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USO DE NANOFIBRAS POLIMÉRICAS COMO VEÍCULO PARA FEROMÔNIO E
INSETICIDAS UTILIZADOS NO MANEJO DE *Grapholita molesta* (LEPIDOPTERA:
TORTRICIDAE)

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USO DE NANOFIBRAS POLIMÉRICAS COMO VEÍCULO PARA FEROMÔNIO E INSETICIDAS UTILIZADOS NO MANEJO DE *Grapholita molesta* (LEPIDOPTERA: TORTRICIDAE)[1]

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RESUMO

Uma das principais pragas de pomares de Rosaceas na região sul do Brasil é a mariposa-oriental (MO), *Grapholita molesta*, (Busck, 1916) (Lepidoptera, Tortricidae). Para o controle deste inseto são utilizados, principalmente, inseticidas, sendo os feromônios uma alternativa para o seu manejo. Dentro deste contexto, feromônios e inseticidas nano formulados podem potencializar o uso destas ferramentas em agroecossitemas. Sendo assim, os objetivos deste trabalho foram: a) avaliar via cromatografia gasosa (CG), a taxa de liberação de (*Z*)-8-acetato de dodecenila (composto feromonal majoritário) impregnado em nanofibras de policaprolactona (PCL) e polietilenoglicol (PEG) (1:1); b) observar as respostas eletrofisiológicas (EAG) de machos de MO frente às nanofibras contendo feromônio sexual específico; c) testar a atratividade de nanofibras com feromônio na captura de MO a campo e d) registrar a mortalidade por contato e a percepção eletroantenográfica de machos de MO em relação a formulação de nanofibras confeccionadas com feromônio sexual e o inseticida Nortox 250 CE (cipermetrina). Em todos os bioensaios de laboratório foram avaliadas nanofibras novas e expostas ao ambiente. Nos testes (EAG) foi avaliada a percepção de machos a nanofibras com 0,01 e 0,001% de feromônio, assim como, a formulação comercial ISCAlure®Grafolita; nanofibras com feromônio e 125mg.L^{-1} de cipermetrina; somente com inseticida e ao controle (sem ambos). Os experimentos de campo foram realizados em pomares de pessegueiro e ameixeira em duas safras consecutivas (2014 e 2015). Na primeira, foram avaliadas a formulação (PCL/PEG) contendo feromônio em diferentes proporções (0,01; 0,1 e 1%), além de armadilhas com fêmeas virgens. Em 2015, foram utilizadas nanofibras com 0,01 e 0,001% de feromônio e estas comparadas com a formulação comercial e o controle (sem feromônio). Ambos os experimentos de campo foram conduzidos ao longo de dez semanas. Para avaliar a mortalidade, foram realizados testes de contato e de gaiola, com nanofibras contendo 0,01% (0,87mg.L^{-1}) de feromônio e 125mg.L^{-1} de cipermetrina. Nas análises em GC foi possível constatar que as taxas de liberação das nanofibras, não variaram entre 21 e 42 dias, no entanto houve uma diminuição na presença do feromônio após os 63, para ambos os tratamentos (0,01 e 0,001%). As respostas eletroantenográficas foram estatisticamente iguais entre os tratamentos contendo 0,01, 0,001% e o septo comercial de feromônio. Também não houve diferenças na percepção entre nanofibras com e sem inseticida. Em 2014 as maiores capturas foram em armadilhas iscadas com 0,01% de feromônio. No ano seguinte, este mesmo tratamento somente atraiu mais insetos nas primeiras cinco semanas, sendo o septo comercial o mais atrativo da sexta a décima. Nos bioensaios de contato tarsal, a mortalidade foi maior do que 87%, após a exposição de 84 dias e variou 28,33 a 56,67% nos testes de atrai-e-mata.

