, visto que os espermatóforos são colapsados no momento em que o esperma é transferido para a espermateca e à medida que as fêmeas vão adquirindo cópulas subsequentes.

Frequência de Cópula

Em Lepidoptera a frequência de cópulas varia desde a estrita monandria até uma ampla poliandria (Drummond 1984). Baseado no fato de que a quantidade de espermatozóides transferidos pelo macho é grande o suficiente para fecundar todos os ovos que uma fêmea possue (Drummond 1984), acreditava-se que a poliandria seria um fenômeno raro. Contudo, a ocorrência regular da poliandria sugere que as aparentes desvantagens das cópulas múltiplas devem ser superadas pelas vantagens desse sistema de reprodução (Thornhill & Alcock 1983). Existem diversas hipóteses utilizadas para explicar os benefícios da poliandria: favorecer a diversidade genética da progênie e evitar incompatibilidade genética (Tregenza & Wedell 2002); reposição espermática, que ocorre quando a primeira cópula não transmite esperma em quantidade suficiente para fertilização de todos os ovos da fêmea, ou pela degradação natural do esperma, devido ao longo tempo de armazenamento dentro da espermateca (Thornhill & Alcock 1983); benefícios materiais, quando as fêmeas copulam para obter mais nutrientes através do espermatóforo do macho, a fim de aumentar a longevidade e fecundidade (Karlsson 1998, Jimenez-Perez *et al.* 2003) e conveniência, quando a recópula é uma forma de minimizar o gasto de energia ao tentar recusar o assédio insistente de machos (Thornhill & Alcock 1983).

A frequência de cópula afeta diretamente a dinâmica populacional de uma espécie. A propensão que uma fêmea tem de recopular afeta diretamente a eficiência de estratégias de manejo de insetos-praga, como a técnica do inseto estéril (TIE). À medida que a frequência de cópulas aumenta e o período refratário diminui, assume-se que as fêmeas tendem a copular com um número relativamente maior de machos, aumetando as chances da fêmea copular com machos estéreis e depois com machos naturais, diminuindo sensivelmente a eficiência do TIE (Kraaijeveld *et al.* 2005), visto que machos selvagens tem maior sucesso de cópula que machos estéreis (Kraaijeveld & Chapman 2004).

Além da iniciativa da fêmea em copular mais vezes, para aumentar seu sucesso reprodutivo através do aumento da progênie, existe a iniciativa por parte dos machos. O sucesso reprodutivo dos machos é alcançado pelo número de fêmeas com quem copula (Thornhill & Alcock 1983), o que favorece sua tendência em obter maior número possível de cópulas. Características como habilidade no vôo, vigor, atividade e persistência são consideradas responsáveis pelo sucesso reprodutivo dos machos em diversas espécies (Rutowski 1979, Fischer 2006, Fischer *et al.* 2008). Mas essas mesmas características podem ser afetadas por outros fatores, como o número de cópulas que este macho já adquiriu ao longo da vida, o que afeta a capacidade do macho de conseguir novas cópulas. Como em cada cópula o macho precisa transferir um espermatóforo cheio de esperma e nutrientes para a fêmea (Bissoondath & Wiklund 1995), é esperado que ele precise de tempo para investir na produção de um novo espermatóforo e realocar mais nutrientes e esperma para a cópula futura. Em muitos casos, machos experientes demoram mais tempo durante a cópula do que machos virgens (Svard & Wiklund 1986, Wiklund & Kaitala 1995, Lauwers & Dyck 2006), devido ao fato de que diante da oportunidade de recopular, o macho não espera o tempo suficiente para produzir um novo espermatóforo tão grande quanto o primeiro para transferir para a fêmea. Isso implica em que haja pressão de seleção favorecendo os machos que consigam produzir espermatóforos grandes e nutritivos em um menor intervalo de tempo a partir da última cópula, para competir com machos virgens, uma vez que machos recentemente copulados não conseguem produzir um espermatóforo tão grande quanto produziram na primeira cópula (Svard & Wiklund 1986, Rutowski *et al.* 1987). Em espécies monândricas, como os machos investem relativamente pouco no espermatóforo, espera-se que o período entre cópulas do macho não afete sua persistência em adquirir uma nova cópula.

As estratégias reprodutivas e a Broca-pequena-do-tomateiro

A broca pequena *N. elegantalis* é considerada uma praga de grande importância devido aos prejuízos causados às Solanaceae em geral, notada especialmente na cultura do tomate, de onde provém seu nome popular. As perdas provocadas pela broca são altas, inviabilizando comercialmente de 45 a 90% dos frutos de tomate produzidos (Leiderman & Sauer 1953, Gallo *et al* 2002). Como Pernambuco atualmente é considerado um dos principais produtores desta hortaliça no Nordeste do país (Costa & Heuvelink 2005) os estudos sobre este inseto devem ser incentivados, para minimizar os danos econômicos resultantes da ação desta broca.

A forma mais popular de controle da broca pequena ainda é a aplicação de inseticidas. Contudo, esse método é muito limitado, visto que os períodos no qual a broca estaria mais vulnerável (entre a eclosão da lagarta do primeiro ínstar e sua entrada no fruto) são de curta duração. Apesar do uso de inseticidas se mostrar eficiente em alguns casos (Lima *et al.* 2001), há diversos fatores que podem alterar a eficiência do produto e consequentemente o regime de aplicação a ser adotado (Lima *et al.* 2001). Estudos sobre inimigos naturais da broca pequena têm sido desenvolvidos em outros países da América Latina (Muñoz *et al.* 1991, Millán *et al.* 1999, Trochez *et al.* 1999), destacando-se a ocorrência do parasitóide *Copidosoma* sp. (Hymenoptera:Encyrtidae) que ocorre naturalmente. Métodos culturais como ensacamento dos cachos é visto como uma alternativa ao ataque da broca, mas restrito somente a pequenos produtores (Jordao & Nakano 2000).

O uso do método de controle comportamental é visto como uma das alternativas mais adequadas para o controle da broca pequena no programa de no manejo integrado desta praga. Graças a estudos do comportamento reprodutivo de *N. elegantalis*, foi possível verificar fatores que dificultam o controle químico, como o local no fruto onde a maioria das fêmeas ovipositam (Blackmer *et al.* 2001), a inviabilização de aplicar inseticidas de forma que atinjam as lagartas, uma vez que o período em que a lagarta fica vulnerável à aplicação de produtos é de curta duração (Eiras & Blackmer 2003), e descobrir os detalhes do chamamento (Eiras 2000) que permitiram identificar os principais componentes do feromônio sexual desta espécie (Cabrera *et al.* 2001) e testá-los em campo (Badji *et al.* 2003). Apesar de o feromônio ser eficiente como atraente dos machos da broca (Cabrera *et al.* 2001, Badji *et al.* 2003, Jaffé *et al.* 2007), estudos realizados na Venezuela atestam que a eficiência do feromônio sintético varia de acordo com fatores diversos, como altitude do plantio (Arnal *et al.* 2006) e que os modelos das armadilhas de feromônio variam no seu potencial de capturarem machos (Mirás *et al.* 1997). O estudo de outros fatores intimamente relacionados com o sucesso reprodutivo desta praga é essencial para a melhoria e elaboração de novos métodos de controle desta praga.

Esta tese tem por objetivo estudar a relação química existente entre a broca pequena do tomateiro *N. elegantalis* e seu hospedeiro mais conhecido, o tomate; estudar as causas que geram o dimorfismo sexual nesta espécie e o potencial de recópula dos machos, de acordo com sua experiência sexual.

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CAPÍTULO 2

PHYSICAL AND CHEMICAL CUES AFFECT OVIPOSITION BY *Neoleucinodes elegantalis*[1]

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ABSTRACT – Recognition and acceptation of a suitable host plant by phytophagous insects requires integration of visual, physical and chemical cues. In this study, we investigated which host cues a specialist insect integrates to optimize oviposition decisions and whether these cues are weighed in a specific way. We tested whether the tomato fruit borer *Neoleucinodes elegantalis* (Lepidoptera: Crambidae), an important pest on Solanaceae in Brazil, shows a preference for oviposition sites that differ in physical cues, chemical cues, or both. We used styrofoam balls as artificial fruits. *N. elegantalis* deposited significantly more eggs on rough artificial fruits than on smooth ones. Hexanic fruit extracts applied to the artificial fruits strongly stimulated female oviposition. Physical and chemical cues also affected the oviposition of females when offered together. Furthermore, certain parts of the artificial fruits were prefered, irrespective of the presence of chemical cues. Both physical and chemical cues affected oviposition decisions, hence the fruit borer relied on cues of different sensory modalities.

KEY WORDS: Artificial fruits, chemical stimuli, egg-laying behavior, physical stimuli, tomato fruit borer

SINAIS FÍSICOS E QUÍMICOS AFETAM A OVIPOSIÇÃO DE *Neoleucinodes elegantalis*

RESUMO – O reconhecimento e aceitção de uma planta hospedeira adequada pelo inseto fitófago requer uma integração de rastros físicos, químicos e visuais. Nesse estudo, foi investigado quais rastros um inseto oligófago utiliza para otimizar as decisões de ovipositar e de que forma esses rastros são avaliados. Foi testado se a broca pequena do tomateiro *Neoleucinodes elegantalis* (Lepidoptera: Crambidae), uma importante praga das solanáceas no Brasil, mostra alguma preferência de oviposição em substratos que diferem em relação aos estímulos físicos, químicos ou quando ambos são oferecidos. Foram usadas bolas de isopor simulando frutos artificiais nesses experimentos. *N. elegantalis* ovipositou um maior número de ovos em frutos artificiais rugosos do que em lisos. Extrato hexânico de frutos de tomate que foram aplicados nos frutos artificiais estimularam fortemente a oviposição. Os sinais físicos e químicos também afetaram a oviposição da fêmea, quando oferecidos juntos. Certas partes dos frutos artificiais foram preferidas, a despeito da presença de sinais químicos. Ambas as pistas físicas e químicas afetaram o estímulo de oviposição. Esses dados mostram que a broca pequena acessa as pistas disponíveis através de diferentes modalidades sensoriais.

PALAVRAS-CHAVE: Frutos artificiais, estímulo químico, comportamento de oviposição, estímulo químico, broca pequena do tomateiro

Introduction

Finding a suitable host plant is one of the critical steps for reproduction of all phytophagous insects. The insect-host association tends to evolve most frequently oviposition preference and larval performance (Thompson & Pellmyr 1991). Because newly hatched larvae are relatively immobile, they are not able to find a better host when their mother has oviposited on a host not suitable for larval development (Renwick & Chew 1994).

Recognition of a suitable host plant requires integration of inputs from physical and chemical senses. Olfaction and vision may be used for long-distance orientation to the host plant. After landing, short-distance judgment requires senses to detect chemical and physical cues, using contact chemo receptors on antennae, mouthparts and ovipositor. Usually, recognition of chemical compounds on the plant surface results in a decision to accept or reject the oviposition site (Ramaswamy 1988, Renwick & Chew 1994).

Physical characteristics of the oviposition site such as surface texture play a critical role in the final decision to lay eggs (Renwick & Chew 1994). Some moths prefer to oviposit on hairy or rough surfaces (Ramaswamy 1988, Rojas *et al.* 2003, Nava *et al.* 2005), other species prefer smooth surfaces (Foster *et al.* 1997 Calatayud *et al.* 2008).