APPLICATION OF POLYMERIC NANOFIBERS AS VEHICLE FOR PHEROMONE AND INSECTICIDE USE IN THE MANAGEMENT OF *Grapholita molesta* (LEPIDIOPTERA: TORTRICIDAE)[1]

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ABSTRACT

One of the main pests of rosacea orchards in southern Brazil is the oriental-fruit-moth *Grapholita molesta* (MO) (Busck, 1916) (Lepidoptera, Tortricidae). To control this insect insecticides are used mainly, pheromones are an alternative to their management. Within this context, pheromones and insecticides nanoformulations can enhance the use of these tools in agroecosystems. Thus, the objectives of this study were: a) to evaluate by gas chromatography (GC), the release rate of (Z) -8-dodecenyl acetate (majority pheromone compound) contained in polycaprolactone (PCL) and polyethyleneglycol (PEG) nanofibers (1:1); b) observe the Electroantenographical (EAG) responses of MO males front of nanofibers containing specific sex pheromone; c) testing the attractiveness of pheromone nanofibers in the capture *G. molesta* in field; d) record mortality by contact and EAG perception MO males compared to nanofibers formulation made with sex pheromone and insecticide Nortox 250 EC (cypermethrin). In all the laboratory bioassays were evaluated nanofibers new and exposed to the environment. In EAG tests the perception of males to nanofibers with 0.01 and 0.001% pheromone, as well as the commercial formulation ISCAlure®Grafolita was evaluated; nanofibers with pheromone and cypermethrin $125mg.L^{-1}$; only with insecticide and control (without both). Field experiments were conducted in peach and plum orchards in two consecutive seasons (2014 and 2015). At the first, the formulations (PCL / PEG) containing pheromone in different ratios (0.01, 0.1 and 1%), and traps with virgin females was evaluated. In 2015, nanofibers with 0.01 and 0.001% of pheromone were used and compared with the commercial formulation and control (without pheromone). Both field experiments were conducted over ten weeks. To assess mortality, contact and cage tests were performed using nanofiber whit 0.01% ($0.87mg.L^{-1}$) pheromone and $125mg.L^{-1}$ cypermethrin. In the analysis in GC was possible to notice that the release rates of pheromone, did not vary between 21 and 42 days, however there was a decrease in pheromone presence after 63 for both treatments (0.01 and 0.001%). EAG responses were statistically similar between treatments containing 0.01, 0.001% and the commercial septum. There were also no differences in perception between the nanofibers with and without insecticide. In 2014 the largest catches were in traps baited with pheromone 0.01%. In the following year, the same treatment only attracted more insects in the first five weeks, and the commercial septum was most attractive from the sixth to tenth. Mortality in tarsal-contact tests was greater than 87%, after 84 days exposure and ranged from 28.33 to 56.67% in attract-and-kill bioassays.

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1 INTRODUÇÃO

Tradicionalmente o controle de insetos pragas é feito através do uso de inseticidas, porém esses apresentam uma série de inconvenientes tais como riscos de intoxicação humana e de animais, resíduos em alimentos, desenvolvimento de resistência das pragas, surgimento de pragas secundárias, redução na população de inimigos naturais e polinizadores, além da contaminação ambiental.

Durante as últimas décadas, novas estratégias no Manejo Integrado de Pragas (MIP) vêm sendo desenvolvidas, a fim de minimizar os efeitos adversos do uso intensivo de pesticidas sintéticos. Dentro das práticas empregadas para o controle de pragas no MIP está o uso de semioquímicos – moléculas utilizadas para mediar à comunicação inseto-inseto (feromônios, aleloquímicos), como alternativas ou complementares ao uso de inseticidas. Estes compostos espécie-específicos não apresentam qualquer efeito negativo relacionado a organismos benéficos e não geram qualquer risco de resistência de insetos pragas, como observado com inseticidas.

Dentre as estratégias utilizadas, empregando o uso de semiquímicos, se destacam o monitoramento, atrai-e-mata e interrupção de acasalamento. Entretanto, devido à atividade biológica e a dispersão no ambiente, é necessária a construção de dispositivos/suportes de libertação lenta para assegurar uma dispersão controlada dos compostos voláteis biologicamente ativos.

Diversas formulações de dispersores vêm sendo utilizadas atualmente, matrizes sólidas, como septos de borracha; formulações líquidas em spray e reservatórios difusores. Muitos destes dispositivos não garantem liberações a uma taxa constante, induzindo uma diminuição da taxa de liberação durante a temporada. Para tanto, estudos utilizando nanomateriais como dispersores vêm sendo realizados, apresentando resultados promissores quando a sua utilização em sistemas agrícolas.