Chemical compounds present on plant surfaces can be volatiles that contribute in long-distance orientation to the host plant, or non-volatile compounds that play a role as oviposition stimulants or deterrents (Renwick & Chew 1994). It is well established that these chemicals cues are very important in orientation and acceptance of oviposition sites in several Lepidoptera species (Ramaswamy 1988, McNeil & Delisle 1989, Honda 1995, Peterson & Elsey 1995, Hora & Roessingh 1999, Reddy *et al.* 2004, Gouinguené *et al.* 2005, Lombarkia & Derridj 2008, Sidney *et al.* 2008).

For both physical and chemical cues, there is a broad or narrow preference among phytophagous insects depending on host suitability, because host plants differ in physical and chemical cues, that insects need to discriminate among even more different cues. Oligophagous insects could concentrate on one or few cues that are specific to their host plant specialists should have high ability to evaluate available cues from host plants to discriminate between oviposition sites (Janz & Nylin 1997). The recognition of these cues depends on the insect sensory system in detecting and decoding signals available in the host (Dethier 1982). However, rejection and acceptance of oviposition sites depend on the analysis of sensory input by the insect's central nervous system and the physiological state of the insect.

The tomato fruit borer *Neoleucinodes elegantalis* (Lepidoptera: Crambidae) is an important pest and oligophagous on Solanaceae. Adults are active at night only. First instar larvae have low mobility and bore inside the fruits within a few minutes after hatching (Eiras & Blackmer 2003). When the fruits are not available, larvae are moribund (Pontes, personnal communication). This suggests that mated females are under strong selection pressure to find the correct site to lay eggs. Hence, mated females should have the ability to detect cues indicating hosts and host quality. *N. elegantalis* also shows preference to lay eggs on specific places of fruits, i.e. under the calix (Blackmer *et al*. 2001). However, is not known what kind of physical and chemical cues determine oviposition choice by *N. elegantalis*, or if they are sensitive to luminescence, as members of the closest family Pyralidae seems to be (Briscoe & Chittka 2001). We therefore tested which physical cues, i.e. surface types, and which chemical cues affect oviposition in *N. elegantalis* females and how these cues of different sensory modality interact.

Material and Methods

Neoleucinodes elegantalis were collected from a commercial tomato crop in Minas Gerais State, Brazil, and reared in the laboratory for two years. Both the rearing and experimental conditions were set at 25 ± 1°C and 71.2 ± 10% relative humidity (RH) and under a light regime set to LD 12 : 12 h. The larvae were reared on *Solanum gilo* (Solanaceae) fruits until pupation. Pupae were sexed and each gender was incubated in separate experimental wooden cages (50 x 50 x 50 cm). At emergence, males and females were put together during 3 days for copulation in another cage with a cotton piece soaked in a honey solution (10%). After about 3 days, the females were used in the experiments, and thereafter dissected to assess the presence of spermatophores in the *bursa copulatrix* (Burns, 1968). To assess oviposition, we used styrofoam balls with a diameter of 3.5 cm as artificial fruits. All these balls were dipped in green melted paraffin to produce a thin wax layer covering the surface. Nine replicates were done for each treatment of each experiment, and each replicate involved the oviposition response of a group of 10 females.

Physical cues. To test the influence of physical cues on *N. elegantalis*, ovipositing females were offered a choice between two different surfaces. We used ten gravid females of 48-h-old. Longitudinal furrows were made with a razor blade on the surface of the artificial fruits to obtain a rough surface. Intact artificial fruits were considered smooth. A rough and a smooth artificial fruit were suspended from the top of the cage with a wire, approximately 25 cm apart. Between replicates, and after 24 h within an experiment, the position of the artificial fruits was interchanged to avoid environmental bias. The females were allowed to oviposit for 48 h. Thereafter, eggs on each artificial fruits were counted. On rough artificial fruits, eggs on the smooth surface and in the furrows were counted separately.

Chemical Cues. Tomatoes fruits, *Lycopersicon esculentum* L. (Solanaceae, variety Sensação, Agrocinco, São Paulo) with a diameter of approximately 1.5 cm were collected in a commercial tomato crop in Minas Gerais State, Brazil, for extraction of chemical cues. The fruits were weighted and immersed in hexane for 30 min. After this period, the solution was reduced to by evaporation of the solvent. The fruit extract was then expressed as gram fruit equivalent per mL solvent and kept in a freezer (-18ºC) until used in the tests.

To access if these fruit extracts affected the oviposition decision of females, a choice test was performed with artificial fruits containing extracts and controls without extract. The fruit extract was applied 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 30 µl of the hexane extract to the filter paper; the same amount of pure hexane was applied to the controls. Groups of ten gravid females were offered a choice between these two artificial fruits for 24 h, after which eggs on each artificial fruit were counted. The eggs deposited on the waxy surface, on the surface of the strip of paper and under the strip of paper were counted separately and compared between treatments.

Visual and chemical cues. To evaluate the effect of chemical and visual cues on oviposition, four treatments were offered to females: both chemical and visual cues; only visual cues; only chemical cues; and neither of the two cues.

To manipulate visual cues, experimental cages were placed under low light intensity (0.11 Lux, measured with a luxometer) providing the minimum light intensity for insect activity and used to rear *N. elegantalis* during the scotophase. Absence of visual cues was mimicked by placing cages in absolute darkness (0.00 Lux). Chemical cues were offered on smooth artificial fruits to which a hexanic extract of tomate fruits was applied as described above. Females were allowed to oviposit for 48 hours. In this experiment, all treatments were repeated five times with 6 females per replicate. Eggs were counted after 24h.

Statistical analysis. A chi-square test was applied to analyze the choice between each treatment in both physical and chemical tests. The mean percentage of eggs on each of the two artificial fruits was calculated.

To test the combined effects of visual and chemical cues on oviposition of females, eggs were counted on each artificial fruit in all treatments and analyzed with generalized linear model with a quasi-poisson error distribution. All analyzes were performed with R statistical system, version 2.4.1. (R Development Core Team, 2006).

Results

Physical cues. *N. elegantalis* deposited significantly more eggs on rough artificial fruits than on smooth ones ($\chi^2 = 22.65$, $p < 0.0001$) (Fig. 1A). More eggs on rough artificial fruits were found inside the furrows ($\chi^2 = 51.12$, $p < 0.0001$) than on the waxy smooth surface (Fig. 1A).

Chemical cues. More eggs were found in treatments with hexane extract than in the control ($\chi^2 = 3.92$, $p < 0.05$) (Fig. 1B). More eggs were laid on the filter paper strips with hexane extracts than on strips with only hexane ($\chi^2 = 8.20$, $p < 0.001$) (Fig. 2A) and more eggs were laid on the wax layer of the artificial fruit with tomato hexane extract than on that with hexane only ($\chi^2 = 8.26$, $p = 0.0040$) (Fig. 2B). Similarly, more eggs were deposited under of the filter paper strip on the artificial fruits treated with tomato hexane extracts ($\chi^2 = 8.27$, $p = 0.0040$) than under the strip on the artificial fruits treated with solvent only (Fig. 2 A and B). Most of the eggs were laid under the paper strip, followed by eggs on the strips and in smaller quantities on the wax layer of the artificial fruits. This pattern was observed both in treatments, i.e. with hexane extracts and with hexane as control (Fig. 2 A and B).

Visual and chemical cues. Significantly more eggs were laid when both visual and chemical cues were available at the same time ($\chi^2 = 321.3$, $P < 0.0001$, df = 3) (Fig. 3). However, no differences in egg numbers were found when at least one of the cues and both of them were removed.

Discussion

The results of the present study show that physical and chemical cues significantly affected the oviposition of *N. elegantalis.* The effect of physical factors on oviposition in Lepidoptera is well known. Some species prefer smooth surfaces (Foster *et al.* 1997, Calatayud *et al.* 2008) while other species prefer rough substrates (Ramaswamy 1988, Rojas *et al.* 2003, Nava *et al.* 2005). A preference for smooth surfaces may help the females to sweep their ovipositor over the surface to taste and thereby to find a suitable site for oviposition (Calatayud *et al.* 2008). However, preference for rough surfaces (Ramaswamy 1988, Fenemore 1988) seems to be more general (Rojas *et al.* 2003).

The large number of eggs found inside the furrows of roughened artificial fruits suggests a preference to oviposit in places that are less exposed to environmental conditions and natural enemies (Janz 2002). For example, in *Pieris rapae crucivora*, not only surface texture is preferred for egg laying, but also plant parts that can provide some protection to eggs (Tagawa *et al.* 2008).

The oviposition behaviour of *N. elegantalis* was observed in some cases: females walked on the surface, thereby contacting the surface of the artificial fruit with their antennae and the tip of the proboscis and dragging the ovipositor over the substrate while walking. This behavior suggests that sensory organs for tactile and chemical cues are located on the antennae, proboscis and ovipositor. Many butterflies and moths prior are known to antennate and drum the plant surface with their ovipositor prior to egg laying, thus probably using contact-chemoreceptors to taste the suitability of chemical compounds and to explore the surface texture of their host plant (Renwick & Chew 1994), using contact-chemoreceptors (Visser 1986, Maher & Thiery 2004). The antennal receptors of other Lepidoptera species show differential sensitivity to host and non-host plants (Mercader *et al.* 2008).

Hexane extract of fruits significantly stimulated oviposition by *N. elegantalis*. All artifical fruits on which tomato extracts were applied were preferred for oviposition. Application of host plant chemical compounds to artificial substrates usually makes these sites more acceptable for oviposition (Foster & Howard 1998, Hora & Roessingh 1999, Heinz & Feeny 2005, Heinz 2008).

The fact that a hexane extract of tomato stimulates oviposition in *N. elegantalis* does not rule out the influence of visual cues on oviposition behaviour; we found that light is required to locate a source of attractive chemicals. These results show that most females lay more eggs in experimental cages when both cues (in this case, visual and chemical) were available. The few eggs that were found on artificial fruits in absolute darkness and without extract (no chemical cue) are perhaps the result of some females that randomly encountered artificial fruits, landed on it and oviposited.

More eggs are laid when chemical stimuli were combined with rough surfaces; when a filter paper treated with extracts was combined with the artificial fruit, the rough surface of the filter paper received the highest humber of eggs. This shows an interaction of chemical and physical cues on oviposition decisions. Volatiles and non-volatile compounds are known to stimulate attraction of gravid females (Piñero & Dorn 2007), and to increase number of eggs layed on the source (Honda 1995, Spencer *et al.* 1999). Our results show that *N. elegantalis* females also use different sensory modalities to assess oviposition sites.

In both treatments, with and without hexane extract, the insects preferred to lay eggs under the strip, near the edge, which is probably similar to their preference to lay eggs in a furrow. In the field, eggs of *N. elegantalis* are found mainly on the underside of the calyx, and this preference was suggested to be due to physical rather than chemical cues (Blackmer *et al.* 2001). Our results showed that *N. elegantalis* preferred furrow-like places on the artificial fruits, irrespective of whether they were offered chemical stimulants or not. However, the presence of hexane extracts significantly increased the total number of eggs laid by females, in a combined effect of physical and chemical stimuli presented together.

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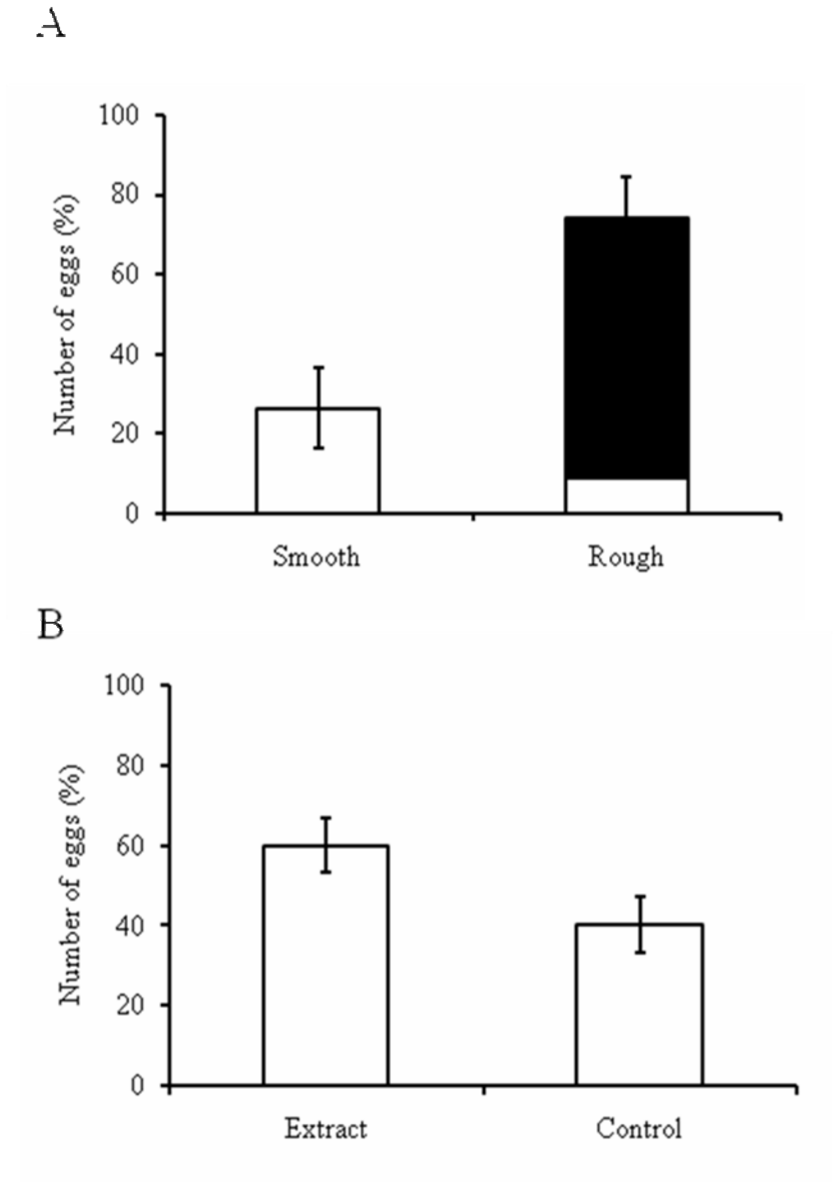

Figure 1. (A) Mean percent of eggs of *N. elegantalis* deposited on smooth and rough artificial fruits when they were offered simultaneously in a two-choice test. On the right bar is shown the amount of eggs layed inside furrows (black) on rough artificial fruits. (B) Eggs deposited on artificial fruits treated with hexane extract of tomato fruits compared with artificial fruits treated with hexane only (control). Error bars indicate standard errors.

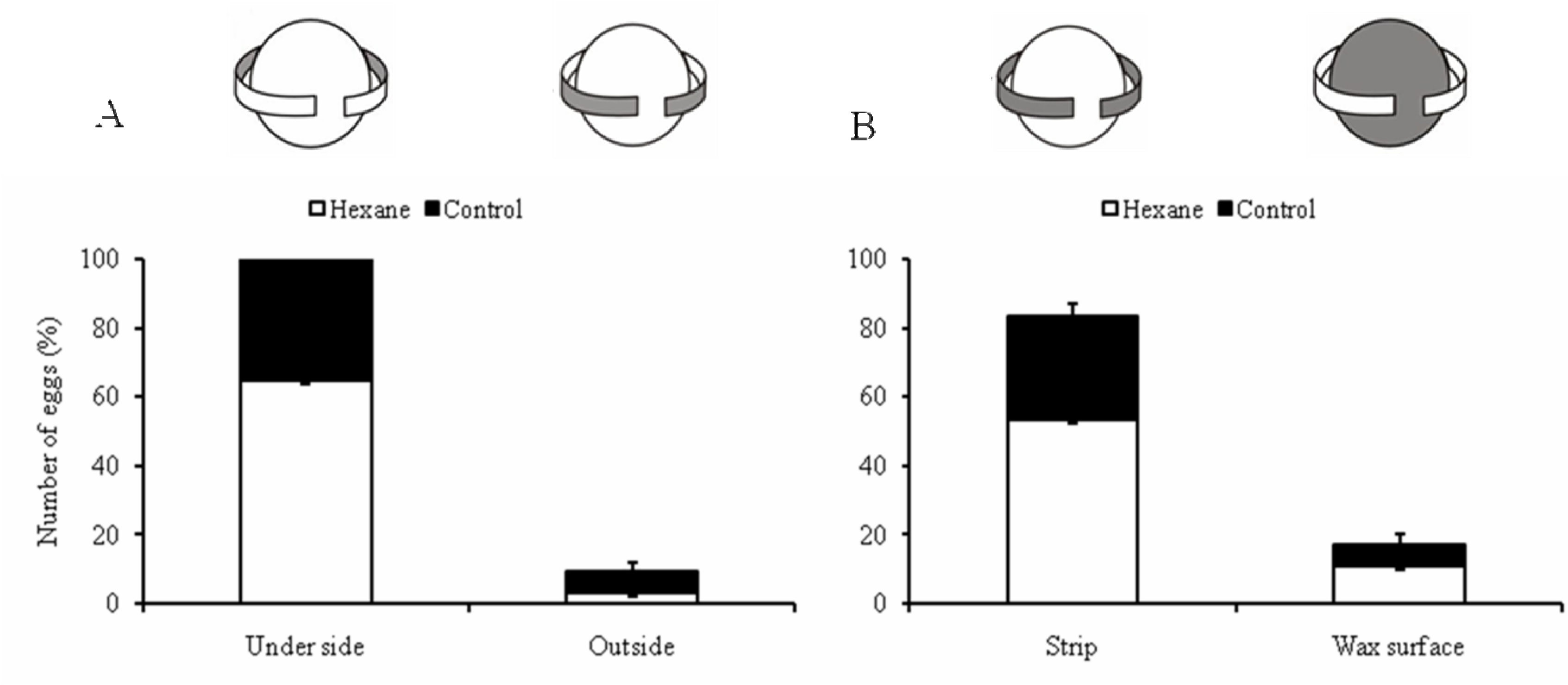

Figure 2. Number of eggs deposited by *N. elegantalis* on artificial fruits with hexane extract (open bars) and only hexane (black bars). Eggs on: (A) under side of the strip and the strip (outside), (B) the whole strip and the wax surface. Egg deposition areas are highlighted in grey.

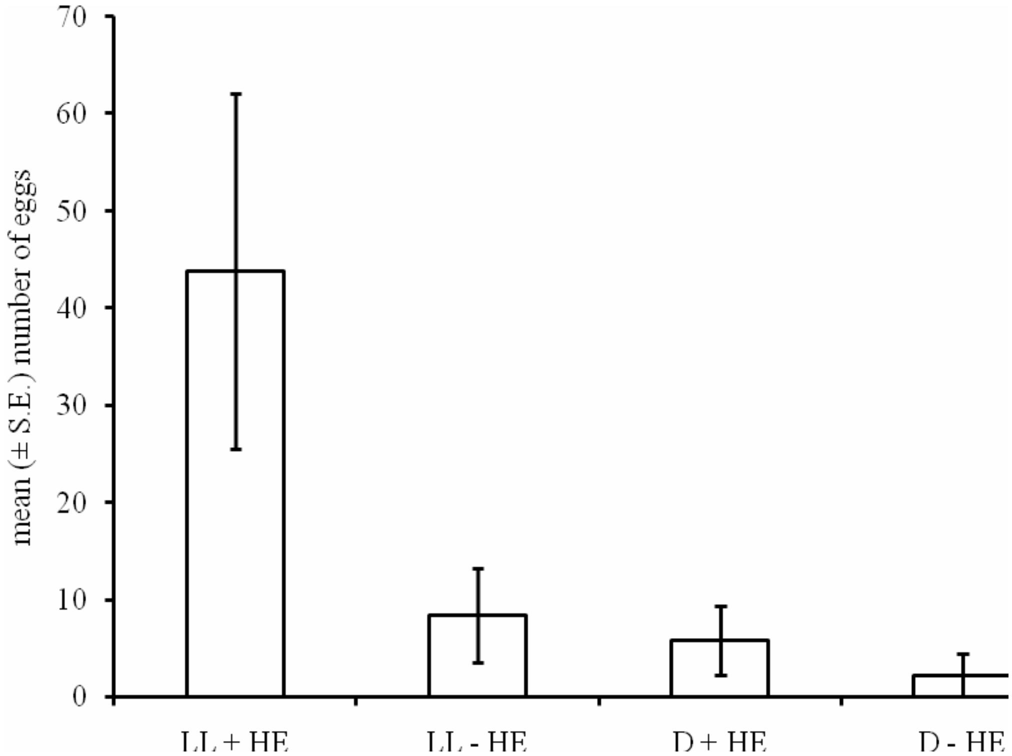

Figure 3. Mean number of *N. elegantalis* eggs on artificial fruits in visual and chemical conditions. (LL: low light intensity; HE: hexane extract and D: absolute darkness). Only the values recorded for the LL + HE treatment are significantly different from the others. Error bars indicate standard errors.

CAPÍTULO 3

DIFFERENCES IN GROWTH RATE EXPLAIN SEXUAL SIZE DIMORPHISM IN *Neoleucinodes elegantalis* GUENÉE (LEPIDOPTERA: CRAMBIDAE)?[1]

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[1] Pontes, W.J.T., E.R. Lima, E.G. Cunha, & R. Barros. Differences in growth rate can explain sexual size dimorphism in *Neoleucinodes elegantalis* Guenée (Lepidoptera: Crambidae). A ser submetido.

ABSTRACT – Sexual size dimorphism is a response to fecundity selection, where the relationship between fecundity and size is greater in one sex than in another. Selection on females which achieve larger size is a result of prolonged growth period or high growth rate, or both. If females increase development time rather than males, protandry is expected to occur. If females increase growth rate, both males and females must eclode at the same time and sexual dimorphism is maintained. The present experiment tested if sexual size dimorphism in the tomato fruit borer *Neoleucinodes elegantalis* is maintained by protandry (maintenance of the same growth rate between the sexes) or by differences in growth rate (resulting in absence of protandry), or by both. We found that sexual size dimorphism is produced by differences in growth rate, when females increased more than males at the same developmental period. Ecological implications are discussed, as potential implications on pest management.