O emprego da nanotecnologia no controle de insetos versa no sentido de promover uma maior durabilidade de inseticidas e/ou dispersão de feromônios sintéticos no campo. Neste sistema, feromônios e inseticidas podem ser nano encapsulados para o manejo de insetos considerados praga na agricultura, podendo propiciar uma série de vantagens, tais como a liberação gradual e proteção contra efeitos climáticos como chuva, radiações, entre outros. Estas características podem permitir uma maior economia de produto e mão de obra, por reduzir a necessidade de repetidas aplicações, uma vez que os ingredientes ativos ficam protegidos nas matrizes de nanométricas, aumentando sua durabilidade. Dessa forma, a nanotecnologia pode se tornar uma ferramenta importante, não agressiva a organismos não alvos e o ambiente. A escolha do inseto-modelo para a realização dos testes [*Grapholita molesta* (Busck, 1916) (Lepidoptera: Tortricidae)] foi baseada em quesitos importantes para o sucesso do estudo, sendo estes: possuir inseticida registrado para seu controle, ter seu feromônio sexual sintetizado e disponível comercialmente; ter atualmente grande parte de seu monitoramento e controle baseado em técnicas comportamentais e ser uma praga-chave na agricultura, visto que a macieira, seu principal hospedeiro, produz um dos frutos destinados à exportação e com alto valor comercial. As pesquisas com nanotecnologia poderão promover o desenvolvimento de novos produtos e trazer inovação, uma vez que poderá ser aplicada para outras espécies-praga, promovendo a capacitação de recursos

humanos nessas duas áreas inovadoras e estratégicas para o país, ou seja, biotecnologia e agricultura.

2   REVISÃO BIBLIOGRÁFICA

2.1 Nanotecnologia – biomateriais e *electrospinning*

A nanotecnologia é um campo multidisciplinar que abrange uma vasta gama de processos, materiais e aplicações englobando física, química, biológica, engenharia e ciências eletrônicas. Centra-se na caracterização, fabricação e manipulação de substâncias em nano escala, aproximadamente entre 1 e 100 nm. O reduzido tamanho das partículas combinado com uma grande área de superfície, apresenta propriedades originais e novas, criando assim um vasto potencial de aplicações (EFSA, 2009; Rashidi & Khosravi-Darani, 2011; Weiss *et al.*, 2006).

Um nanomaterial é definido como qualquer material que tem uma ou mais dimensões na nano-escala, enquanto uma nanopartícula é uma entidade discreta, que tem todas as três dimensões em escala nano (Food and Agricultural Organisation of the United Nations (FAO)/World Health Organisation (WHO), 2010).

Nanomateriais e nanopartículas podem abranger as seguintes nanoformas que derivam seus nomes de suas formas e dimensões individuais, ou seja, os nanotubos, nanofibras, nanofilmes, nanofolhas entre outros (Cushen *et al.*, 2012). O uso da nanotecnologia vem quebrando paradigmas, mostrando-se como uma ferramenta para diversos setores como em materiais de construção para pisos, paredes e máquinas, novos dispositivos e técnicas em eletrônica, cosméticos, equipamentos esportivos,

tratamento de águas residuais, medicina, e mais recentemente na agricultura e indústria alimentar (Doyle, 2006).

Nanotecnologia emergiu como o avanço tecnológico para desenvolver e transformar o sector agro-alimentar inteiro, com o potencial de aumentar a produção global de alimentos, além do valor nutricional, qualidade e segurança dos alimentos (Mousavi & Rezaei, 2011). Suas aplicações podem ser classificadas como "nano-inside" (isto é, no produto alimentar como na produção primária ou de processamento de alimentos) e "nano-outside" (embalagens de alimentos) (Henchion *et al.*, 2013). Nanosensores e sistemas de distribuição inteligentes baseados em nanotecnologia são algumas das aplicações da nanotecnologia que estão atualmente empregados na indústria agrícola para ajudar no combate de patógenos de culturas, bem como para aumentar a eficiência de agroquímicos em taxas de dosagem mais baixos (Mousavi & Rezaei, 2011).

Os biomateriais têm como base conhecimentos das áreas de ciências e engenharia de materiais. No grupo de biomateriais, existem os polímeros biodegradáveis e biocompatíveis utilizados na fabricação das matrizes de nanofibras, os quais são inofensivos ao meio ambiente e a saúde humana. Estes materiais podem ser utilizados na fabricação de suportes ou moldes, construídos dentro das mais modernas tecnologias que permitem a liberação programada de substâncias ou organismos, fertilizantes, antibióticos, vírus, células, bactérias, proteínas, feromônios e inseticidas (Dualibi *et al.*, 2004; Boudriot *et al.*, 2005; Hellmann *et al.*, 2009; Ghormade *et al.*, 2011; Gonzalez *et al.*, 2014)

Nanoformulações à base de polímero permitem que uma vasta gama de objetivos seja alcançada, e também combinada (por exemplo, com libertação lenta, proteção contra degradação e baixa solubilidade do AI), o que os torna adequados para um

grande número de diferentes aplicações. Ao longo dos últimos dois anos, testes de eficácia que incluem ambos, ensaios de campo e comparações com formulações comerciais foram demonstrados, fornecendo informações valiosas para possíveis desenvolvimentos futuros (Kah & Hofmann, 2013).

As matrizes de nanofibras podem ser desenvolvidas com base em substâncias naturais ou sintéticas (Barbanti *et al.*, 2005; Boudriot *et al.*, 2005). Polímeros biodegradáveis apresentam uma série de vantagens sobre outros materiais para o desenvolvimento de *scaffolds* ou matrizes, pois permitem uma combinação adequada entre propriedades físicas, biológicas, biomecânicas e degradabilidade (hidrolítica ou enzimática) (Lutz & Börner, 2008). Os nanomateriais podem também ser preparados de diferentes maneiras (*electrospinning*, prototipagem, *foaming*, entre outros) gerando formas mais ou menos porosas, de acordo com a necessidade e as propriedades dos produtos a serem encapsulados (Lutz & Börner, 2008).

A técnica de *electrospinning* tem sido considerada como a abordagem mais promissora para produzir nanofibras em grande escala e o diâmetro da fibra pode ser ajustado de nanómetros a micrómetros (Li & Xia, 2004). Além disso, a técnica permite a preparação de polímeros bioconjugados, como por exemplo, com proteínas ou outros produtos covalentemente ligados (Lutz & Börner, 2008). Este método funciona pelo princípio eletrostático para transformar soluções de polímeros, em diferentes solventes, em filamentos finos. A configuração básica do *electrospinning* consiste em um bico de agulha, uma fonte de alimentação de alta tensão, um recipiente para a solução do polímero e um eletrodo coletor. Quando um fluido viscoso é carregado com uma tensão elevada, a força eletrostática puxa o fluido, que se transforma em um jato líquido. A evaporação do solvente resulta em filamentos de fibras sólidos. Na maioria dos casos, o depósito de

fibras ocorre aleatoriamente no eletrodo coletor formando uma superfície de nanofibras (Theron *et al.*, 2001, Li *et al.*, 2004).

Na indústria farmacêutica, muitas classes de polímeros têm sido usadas em sistemas de liberação controlada de fármacos devido as suas diferentes permeabilidades, aumentando a eficácia e a especificidade do produto e reduzindo tanto a necessidade de doses elevadas, quanto os riscos de danos ao meio ambiente (Villanova *et al.*, 2010). O óxido de polietileno (PEO) também conhecido como polietilenoglicol (PEG) dependendo da forma como for sintetizado, é o polímero sintético mais usado para bio-conjugação (Duncan, 2003). Este tem sido amplamente utilizado em sistemas de liberação de fármacos como um material de proteção para as substâncias ativas (Zalipsky, 1995; Greenwald et al , 2003). PEG é um polímero solúvel em água, não tóxico, não imunogênico, sem carga e, por conseguinte, um material ideal para proteger biomoléculas ativas (Pasut & Veronese, 2006). Outros polímeros que tem sido amplamente utilizados, segundo Yang *et al.* (2007) em sua revisão são: ácido polilactico (PLA), poli (Ɛ-caprolactona) (PCL), poli (p-dioxanona) (PPDO), poli (butileno succinato) (PBS), além de polímeros naturais renováveis tais como amido, celulose, quitina, quitosana, lignina, e proteínas.