KEY WORDS: Developmental time, eclosion, sexual size dimorphism, tomato fruit borer

DIFERENÇAS NA TAXA DE CRESCIMENTO EXPLICAM O DIMORFISMO SEXUAL EM *Neoleucinodes elegantalis* (LEPIDOPTERA: CRAMBIDAE)

RESUMO – O dimorfismo sexual é uma resposta à seleção sobre a fecundidade, onde a relação entre fecundidade e tamanho é maior sobre um sexo do que sobre outro. A seleção sobre as fêmeas para alcançarem um maior tamanho pode ser o resultado de uma prolongada fase de desenvolvimento larval, de uma alta taxa de crescimento, ou ambas. Se as fêmeas aumentam o tempo de desenvolvimento larval em relação aos machos, é esperado que ocorra protandria. Se as fêmeas tem uma maior taxa de crescimento, machos e fêmeas devem eclodir ao mesmo tempo e o dimorfismo sexual deve ser mantido. O presente trabalho testa se o dimorfismo sexual na broca pequena do tomateiro *Neoleucinodes elegantalis* resulta em protandria (isto é, ambos os sexos possuem a mesma taxa de crescimento), ou é o resultado da diferença entre taxas de crescimento (ausência de protandria), ou se é devido a ambas as causas. Os resultados mostram que o dimorfimso seuxal é produzido pela diferença da taxa de crescimento, onde fêmeas crescem mais que machos no mesmo período de desenvolvimento larval. Implicações ecológicas desse resultado é discutido, bem como sua potencial implicação no manejo de pragas.

PALAVRAS-CHAVE: Duração do período larval, emergência, dimorfismo sexual, broca pequena do tomateiro

Introduction

Sexual size dimorphism is a widespread phenomenon among animals (Teder & Tammaru 2005), including insects (Blanckenhorn *et al.* 2007, Gotthard 2008). One of the forces that acts on the evolution of sexual size dimorphism is natural selection, which acts differently on males and females in consequence of differences in their sexual strategies (Rutowski 1997). Female size is a response to fecundity selection, where the relationship between fecundity and size is greater in females than in males, leading to a female biased sexual size dimorphism (Wiklund & Karsson 1988). Female fecundity in insects has a strong positive relationship to adult body mass (Honek 1993) especially in proovigenic females whose egg production is dependent of nutrients obtained in larval stage. Selection on females to larger size can be achieved through prolonged growth period or high growth rate (Gotthard 2008). If females can increase their fitness through achieving larger size by longer development, and males do not invest in growth rate at the same extent, protandry also is expected to occur (Singer 1982). Some inferences are needed to link protandry with sexual size dimorphism, as differences in larval developmental time between sexes (Singer 1982) and the degree of polyandry or monandry in the species (Wiklund & Forsberg 1991).

In monandric or monogamic Lepidoptera, females are known to receive small male resources such as protein in ejaculate (Bissoondath & Wiklund 1995, Bissoondath & Wiklund 1996) and male investment in longevity and fecundity of females and fertility of offspring is relatively small (Svard 1985, Oberhauser 1997). Hence big ejaculates as male investiment in monandric systems should be weak and females will count only with their own resources achieved in larval stage to invest in reproduction. In monandric and monogamic systems, selection for male early emergence, and not for large size, is strong (Wiklund & Forsberg 1991, Zonneveld 1996a, Zonneveld 1996b, Wiklund & Solbreck 1982). Positive correlation between sex size dimorphism and the degree of female polygamy suggests that sexual selection favors female's large size only when females are relatively monandrous (Wiklund & Forsberg 1991). But males also can achieve higher size in absence of protandry (Nylin *et al.* 1993), by increasing at the same rate as females increase, resulting in same larval development time (absence of protandry) and permanence of sexual size dimorphism female biased (Nylin *et al.* 1993, Gotthard *et al*. 1994).

The tomato fruit borer, *Neoleucinodes elegantalis*, a well-known Solanaceae insect pest in Brazil, shows both sexual size dimorphism female biased and apparent monogamy (Jaffé et al. 2007). Thus, is expected that sexual selection could act on this species to maintain size dimorphism by: (1) protandry, when males should develop at the same rate as females but is selected to emerge earlier, or by (2) differences in growth rate between the sexes, resulting in absence of protandry but maintenance of sexual size dimorphism. The aim of this article is test if one of the above mentioned explanations, or both, can explain the sexual size dimorphism in *N. elegantalis*.

Material and Methods

The insects used in this study were from laboratory rearing. The rearing conditions were 12L:12E, 25 $^oC \pm 1^oC$ and 71,2 ± 10% UR. All larvae originated from adults reared in laboratory were placed on jilo (*Solanum gilo*) fruits in the proportion of six larvae per fruit. The fruits were inspected every day to collect last larval instars that left the fruits. Larval duration was recorded. All larvae were allowed to pupate individualized in separate containers. Pupae were removed and weighed on the second day after pupation, in an analytical balance (Precisa 262 SMA-FR) and individualized into plastic vials. Pupal weight was taken as a good indicator of body mass (Bissoondath & Wiklund 1995, Wiklund *et al.* 1991). Overall 192 pupae are used in this experiment. At eclosion, adults were sexed by comparing abdominal tips. Males have lighter abdomen and thinner tips compared to females. We registered eclosions daily since the first adults appeared until the day when no more adults ecloded, and all the remaining pupae are discarded.

The growth rate of both sexes was calculated according to the formula: Growth rate = [ln(Pupal weight) - ln(hatchling weight)]/larval time (Gotthard *et al.* 1994). Since *N. elegantalis* at hatching are small, we weighed 10 samples of 15 larvae and used the mean weight (0.019 mg) as hatchling weight for both sexes.

The analyzes were performed with the Statistic Software R (version 2.4.1). The data were analyzed using generalized linear models (Crawley 2005). To analyze sexual size dimorphism, mean emergence day and comparison of growth rate between sexes, one-way ANOVA was used. To evaluate the time of eclosion differences between males and females for each day, we used a test for survival used to compare differences between two or more curves (Harrington & Fleming 1982).

Results

Of all pupae weighted and individualized (n=192), just 111 moths emerged (57.8%) and these were used in the analysis. Sexual size dimorphism was significant ($F_{1,109}$=82.463, P<0.001), where females were heavier (50.37±0.89 mg) than males (38.80±0.85 mg) (Fig. 1A).

There was no difference in time of eclosion between the sexes ($F_{1,109}$=3.289, P=0.072); males (6.46 ± 0.29 days after pupation) and they did not emerge significantly earlier than females (5.79 ± 0.22 days after pupation) (Fig. 1B).

When evaluating cumulative number of moths emerged on the following days (Fig. 2A) slightly more males emerged before females in the first four days, from which the number of females that ecloded was higher than males, and continued until the end of all eclosions. This difference was, however, not significant (χ^2=2,6,df=1,P=0.106).

Differences in growth rate between the sexes were significant. Females increased more per unit of time (3.75 ± 0.04 mg/day) than males (3.52 ± 0.03 mg/day) ($F_{1,49}$=20.782, P<0.001) (Fig. 2B), in the same larval development time.

Discussion

The present results suggest that maintenance of sexual size dimorphism in *N. elegantalis* can be explained by differences in growth rate between the sexes and that early male emergence and it does not occur. Protandry only makes sense when the generations are discrete. When females are always available, early eclosion of males in order to increase the probability to find virgin females should not be selected for (Singer 1982). Because Solanaceae fruits are available throughout the year (Albuquerque *et al.* 2006) being available for infestation by *N. elegantalis* during all time, overlap of generations probably occurs. This is a trait that should exclude protandry (Wiklund 1977, Singer 1982). Another trait that certainly concurred to absence of protandry is the fact that *N. elegantalis* mating peak is two and three days after eclosion (Eiras 2000). All models that explain protandry preclude that females should mate in the first day of eclosion (Wiklund 1977, Fagerstrom & Wiklund 1982, Zonneveld 1992). Even in absence of overlap of generations, early male emergence should not occur because if both sexes will eclode at the same time, most of the females only will mate two days before eclosion. These traits seem to be under stronger selection than protandry should be, and developed as a result of that.

Variation in eclosion period reflects differences in larval growth rate or larval development time. The results showed that *N. elegantalis* females have higher growth rate than males, which can be a coherent explanation for the sexual size dimorphism in absence of protandry. Increase in larval growth rate is shown to be an alternative to compensate the trade-off of small size to achieve early emergence in protandric species. Early emergence precludes small males, if the growth ratio is the same for both sexes. But in polyandry, selection for larger males should be strong, producing larger ones. Because selection on early male emergence counterbalance with larger size, another way to achieve protandry and be larger is increasing male growth rate. Some evidences suggest that this strategy is used in species where large size and polyandry are under strong selection, and can co-exist when males can increase growth ratio to emerge earlier and achieve larger size simultaneously (Blanckenhorn *et al.* 2007). In *N. elegantalis*, female high development rate can be explained by the strong selection on female larger size, what means high fecundity, especially in monandric species where smaller contribution of males on female reproduction output is expected (Gotthard 2008). Absence of protandry shows that emergence time of both sexes is equal. Thus, increasing female growth rate is a way to achieve larger adult size. Females of aseasonal populations of *Pararge aegeria* can grow faster and reach larger size without the need to have a longer developmental period compared to males (Gotthard *et al.* 1994).

In absence of protandry, why males do not increase at the same rate as females, achieving larger size? Although male body size could be important in male-male competition (Wiklund & Forsberg 1991), in sexual selection (Phelan & Baker 1986) and mobility (Tammaru *et al.* 1996), no further advantage is seem to favor male larger size. High growth rate could shorten larval development time, reducing the most commonly cost assumed by larval development time, that is decreasing risk of mortality in immature stage (Gotthard 2000). However, increasing growth rate have a trade-off of physiological costs (Gotthard *et al.* 1994, Fischer *et al.* 2004) and increasing predation risk (Gotthard 2000), although predation risk during larval stage is minimized in *N. elegantalis*, a fruit borer that stays almost all larval stage protected inside fruits. Despite all advantages in achieve larger size, the fact that females of most insects are larger than males suggests that the relation between fecundity and size is an advantage more important for female fitness than for males, if they could achieve larger size (Nylin & Gotthard 1998).

When females are heavier and protandry is absent, it has been suggested that female growth ratio is closer to maximum than males (Nylin & Gotthard 1998). This plasticity between the sexes has recently received many attentions as a result of studies in life history and growth plasticity in insects, especially butterflies (Gotthard *et al.* 1994, Nylin & Gotthard 1998, Fischer & Fiedler 2000, Fischer & Fiedler 2001a, Gotthard 2004, Teder & Tammaru 2005). Many results suggest that insects can increase growth rate to optimization and not maximization, once females and males have the potential to grow more than they really grow (Tammaru *et al.* 2004). The proportion of plasticity in life histories among sexes will depend of selection pressures acting differently in both males and females (Fischer & Fiedler 2001b), suggesting that large size can be more important to be achieved for either sex, depending on the selection forces acting on each sex, and in each species.

These results can be used as helpful information for pest management. The strong female biased sexual dimorphism suggests that probably all potential of fecundity in *N. elegantalis* is obtained from larval stage. It is possible to expect that changes in distribution of hosts in time and space can directly affect female reproductive output, hence affecting the population size. Absence of protandry in *N. elegantalis* also may be useful. When monitoring pest populations, it is important to know the temporal patterns of population dynamics, like protandry (Nylin 2001). For example, in protandric species, high incidence of males caught in pheromone traps can be understood that female emergence will occur some days after, and implementation of mass trapping could be an efficient strategy to control insect pests, removing males from field.