No que concerne à agricultura, segundo Bansal (2012), uma característica importante para o uso das nanofibras é a biodegradabilidade desses materiais e a possibilidade desses veículos apresentarem capacidade de liberação controlada. Em trabalho recentemente publicado por pesquisadores da UFRGS em parceria com a empresa TECNANO, Damasceno *et al.* (2013), verificaram que plântulas de soja provenientes de sementes recobertas com esporos de bactérias do gênero *Rhizobium* imobilizados em nanofibras de álcool polivinilico (PVA) tiveram maior produção de nódulos do que aquelas inoculadas com *Rhizobium* sem cobertura de PVA. Dessa

forma, comprovaram que a técnica de *eletrospinning* pode ser uma ótima alternativa para inocular e controlar a liberação de bactérias fixadoras de nitrogênio.

2.2 Feromônios de insetos e inseticidas

Os semioquímicos nos insetos são os principais responsáveis pelo comportamento reprodutivo, localização e seleção do hospedeiro, do habitat e, no caso de insetos sociais, da organização da colônia. Estes compostos uma vez liberados no ambiente, provocam uma mudança fisiológica e/ou comportamental em outro organismo, podendo ter ação intraespecífica (feromônio) ou interespecífica (aleloquímico) (Norin, 2007).

Os feromônios são de grande importância para o manejo e controle de insetos de importância agrícola. Estas substâncias têm a vantagem de apresentar elevada seletividade para uma determinada espécie ou para um número limitado de espécies similares (pertencentes à mesma família), sendo que seu uso não prejudica a fauna e o meio ambiente, em comparação aos métodos tradicionais, fundamentados no uso de inseticidas (Vilela & Mafra-Neto, 2001).

Estes compostos feromonais podem ser utilizados em sistemas agrícolas com o objetivo de fazer monitoramento de insetos, ou seja, um acompanhamento sistemático da população da praga, como um importante subsídio para avaliar a presença do inseto no campo, ou mesmo para fazer o controle por meio de coleta massal. Outra forma de utilizar esta ferramenta é através da confusão sexual ou interrupção do acasalamento, a qual consiste no excesso de estímulo químico no campo com o objetivo de desorientar e impedir a cópula. Uma variável destas técnicas é denominada de "atrai-e-mata". Neste caso é incorporado às iscas de feromônio um inseticida, sendo assim os insetos que entram em contato com este produto acabam morrendo e como resultado tem-se a

diminuição do uso de agrotóxicos (Cardé & Minks, 1995; Vilela & Mafra-Neto, 2001, Bento, 2007, Santos & Borges, 2008).

A aplicação da nanotecnologia na formulação de dispersores de feromônios teve como base o trabalho pioneiro, realizado na Alemanha por Hellmann *et al.* (2009). Os autores usaram a técnica de *eletrospinning* para produzir nanofibras de poliamida e de acetato de celulose contendo o feromônio sexual sintético da traça-da-uva, *Lobesia botrana* (Denis & Schiffermuller, 1775) (Lepidoptera, Tortricidae) e posteriormente utilizaram-nas em bioensaios de campo, através da técnica de interrupção de acasalamento. Os resultados dos experimentos evidenciaram a eficácia das nanofibras, vislumbrando uma possível aplicação desta técnica no manejo comportamental de *L. botrana* em videiras.

Além dos semioquímicos, as nanoformulações também podem ser utilizadas para incorporação de inseticidas, aumentando a eficiência de ingredientes ativos (IA) no controle e manejo de pragas (Barik *et al.* 2008; Gajbhiye *et al.* 2009). As vantagens da nanotecnologia dentro deste contexto estão relacionadas ao desenvolvimento de mecanismo de liberação de pesticidas de forma segura e eficaz, evitando perdas para o ar durante a aplicação e também por deriva, afim de reduzir os danos ambientais e custos de aplicação (Stephenson, 2003; Ghormade *et al.*, 2011). Esta tecnologia também pode ser importante na redução da concentração de IA a ser aplicado (Ghormade *et al.*, 2011; Gonzalez *et al.*, 2014).

Um dos exemplos de avanço no uso de nanoformulações com inseticidas foi demosntrado por Boehm *et al.*, (2003) utilizando o produto Etiprole, contendo fenilpirazole, que bloqueia a neurotransmissão do ácido γ-aminobutírico (GABA). Este produto apresenta foto inativação durante aplicações de campo (Caboni *et al.*, 2003). Segundo os autores, o tempo de ação associado à liberação controlada das formulações

de nanoesferas propiciou um eficiente controle de afídeos em algodoeiro (Boehm *et al.*, 2003) A utilização de nanoparticulas de polietilenoglicol contendo óleo essencial de alho também resultou em eficiente controle de *Tribolium castaneum* (Herbst, 1797), isto indica que é possível utilizar as nanopartículas de revestimento de PEG carregados com óleo essencial de alho para controlar pragas em produtos armazenados (Yang *et al.*, 2009).

Diversos estudos demonstram que a eficiência de nanopartículas (NPs) como transportadores de inseticidas, tanto para liberação imediata como por períodos prolongados (Park *et al.* 2006; Kumar & Yadav 2009; Prasad *et al.* 2011; Swamy & Prasad, 2012; Prasad & Swamy, 2013). Uma das empresas líder no comércio de agrotóxicos e fertilizantes, Syngenta, introduziu com sucesso no mercado o pesticida Karate®ZEON nanoemulsificado, contendo lambdacialotrina (piretroide), esta formulação *Gutbuster* libera o IA de sua forma encapsulada quando encontra ambientes alcalinos, como no estômago de alguns insetos, o que aumenta a eficácia do produto (Joseph & Morrison, 2006). Bhattacharyya *et al.* (2010) em sua revisão enfatiza que a nanotecnologia iria revolucionar a agricultura em um futuro próximo.

2.3 *Grapholita molesta*: importância e manejo

A mariposa-oriental, *G. molesta* é considerada uma espécie cosmopolita, distribuída em diversas regiões agrícolas do mundo, onde se cultivam espécies frutíferas de Rosaceae (Salles, 2000; Botton *et al.*, 2005) e tem sua origem no continente asiático (Gonzalez, 1989). No Brasil, é referida como praga principalmente na região centro-sul do país, nas culturas de pessegueiro (Salles, 1998; Campos & Garcia 2001; Nunes *et al.*, 2003; Botton *et al.*, 2005) e macieira (Kovaleski & Ribeiro, 2003;. Pastori et al, 2008; 2012). Sendo o estado do Rio Grande do Sul responsável por 80% da produção de

pêssego da região Sul do país e por 50% da produção de maçã, junto com o estado de Santa Catarina (IBGE, 2014).

Os adultos de *G. molesta* possuem aproximadamente 12 mm de envergadura, coloração pardo-escuro-acinzentada, com algumas estrias de cor branca (Monteiro & Hickel, 2004). A longevidade média de fêmeas e de machos, segundo Alfonso & Marin (2004), é de 16,18 e 14,08 dias, respectivamente. Os ovos medem aproximadamente 0,7 mm de diâmetro (Monteiro & Hickel, 2004). A postura é feita, preferencialmente, na face inferior das folhas novas, nas brotações e nos frutos, com um período de incubação entre 2 a 6 dias (Grellmann *et al.*, 1992).

A lagarta perfura os brotos novos, não lignificados, ou o pecíolo da folha, migrando para os ramos principais, onde constroem galerias, através do consumo da parte interna do ramo, provocando murchamento e morte dos ponteiros (Fachinello *et al.*, 1996). A fase jovem pode atacar de dois a quatro ponteiros (Salles, 2000). As lagartas se alimentam fazendo uma galeria de cima para baixo e é possível perceber sua presença pelos excrementos deixados na entrada desta. Nos frutos, sua penetração ocorre próximo à cavidade peduncular, perfurando-os em direção ao centro. A galeria, resultante da alimentação destas, contém excrementos envoltos em fios de seda aderidos a uma goma de resina, apresentando um aspecto de teia (Embrapa, 2005).

Os principais danos são observados nos frutos, uma vez que as lagartas danificam a polpa, pela construção de galerias em direção ao caroço (Salles, 2000), podendo causar quedas prematuras (Monteiro & Hickel, 2004; Botton *et al.*, 2005). Além disso, as lesões causadas pelo inseto na epiderme do fruto podem facilitar a entrada de doenças, como a podridão-parda (*Monilinia fructicola*), resultando em prejuízos adicionais (Botton *et al.*, 2001; Afonso *et al.*, 2002).