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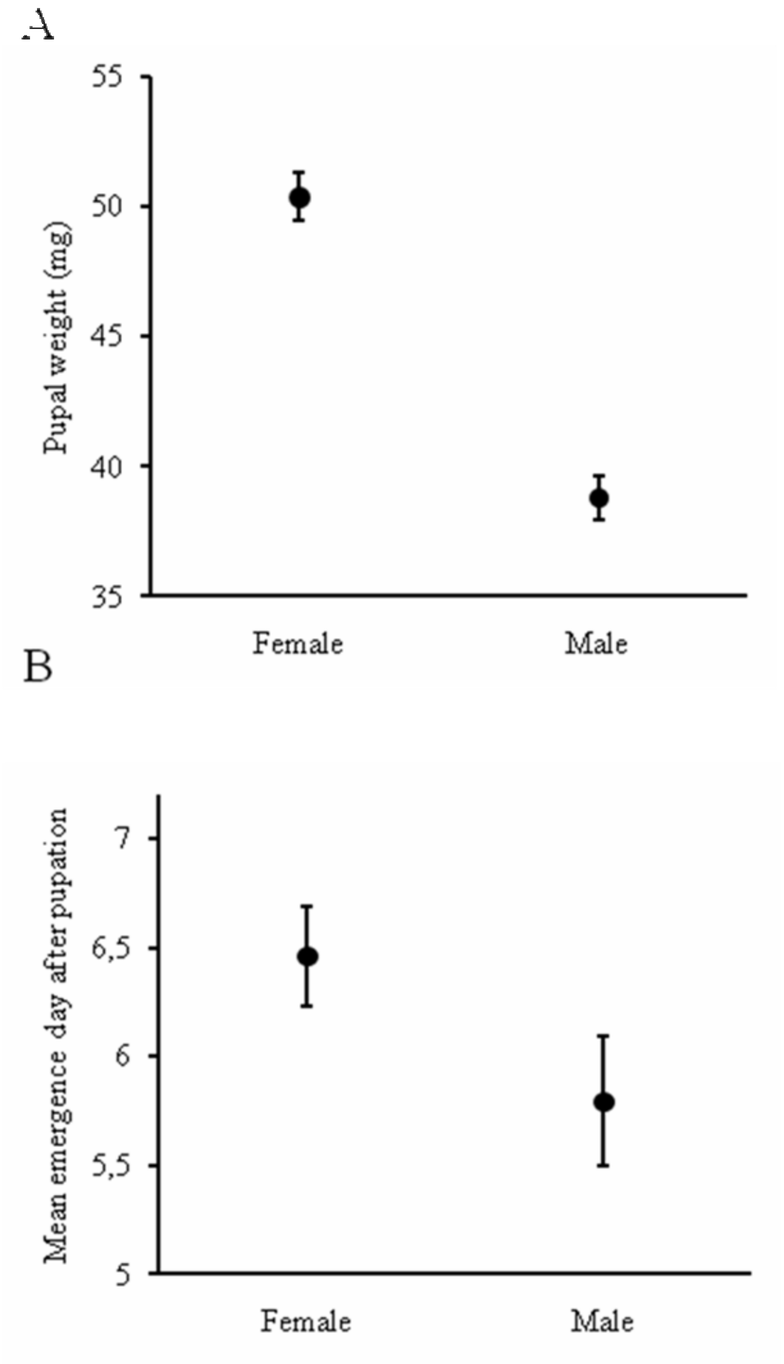

Figure 1: (A) Pupal weight (± S.E.) differences between the sexes in *Neoleucinodes elegantalis*, showing the occurrence of sexual size dimorphism (n=111) (P<0.001) and (B) mean emergence day (± S.E.) of males and females of *Neoleucinodes elegantalis* after pupation, showing the absence of protandry. Data analyzed on basis of Julian Calendar (n=111).

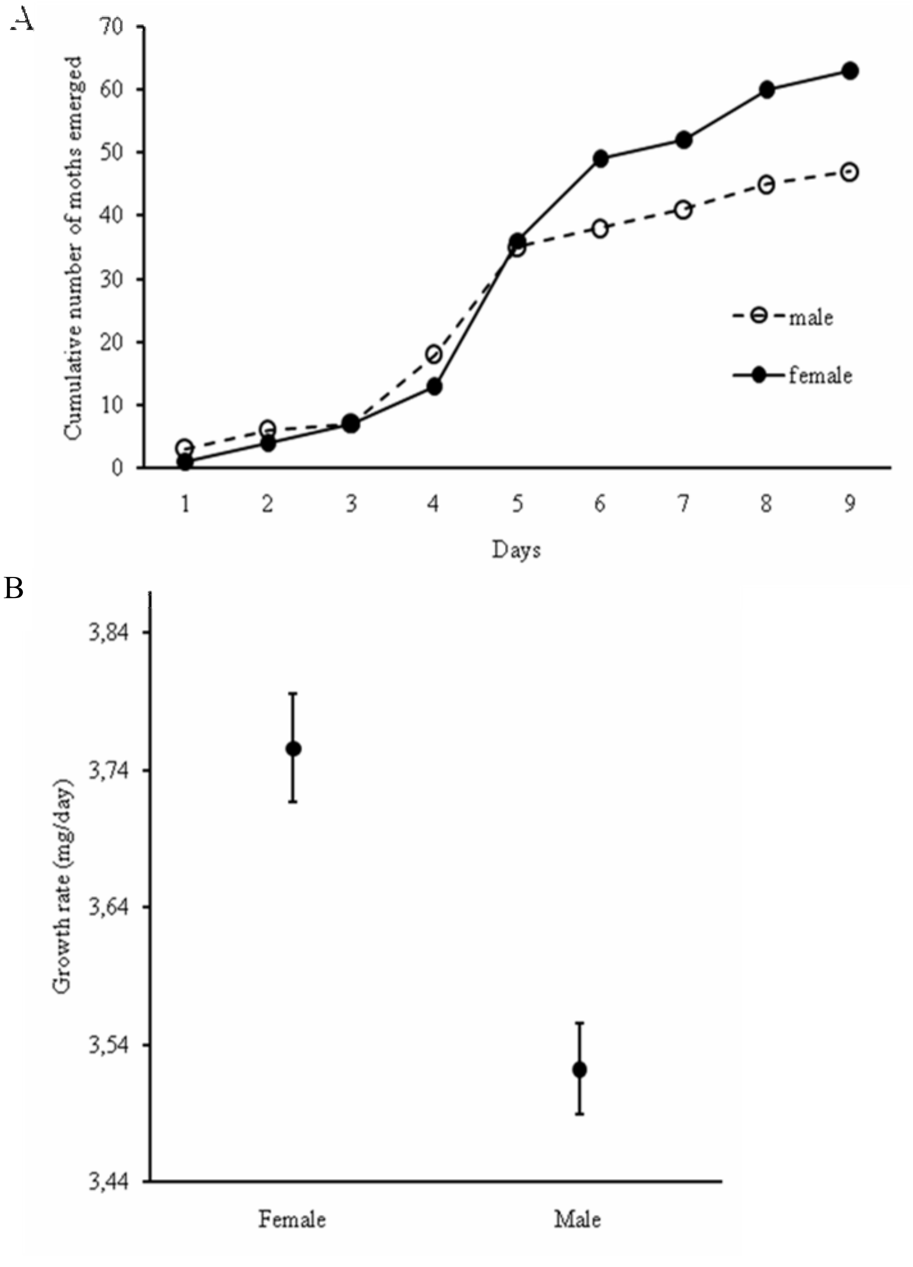

Figure 2. (A) Cumulative number of *N. elegantalis* moths eclosed from the first to last day of eclosions (n=111) and (B) growth rate of males and females of *Neoleucinodes elegantalis*, showing that females grow more per unit of time than males (P<0.01) (n=51).

CAPÍTULO 4

ESTIMATING THE INITIAL VOLUME OF SPERMATOPHORES FROM *Neoleucinodes elegantalis* GUENÉE (LEPIDOPTERA: CRAMBIDAE) BASED ON MEASURES OF COLLAPSED FRAGMENTS[1]

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[1] Pontes, W.J.T., E.R. Lima, & R. Barros. Estimating the initial volume of spermatophores from *Neoleucinodes elegantalis* Guenée (Lepidoptera: Crambidae) based on measures of collapsed fragments. A ser submetido.

ABSTRACT – To assess mating rate in field, butterflies and moths are good models. Males inseminate females through a quitinous sac that contains sperm. This sac is called spermatophore, and a male can transfer one spermatophore in each copulation. The number of spermatophores found in females shows how many times they have copulated in field. However, because spermatophores are collapsed after copulation, it is not possible to evaluate the real male contribution, that only can be assessed based on measures of intact spermatophores. We propose here a formula based on measures of collapsed and deformed spermatophore fragments from the tomato fruit borer *Neoleucinodes elegantalis*, that should help estimate the initial volume of fragmented spermatophores found inside female reproductive tract.

KEY WORDS: Spermatophore fragment, Lepidoptera, equation, tomato fruit borer

ESTIMANDO O VOLUME INICIAL DE ESPERMATÓFOROS DE *Neoleucinodes elegantalis* GUENÉE (LEPIDOPTERA: CRAMBIDAE) BASEADO NAS MEDIDAS DE FRAGMENTOS

RESUMO – Para avaliar a frequência de copula na natureza, borboletas e mariposas são bons modelos. Os machos inseminam as fêmeas através de um estojo quitinoso contendo esperma. Esse estojo é chamado espermatóforo, e um macho é capaz de transferir um único espermatóforo durante cada cópula. O número de espermatóforos encontrados no corpo das fêmeas mostra quantas vezes ela copulou na natureza. Contudo, pelo fato do espermatóforo se colapsar depois da cópula, não é possível avaliar a contribuição do macho no momento da cópula, o que somente pode ser verificado através da medida de espermatóforos intactos. Este trabalho propõe uma fórmula baseado na medida de espermatóforos deformados e fragmentados da broca pequena do tomateiro *Neoleucinodes elegantalis* que ajude a estimar o volume inicial do fragmento de espermatóforo encontrado no trato reprodutivo da fêmea.

PALAVRAS-CHAVE: Fragmento de espermatóforo, Lepidoptera, equação, broca pequena do tomateiro

Introduction

Butterflies and moths are good models to study mating frequence because in most species, spermatophore fragments remain in the reproductive trait of females during all life (Burns 1968, Wedell *et al.* 2002). This helps to estimate the mating rate of wild-caught females, simply be counting the number of fragments of spermatophores inside their reproductive organ (Arnqvist & Nilsson 2000). Spermatophores also are important to know the real contribution of males in copulation, by transferring not only sperm to females, but also protein, lipids and nutrients that can be used to increase female lifespan and to increase egg female production. Many ways are used to estimate the male contribution by measuring spermatophores. This can be made be weighting (Oberhauser 1997, Wedell 2006, Wedell & Cook 1999, Marcotte *et al.* 2007) or comparing the spermatophore to any spatial geometric form and made measures to obtain the volume of the respective form (Rutowski 1980, Royer & McNeil 1993, Jimenez-Perez *et al.* 2003). However, this can only be used when spermatophores are intact, only a little time before spermatophore collapse.

In *Neoleucinodes elegantalis*, the spermatophore has a shape very close to a sphere (Fig. 1A), and after 24h it is cup-shaped collapsed and also deformed like an ellipsoid (Fig. 1B). Only before 24h after mating it is possible to calculate male contribution be measuring the volume of intact spermatophores. Here it is proposed a calculation to estimate the initial volume of spermatophores based on measures of length and width of fragments present in the bursa of *N. elegantalis*.

Material and Methods

Twenty two intact spermatophores were obtained from females just mated with virgin males in rearing cages (50 x 50 x 50 cm) and twenty two collapsed spermatophores were obtained from females that were allowed to mate *ad libitum* until three days in rearing cages. The measure of diameter of an intact spermatophore was made to estimate the volume (mm^3) based on the calculation of a sphere:

$$V = \frac{4}{3}\pi R^2 \qquad \text{(equation 1)}$$

In collapsed spermatophores, both length and width for each fragment were measured. Lenght and width were considered to be the major and minor diameters of a fragment, respectivily. Only measurable fragments were used, once some of them were highly diggested. To see if the deformation of fragments changes significantly from measures obtained from intact spermatophores, lenght and widht of the fragments were used to estimate initial volume, considering length and widht as diameters and applying the measures in formula to calculate a sphere volume. Because the data of lenght and widht are so different from that of intact diameter ($F_{3,84}=17.59$, $P<0.0001$) (Fig. 2), we propose a transformation to achieve proximity in estimation close to the data from intact spermatophore measures, considering that:

$$R = \frac{L - (L - W)}{2} \qquad \text{(equation 2)}$$

Where R is the radius, L and W are length and width of the fragment, respectively. This measure was applied in the formula to calculate the volume of a sphere. The data were analyzed by ANOVA and Generalized Linear Model with normal distribution. Model simplification was made by the analisys of contrast extracting significant terms ($P<0.05$) until achieving the simplest model, considered to be all non significant terms, similar to those of intact spermatophore volume.

Results

The results showed that the estimation based on the formula using the diameter of fragments is not significantly different from the calculation based on the diameter of an intact spermatophore (Fig. 2). Calculations using length of fragments as the diameter of a sphere shown that the obtained volume is higher (0.488 mm^3) than that obtained from the diameter of intact spermatophores (0.3308 mm^3) (F=10.83, P<0.0001). Calculations using fragment width also showed significant difference, estimating a lower volume than intact ones (0.217 mm^3). However, when using the transformation, the volume obtained is similar to that of an intact spermatophore (0.3302 mm^3) (F=0.004, P=0.94).

Discussion

According to that, it is possible to estimate a volume of an intact spermatophores based on the measurable fragments in the bursa copulatrix of females of *N. elegantalis*. Intact spermatophores were obtained from no-experienced males, however male mating status is not controled when obtaining collapsed ones, when all insects in mating cages where alowed to mate for three days. Few males could remate in this period of time, which could affect fragments size measured after female dissecation. However, although significant differences between spermatophore size are shown to vary according to male mating history (Royer & McNeil 1993; Wedell & Cook 1999), spermatophore size is seemed to have little or no variance in size in monandric species (Svard & Wiklund 1989, Bissoondath & Wiklund 1995) and in *N. elegantalis* it was observed that there is no relationship between adult size and spermatophore size (Pontes, unpublished), what is expected in polyandrous butterfly where variation in spermatophore size is positively correlated with male size (Wiklund & Kaitala 1995). That reinforces evidences suggesting that *N. elegantalis* is considered to be monogamic (Jaffé *et al.* 2007). Thus, it is assumed that all spermatophores obtained in this experiment of three days have similar sizes because probably they were produced by virgin males.

This finding provides a way to estimate the male contribution in females collected in field, in a situation that spermatophore fragments could be measured. This information can help to ellucidate some questions of interest as well as evaluate natural male nutritional conditions based on spermatophore fragments found inside female reproductive tract. Previous knowledge of spermatophore size varying between males of different sizes or different mating histories in natural conditions also could be measured from females captured, what could help to understand some aspects of sexual selection occurring in field. However, further research with another species that shows spherical spermatophore and deformation after collapse must be done to see if this pattern can be repeated and if this same transformation from available fragments could also be applied on other species.

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Figure 1. Intact and fragmented spermatophores of *Neoleucinodes elegantalis*. (A) Ball-shaped intact and cup-shaped fragments; (B) geometric forms drew on photos, where intact is ball-shaped and fragment is cup-shaped. (Photo: I.A. Santos).

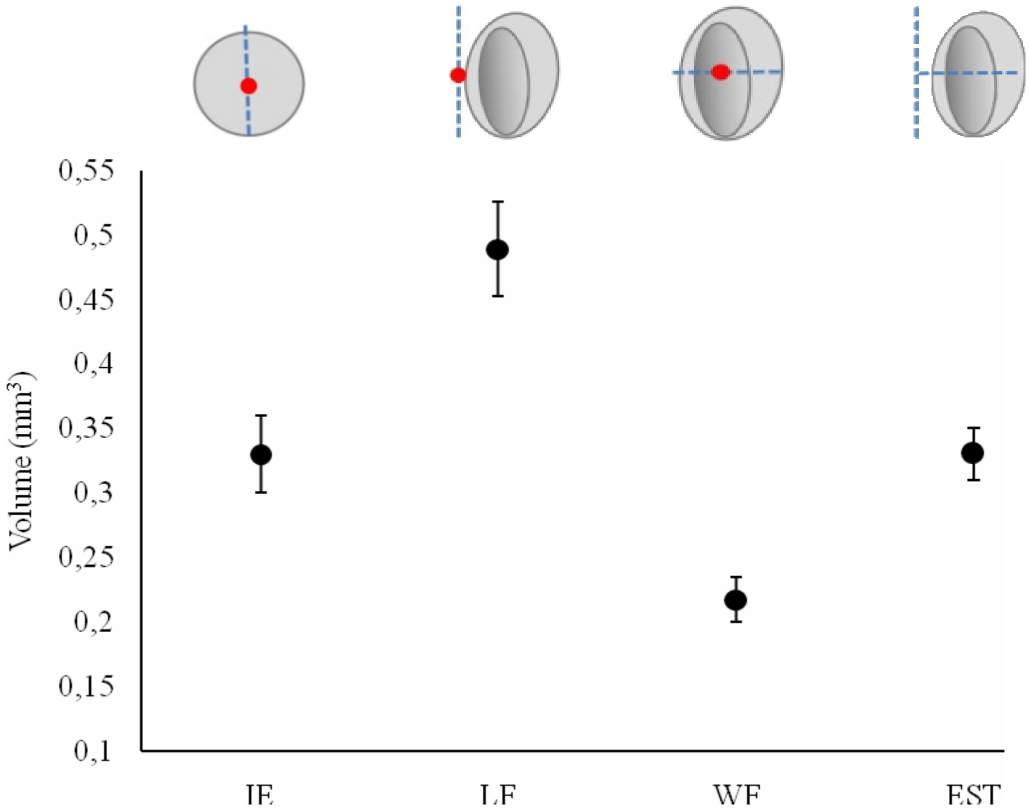

Figure 2. Analyzes of sparmotophore volume (mm^3) from *Neoleucinodes elegantalis*: (IE) intact spermatophore, calculation based on measures of the diammeter; (LF) volume based on measures of the lenght of fragments as the diammeter; (WF) volume based on widht of fragments as diammeter and (EST) estimation based on the calculation from lenghts of fragments (equation 2). Only volume from the estimation is non-significantly different from volume calculated from intact spermatophores.

CAPÍTULO 5

VIRGIN AND RECENTLY MATED MALES OF *Neoleucinodes elegantalis* GUENÉE (LEPIDOPTERA: CRAMBIDAE) ARE EQUALLY ABLE TO ACHIEVE NEW MATINGS?[1]

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[1] Pontes, W.J.T., E.R. Lima, E.G. Cunha, H.D. Araújo, J.N. Curtinhas & R. Barros. Virgin and recently mated males of *Neoleucinodes elegantalis* Guenée (Lepidoptera: Crambidae) are equally able to achieve new matings. A ser submetido.

ABSTRACT – In *N. elegantalis* heavier males should be the first to achieve mates because they should respond more readily to pheromone emission by females and copulate. Direct female choice is not observed in this specie, and it is not expected that females should discriminate between virgin and previous mated males. In the absence of female choice, male ability to mate should be superior of male intrinsic characteristics and can be a result of experience from first mating. We predict that males which achieved matings in the first opportunity also should be the first to remate, when compared to males that did not achieve mating in the first opportunity. To ensure any cost of remating, the time spent in copula was recorded. To analyze male activity, the time spent at the beginning of the experiment until copula was considered as index of male activity, and if male remating ability is related with thorax, abodmen and total body mass. Recently mated males remated at the same ratio as virgin ones that did not mate in the first opportunity. Copula duration and time taken to copulation did not vary between virgin and experienced males.

KEY WORDS: Mating rate, male persistence, tomato fruit borer

MACHOS VIRGENS E COPULADOS DE *Neoleucinodes elegantalis* GUENÉE (LEPIDOPTERA: CRAMBIDAE) SÃO IGUALMENTE CAPAZES DE OBTER NOVAS CÓPULAS?

RESUMO – Em *N. elegantalis* machos mais pesados geralmente são os primeiros a conseguir cópulas, uma vez que são os primeiros a responder mais prontamente à emissão de feromônio da fêmea. Seleção sexual exercida pela fêmea não é observada nessa espécie, e por isso não é esperado que fêmeas sejam capazes de discriminar entre machos virgens e copulados. Na ausência da seleção exercida pela fêmea, a habilidade do macho em copular deve ser superior às suas características intrínsecas e deve ser o resultado da experiência obtida na primeira cópula. Espera-se que machos que consigam copular na primeira oportunidade disponível também sejam os primeiros a recopular, comparados com machos que não copularam quando tiveram oportunidade para isso. Para verificar se há algum custo na recópula, a duração da cópula foi registrada. Para analisar a persistência do macho, o tempo desde o início do experimento até a cópula foi considerado como um índice de persistência do macho, e se essa capacidade de recópula está relacionada com a massa do corpo, do tórax e do abdomen. Machos recentemente copulados recopularam na mesma proporção de machos que não copularam na primeira oportunidade. A duração da cópula e o tempo até conseguir uma cópula não variou entre machos virgens e recopulados.

PALAVRAS-CHAVE: Frequência de cópula, persistência do macho, broca pequena do tomateiro

Introduction

The tomato fruit borer *Neoleucinodes elegantalis* is a well-known brazilian pest of Solanaceae crops. The way to monitor this species is by the use of pheromone traps in Brazil and

Venezuela using synthetic compounds previously identified (Cabrera *et al.* 2001). However, little information is known from mating frequence and sexual selection of *N. elegantalis*. Both traits are essential to understand sexual behavior, and thus, help to interpret data obtained in pheromone traps when monitoring population of pest species in space and time (Boake *et al.* 1996). The knowledge of the percentage of males that is able to remate is important to predict how many males should be removed from the population to achieve an effective control of the pest (Jimenez-Perez & Wang 2004b).

In *N. elegantalis* heavier males should be the first to achieve mates because they should respond more readily to pheromone emission by females and copulate (Jaffé *et al.* 2007). Time taken to copulation is considered to reflect male ability to find matings (Kaitala & Wiklund 1995, Fischer 2006). As body size is directly related to mating success in *N. elegantalis* because heavier males should respond readily for calling females, males that can achieve the first mating because they are more active than others probably should achieve the second mating first than lighter ones that did not achieve mating previously. Direct female choice is not observed in this species (Jaffé *et al.* 2007), and it is not expected that females should discriminate between virgin and previous mated males. In the absence of female choice, male ability to mate should be superior to its intrinsic characteristics or to experience from first mating (Schlaepfer & McNeil 2000). Individual intrinsic traits as ability, vigor, activity and persistence in courtship seem to be the cause of male mating success in some species (Rutowski 1980, Kaitala & Wiklund 1995, Tammaru *et al.* 1996, Fischer 2006, Fischer *et al.* 2008). Although male mating success should depend of male activity, male mating history should also affect cost of copulation and male ability to find subsequent matings.