Segundo Carvalho et. al. (1990) as cultivares de pessegueiro com maturação precoce geralmente são menos afetadas por *G. molesta*, em função das baixas populações do inseto durante o período que o fruto permanece no campo. Entretanto, em cultivares de ciclo de maturação médio e tardio, há maior suscetibilidade dos frutos em relação à presença da mariposa oriental (Afonso *et al.,* 2002; Monteiro & Hickel, 2004). Botton *et al.* (2001) mencionam danos de até 5% em frutos de variedades tardias no Rio Grande do Sul.

O comportamento reprodutivo de *G. molesta* é mediado por um complexo de sinais (feromônios) liberados principalmente pelas fêmeas o qual atrai os machos para cópula (Dustan, 1964). O feromônio sexual feminino de *G. molesta* foi caracterizado por Roelofs *et al.* (1969), sendo que seus três principais componentes são o *Z-* e *E-*8-acetato de dodecenila e *E-*8 dodecenol. Porém, outros compostos químicos também compõem a mistura feromonal, liberados pelas fêmeas desta espécie (Roelofs *et al.*, 1969). Inúmeras são as formas de dispersores empregados com este feromônio hoje no campo, com destaque para a pasta SPLAT® (Specialized Pheromone and Lure Application Technology), saches, ampolas, microencapsulados entre outros. Estes se diferenciam, basicamente com relação ao tempo de liberação e, consequentemente, durabilidade no campo (Botton *et al.,* 2011).

A utilização de armadilhas com feromônio sexual, associada à amostragem visual de frutos e ponteiros, é a técnica mais eficiente para estimar a população e o percentual de danos causados por *G. molesta* (Arioli *et al.*, 2013). No caso da mariposa-oriental, são empregadas duas formulações de feromônios sintéticos: Biografolita® (Biocontrole, 2016) e Isca Lure Grafolita® (Isca, 2016).

Em pomares pequenos de pessegueiro, de até três hectares, como é o caso da maioria dos cultivos do Rio Grande do Sul, são recomendadas, no mínimo, duas

armadilhas por pomar. Nos maiores, a recomendação é de uma armadilha a cada três a cinco hectares. As mesmas devem ser colocadas a 1,7 m de altura (Botton *et al.*, 2011) e o septo deve ser substituído em, no máximo, 60 dias (Arioli *et al.* 2013). As armadilhas devem ser instaladas no início do ciclo vegetativo, em função da preferência de *G. molesta* pelas brotações e tecidos novos e tenros dos ponteiros (Botton *et al.*, 2001; Arioli *et al.*, 2013). O nível de controle preconizado para a cultura do pêssego e maçã é de 20 a 30 machos/armadilha/semana (Salles, 1991; Nunes *et al.*, 2003; Arioli *et al.*, 2013).

No Brasil, a eficácia do método da interrupção de acasalamento para *G. molesta* foi constatada em alguns estudos, como os de Arioli *et al.*, (2007); Monteiro *et al.*, (2008); Pastori *et al.* (2008); Ribeiro (2009); Pastori *et al.* (2012), sendo a técnica utilizada em aproximadamente 30% dos pomares do Brasil, com o emprego de feromônio sexual sintético comercial Biolita® (Biocontrole, 2016) e Splat Grapho® (Isca, 2016). Em pomares de macieira no Rio Grande do Sul com *G. molesta* foi constatado que tanto a utilização da formulação com feromônio (Splat Grapho®) quanto à mistura deste a um inseticida (SPLAT Cida Grafo Bona®), proporcionaram redução significativa na captura de machos nas armadilhas, comparativamente ao controle. No entanto, os danos nos frutos durante o período de exposição não variou entre as áreas com e sem a presença de feromônio (Arioli, 2007). Apesar deste estudo, a técnica atrai-e-mata, é ainda pouco utilizada no Brasil, devido às questões relacionadas à sua eficácia a campo, como potencial atrativo, durabilidade e custos de aplicação (Arioli *et al.*, 2013).