Time spent in copula for mated males is higher than that with minor experience or virgin ones (Svard & Wiklund 1986, Kaitala & Wiklund 1995, Wiklund & Kaitala 1995, Lauwers &

Dyck 2006). Mainly in polyandric species, where production of ejaculates with high quantity of protein and lipid contents (Bissoondath & Wiklund 1995, Bissoondath & Wiklund 1996b) should need more time to be allocated and transferred in a second mating. In monandric mating system less investiment is made be males through ejaculate (Svard 1985, Oberhauser 1997) and thus, minor time should be needed for males to be able to mate again. Although recently mated males do not provide a second ejaculate as big as the first (Svard & Wiklund 1986, Rutowski *et al.* 1987), in monandric mating system this difference between the size of the first and second spermatophore is expected to not being high (Svard & Wiklund 1989) or even inexistent (Lauwers & Dyck 2006). In some monandrous evidence has shown that the cost of first mating may not affect male stimulus to recopulate (Kaitala & Wiklund 1995, Tammaru *et al.* 1996, Schlaepfer & McNeil 2000). However, the results are conflicting concerning investiment of monandrous males in producing ejaculate. In some cases, male mating history is shown to affect copula duration in some monandric species (Hughes *et al.* 2000, Lauwers & Dyck 2006) and the cost for producing spermatophore was considered to be non-trivial for males (Svard 1985).

Body size is known to be a physical traits that can be recognisable as an indicator that the individual has good genes, and thus size is selected for directly (Thornhill & Alcock 1983, Jimenez-Perez & Wang 2004a) or indirectly (Jaffé *et al.* 2007). However, body size, as the sum of thorax and abdomen size, is the product of allocation pattern on each of these structures. Thorax mass is correlated with flight performance and hence with male mating location (Wickman 1992b). Abdomen is considered to be an energetic reserve of fat body (Nation 2008) and reserve of reproduction by allocation of nitrogen content, positively correlated with body size (Boggs 1981, Wickman & Karlssonf 1989). Allocation for energy reserves in the abdomen varies with the mating system, where poliandric species invests more in reproductive resource in abdomen that will provide more and nutritious ejaculate to transfer to females (Karlsson 1995).

On the other hand, the opposite pattern was shown for monandric non-investing males, with lighter abdomen mass compared to that of poliandric ones (Karlsson 1995). In poor growing conditions, males can allocate more in the thorax, in flight muscles, and invest less in their abdomens in monandric mating system (Karlsson *et al.* 1997) because monandric males do not need to allocate for reproduction. Although these results have showed that monandric males should invest minor in abdomens than poliandric ones, and that in *N. elegantalis* body mass is related with male mating acquisition, it is not known if allocation of resources in thorax or abdomen should vary on males of *N. elegantalis* related with rematings.

Thus, we predict that males that achieved matings in the first opportunity should also be the first to remate, when compared to males that not achieved mating in the first opportunity. The propose of this paper is to see if males of *N. elegantalis* that were the first to mate in a previous mating experiment will be also the first to remate, when compared to males that not achieve matings previously. To ensure any cost of remating, the time spent in copula will be recorded. To analyze male activity, the time spent at the beginning of the experiment until copula will be recorded for both mated and virgin males, and finnaly we will se if male remating ability is in some way related with thorax, abodmen and total body mass.

Material and Methods

N. elegantalis used in our experiment were reared in the laboratory for 14 generations. Both the rearing conditions and experimental chambers were set at 25 ± 1°C and 71.2 ± 10% RH and had a light regime set to 12L:12D. Larvae were reared on *Solanum gilo* until pupation. Pupae were sexed and separated in experimental cages (50 x 50 x 50 cm) until eclosion.

In trials to obtain first mated males, all females and males that ecloded in the previous day were released into experimental cages from the 5th to 10th hours of scotophase in such way that no more than 74 insects could be placed in each cage. The time given to copulation was chosen because that is known to be the better time to obtain more matings (Eiras 2000). Cages were observed in an interval of 30 min which is considered to be less than the minor time registered for copula duration (Eiras 2000). Because in some days the number of ecloded females was the same or close to ecloded males, pairs collected were always the half of available females inside the cage. As the way to measure ability, considering the time taken to copulation (Kaitala & Wiklund 1995, Fischer 2006), the first males to achieve matings were considered to be the most "active". Each copulating pairs were removed from the cages and kept isolated in plastic vials. Mating trials were finished when half of the females available in each cage achieved copula, then removing the remaining unmated females from the cages. Males that did not copulate were considered to be less active than mated ones and were remained in the cages and fed with honey solution (10%) until the next mating opportunity. Only healthy males (mated and virgin), considered to be males that could fly to the top of cages without any difficulty, were used for the second mating opportunity.

Mated males were marked with a small red spot of water-soluble ink with a fine paintbrush on the forewing. A preliminar experiment showed that red spots on the forewing did not affect male mating success (x^2=0.027, df=1, P=0.869, n=60). After painting, mated males were isolated in plastic vials and fed with honey solution (10%) until the beginning of the second part of the experiment. After 24 hours, experienced and virgin males were released together into experimental cages and allowed to mate with virgin females ecloded between 24 or 48h before (sex ratio always 2:1) in the same conditions as described above. For each pair, time taken to copulation and copula duration were recorded. Mated and virgin males that did not mate and that remained in the experimental cages were counted and separated. All trials for first and second matings were repeated nine times.

All mated and virgin males were separated in the following categories of male mating history: 1) Males that remated (Twice mated); 2) Males that mated in the first opportunity, but not in the second (Not remated); 3) virgin in the first mating opportunity, but that mated in the second opportunity (First mated) and 4) unmated males in all the opportunities provided (Virgin), and frozen in -8^oC until dissecation. Thorax (without legs, head and wings) and abdomens were dried at 80^oC in constant for 48 hours and weighted to the nearest 0.01mg in an analytical balance (Precisa 262 SMA-FR).

A chi-square test was used to analyze if first mated males have more probability to remate against males that did not mate in the first opportunity. Time taken to copulation and copula duration was analyzed using an analysis of variance (ANOVA) with logit link, for twice mated and first mated males. Thorax, abdomen and total body mass were also analyzed with ANOVA for all mating history categories described above.

Results

Mated males do not have more probability to remate as virgin ones, as expected (x^2=2.24, *df*=1, *P*<0.134) (Table 1), although a slitghly more number of males remated (n=65) than males that mated only in the second opportunity (n=49). The percentage of remating was low (37.79%), but was slightly higher than the percentage of individuals that mated the first time in the second opportunity (28.34%). A total of 107 males did not mate again when it was given a second opportunity, 24h after they had copulated the first time (62.20%). A high number of males still unmated in the two opportunities (124 males, 71.67%). Although a considerable little number of males remated in at least 24h of interval between the matings, this percentage should increase as the time between the first and second mating increases. Thus, it is expected that more males should be able to remate in natural conditions.

Male mating courtship is seen in many of the replicates: males approach to females, fluttering the wings that were held vertically with abdomen extended and claspers extruded. Close to the female, the courting male extends his abdomen in her direction. Females may fly or walk away when they refuse males, or accept the courtship and mate.

Copula duration and time taken to copulate were not different between experienced and virgin males (Fig. 1). Copula duration was not longer for mated males than virgin ones ($F_{1,112}$ $=0.75$, $P=0.38$). Virgin males initiated copulation at the same time as experienced ones, both around 90 minutes after the beginning of the experiment ($F_{1,112}=0.317$, $P=0.57$). These results suggest that recently mated males seems to not suffer a significant cost to reallocate reproductive resources to produce a second spermatophore, and that some of them are able to remate in a interval of 24h after the first copula.

In the body mass analysis, thorax mass ($F_{3,241}=2.269$, $P=0.081$) and total body mass ($F_{3,241}$ $=2.408$, $P=0.067$) were not different from all the categories of male mating history (Fig. 2). However, a little difference were found when comparing abdomen mass. Males that did not remate twice when given an opportunity had heavier abdomen than all the others ($F_{1,253}=11.630$, $P<0.001$) (Fig. 2).

Discussion

Males that were the first to mate not were the first to remate when given an opportunity for that. Our results did not show any difference in male mating history affecting male success

(Table 1). The previous experience of males seems to be the cause of remating success when female choice is not evident Schlaepfer & McNeil (2000). In *N. elegantalis*, where females are apparently no-choosy, this pattern was not repeated and both previous mated and unmated males achieved matings at the same rate. Although male activity should be considered a cause of mating success, that varies (Rutowski 1979) or not (Kaitala & Wiklund 1995) between virgin and mated males. This means that male activity is not fundamental for mate success, and should vary between species. Our results showed that, in *N. elegantalis*, more active males were not more able to remate as virgin ones.

Vigor and persistence in courtship is considered to be related with male mating history, in a way that recently mated males not should be so active to achieve a second mating, and females should be able to recognize males that recently mated and cannot transfer a second nutritious ejaculate as big as the first one (Rutowski 1979, Rutowski 1980). This rationale only makes sence according to mating systems. As monandric males would not invest paternally in nutritious substances by ejaculate, they might be more able to reallocate sperm in a small (more rapidly produced) spermatophore to mate again, in the next oppotunity. As females do not seem to be choosy (Jaffé *et al.* 2007) male mating vigor and persistence for a second mating should be the same as for the first mate in an interval of 24 hours, as observed in the present result.

Copula duration between remated and virgin males did not change. Differences in mating systems could explain this similarity. Poliandric males should be more prepared to remate than monandric ones. In monandric mating systems, males should invest only in one mating to ensure his paternity, once females should not remate. In poliandric mating system, males should be more able to allocate more investiment in many spermatophores, because they need to be the last partner of each female they had mated, by increasing the period which females are unwilling to remate and ensure paternity. The production of sperm and accessory substances to be transfered in spermatophores is costly and thus more time is needed for males to be able to transfer an ejaculate as big as the one transfered in previous mate (Svard & Wiklund 1989, Bissoondath & Wiklund 1995). When poliandric males have opportunity to remate in an interval of time that was not enough to produce a bigger ejaculate, they nevertheless will remate, having or not a available spermatophore, and the consequence is a long copula duration (Thornhill 1976). In monandric mating systems, males only need to invest in sperm and not in costly content ejaculates, as protein and accessory substances (Bissoondath & Wiklund 1995). Thus, monandric males should be more able to remate in a little interval of time between matings because they dont need to allocate nutritious resources in spermatophores. The present results agree with that, where recently mated males did not spend more time to transfer ejaculates as expected in recently mated males in poliandric mating systems (Svard & Wiklund 1986). However, the present result did not show if there were differences in the size of spermatophores between mated and virgin males to see if male investiment in this monandric species should vary with male mating history. Despite this, in monandric systems, it is known that males do not need to invest in highly elaborated ejaculate to ensure only paternity in a second mate, and thus they will not be constrained to wait until to be able to transfer a second spermatophore as big as the first. Many results showed that the contribution of monandric males not affect female reproductive output in longevity and fecundity (Oberhauser 1997, Torres-Vila *et al.* 2004, McNamara *et al.* 2007).

Prolonged copulation may be counterbalanced by opposing selective forces. Time loss in copula may limite the time spent searching for oviposition sites (Wiklund & Persson 1983) and increase predation risk for both sexes (Bissoondath & Wiklund 1996a). Shortening of copula duration seems to be an important strategy to avoid these risks, favoring males that can transfer a competitive amount of sperm and accessory substances in minor time.

Physical and behavioral traits can be under selection by female choice or male-male contest. Body size is one of them, when males are preferred when they are bigger (Thornhill & Alcock 1983, Jimenez-Perez & Wang 2004a), when they have an intermediate size (Mason 1969, Jimenez-Perez & Wang 2004b), when they are smaller (Singer 1982), or when body size is not under selection (Hernandez & Benson 1998, Kemp 2000, Kemp & Wiklund 2001). In territorial contest, heavier males are not winner males and thus size is not related with mating success (Hernandez & Benson 1998, Kemp 2000, Kemp & Wiklund 2001). For *N. elegantalis* male body size is important for first mating success, but there were no evidence in the present experiment that thorax mass affected mating or remating. Although variables that are related with flight performance should be under sexual selection (Wickman 1992b) in *N. elegantalis* that parameter not is related with male mating success. Differences between male behavior of patrolling and perching to achieve matings are related with some physical traits, as wing loading, aspect ratio and abdomen size, but thorax mass did not change according to male sexual behavior (Wickman 1992b). No changes in thorax mass between unmated, once mated and remated males have shown that this physical trait is not important for remating acquisition in the present experiment.

Our results showed that only first mated males had heavier abdomens compared to all other categories. Heavier abdomens in monandric mating system should be unnecessary, where little or no investiment by males is given in matings. The additional weight of a higher abdomen should be the cost that probably affect male persistence in achieve a new copula, and this can affect aerodynamics of flight performance (Wickman 1992b). The effort to taking flight and developing courtship for the first time should not be the same to achieve a remating in an interval of 24h. In a monandric system, males that mate once do not seem to invest more time and energy to remate as soon as able, mainly in a system where overlapping of generations provides males always with females available for copulation, as occurr in *N. elegantalis* (Pontes et al, unpublished). On the other hand, short lifespan is an important trait that can stimulate males to copulate more in minor time (Scott 1973). The short lifespan of one to six days of *N. elegantalis* (Jaffé *et al.* 2007, Marcano 1991b, Marcano 1991a) acts as a strong pressure to remating behavior as soon as possible, and probably it is the cause of less discrimination toward males (Wickman 1992a). However, only lifespan is not enough to explain monandry, when long lived females mated only once and never get an addicional mate (Wiklund & Persson 1983).

The present results showed that in the interval of 48h most of the males did not mate in the two given opportunities. This implies a question: Why remaining males did not mate when given two opportunities to do that? Maturation of male reproductive organs should be an explanation that males only would be able to copulate after some days. However this is not the case in the present study, where a high number of males copulated in the first day of experiments, showing that males probably had already ecloded with all reproductive organs developed. Constant availability of females in field can make males "not so rapid" to achieve copulations. Overlap of generations in *N. elegantalis* should provide males with females of different ages and mating histories for all their life, and thus copula interest is not so high to stimulate males to spent time and energy in a new courtship and mating attempt in a short interval of time after the first mating. However lifespan could be a constraint for males achieve matings as soon as possible in a species where individuals could stay alive least a week. Absence of direct female choice could be a result of strong pressure for copulation, where all males that respond firstly to calling females should be accepted. Then, sexual selection could drive male mating success toward male ability to achieve matings faster. In first copula, it was observed and related with body size (Jaffé *et al.* 2007) however, the same does not show to be so important for remating, as the present results indicate. This can explain why mated males were not the same to remate. They had already assured the paternity of the next generations in the first mating, and thus eventual new copulation would be only an advantage.

In conclusion, the present results showed that recently mated males remated at the same ratio as virgin ones that not mated in the first opportunity. Copula duration and time taken to copulation did not vary between virgin and experienced males, indicating that male mating history does not change male ability to achieve matings or time to transfer spermatophores. Physical traits, as thorax, abdomen and total body size are not related with male remating propensity, but abdomen size is positively related with males that mated in the first opportunity but not in the second.

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Table 1. Outcome of 9 mating trials when experienced and virgin males of *Neoleucinodes elegantalis* were placed with virgin females (sex ratio 2:1) in experimental cages. Percentage of matings in parenthesis.

Twice mated	First mated	χ^2	P
65 (37.79%)	49 (28.32%)	2.24	0.134

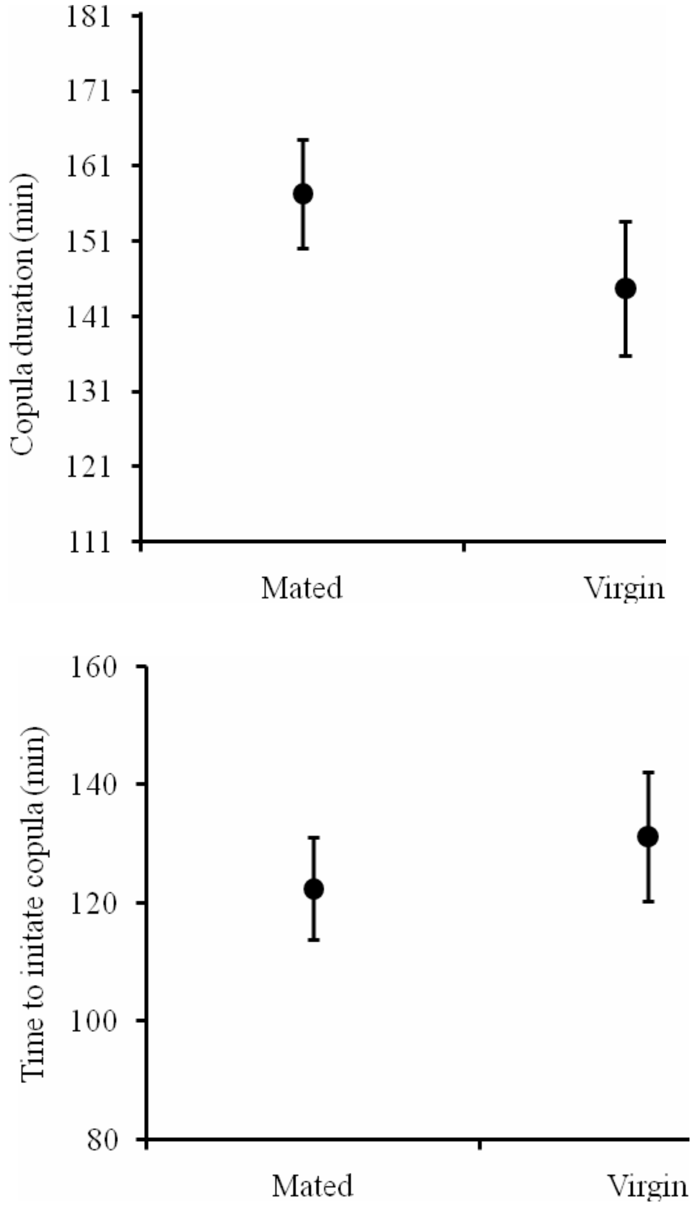

Figure 1. Mean of copula duration and time taken to copulation (± S.E.) for experienced and virgin males of *Neoleucinodes elegantalis*. Mating history did not affected copula duration and time taken to copulation.

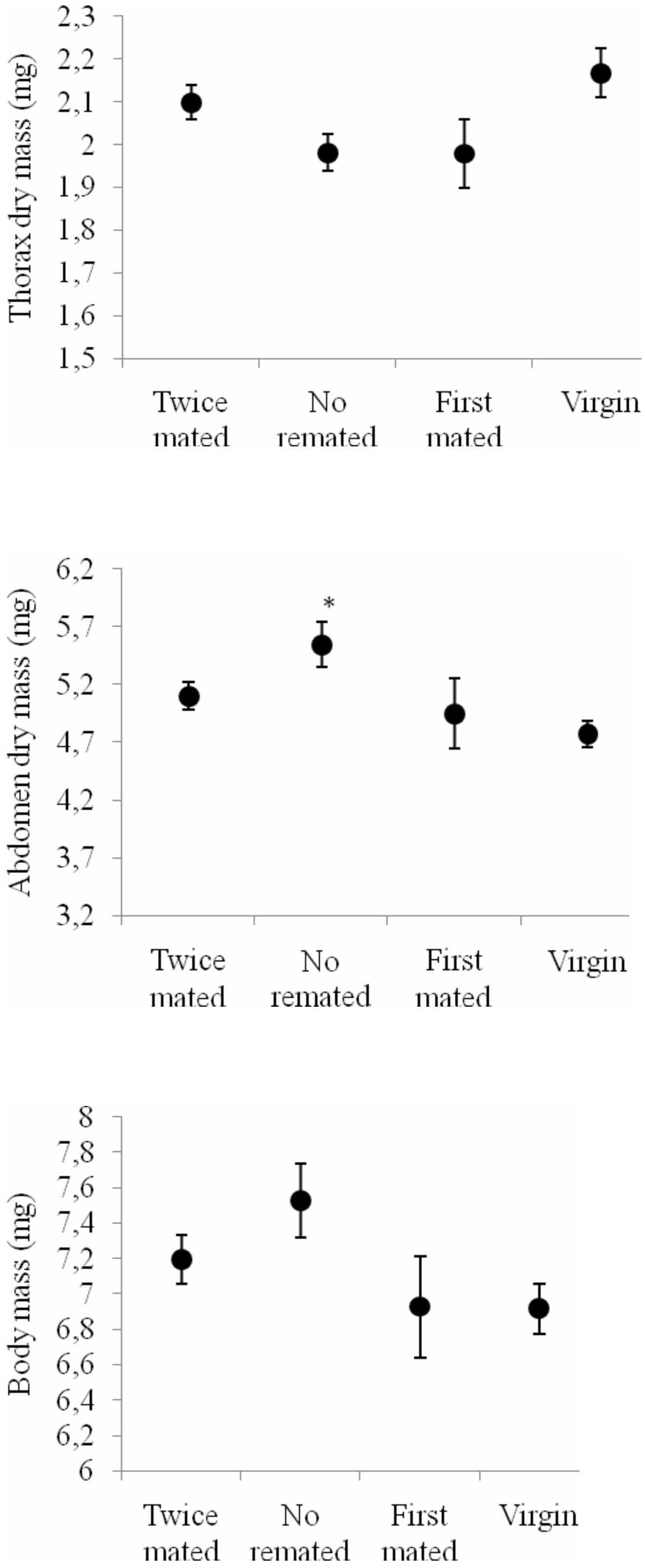

Figure 2. Mean dry mass (mg) (± S.E.) of thorax, abdomen and total body mass (thorax+abdomen) of *Neoleucinodes elegantalis* from four categories of mating history: 1) Males that remated (Twice mated); 2) Males that mated in the first opportunity, but not in the second (Not remated); 3) virgin in the first mating opportunity, but that mated in the second opportunity (First mated) and 4) unmated males in all the oportunities provided (Virgin). Thorax and total body mass did not varied between all the categories, however abdomen mass was heavier in males that did not remated in the second opportunity than all the others. * means $P < 0.05$.