Para o controle de *G. molesta*, os produtores normalmente empregam inseticidas sintéticos seguindo um calendário pré-definido (a cada 10 a 15 dias) levando em consideração principalmente informações meteorológicas e o estádio de

desenvolvimento da cultura, sem considerar a flutuação populacional da praga nos pomares (Arioli *et al.*, 2005). Este manejo utilizado pelos fruticultores está se tornando cada vez mais inviável, dadas as novas exigências da sociedade por frutas de qualidade, obtidas por meio de sistemas de produção que protejam o ambiente e os consumidores (Normas, 2003).

Os resultados utilizando inseticidas para a supressão de pragas têm sido insatisfatórios e às vezes totalmente ineficientes. Esse problema pode estar relacionado com resistência dos tortricídeos a alguns produtos e/ou com a época de aplicação, como demonstraram Bouvier *et al.* (1995), Sauphanor & Bouvier (1995) e Charmillot (1995) para *Cydia pomonella* (Linnaeus, 1758) (Lepidoptera: Tortricidae).

Os inseticidas fosforados e piretroides, que apresentam amplo espectro de ação, ainda são os mais empregados para o controle da mariposa-oriental (AGROFIT, 2016). Esses são letais a diversos inimigos naturais, favorecendo o aumento de populações de pragas secundárias, como ácaros (*Panonychus ulmi* (Koch, 1836)*; e *Tetranychus urticae* **(**Koch,1836), cochonilhas (*Pseudaulacaspis pentagona* (Targioni-Tozzetti, 1885) e pulgões (*Brachycaudus persicae* (Passerini, 1860), além de selecionar populações resistentes, devido ao uso frequente dos mesmos grupos químicos (EMBRAPA, 2005). O clorantraniliprole ou rynaxypyr, o lufenurom e o novalurom foram recentemente registrados para uso no controle da mariposa-oriental na cultura do pessegueiro. Esses inseticidas seriam mais seletivos a inimigos naturais e polinizadores, além de apresentarem reduzida toxicidade a mamíferos (AGROFIT, 2016).

3 OBJETIVOS

3.1 Geral

Avaliar nanofibras como veículo para incorporação de feromônios e inseticidas e o uso destas no controle e monitoramento de *G. molesta*.

3.2 Específicos

a) Avaliar via cromatografia gasosa, as taxas de liberação de (Z)-8-acetato de dodecenila (composto majoritário do feromônio de *G. molesta*) pelas nanofibras (PCL/PEG) após exposição ao ambiente por diferentes períodos.

b) Observar as respostas eletroantenograficas (EAG) de machos de *G. molesta* frente à nanofibras poliméricas (PCL/PEG) contendo feromônio sexual específico, expostas ao ambiente por diferentes períodos.

c) Registrar a captura, em pomares comerciais, de machos de *G. molesta* em armadilhas contendo feromônio impregnados em nanofibras (PCL/PEG).

d) Avaliar a formulação de nanofibras confeccionadas com PCL/PEG juntamente com feromônio sexual e cipermetrina em relação à mortalidade por contato e à percepção eletroantenográfica em machos de *G. molesta*.

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5 **5 ARTIGO 1**

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7 **Electrospun nanofibers for *Grapholita molesta* (Lepidoptera, Tortricidae)**

8 **pheromone releasing: laboratory and field bioassays[2]**

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27 **Key words:** Nanofibers, Pheromone, Insects, Agriculture

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Abstract – Laboratory bioassays and field trials were conducted to evaluate the attractivitveness and the release rates of (*Z*)-8-dodecenyl acetate (majority pheromone compound) of *Grapholita molesta* (Lepidoptera: Tortricidae) from polymer carriers, specifically from nanofibers of polycaprolactone (PCL) and poly ethylene glycol (PEG). In laboratory bioassays, electroantennogram (EAG) studies were performed to evaluate males antennal response to nanofibers containing pheromone that had been exposed during five different periods and compared with a commercial septum (ISCAlure® Grafolita) used to monitor this species. Field experiments were conducted in peach and plum orchard in two consecutive years (2014 and 2015). At first, (PCL/PEG) formulation containing pheromone in different ratios (0.01, 0.1 and 1%) and traps with virgin females were evaluated. In 2015, we compared the number of male caught in trap baited with nanofibers (0.01 and 0.001%) and control (without pheromone) to the commercial formulation. Both field experiments were conducted over ten weeks. GC analyses reveal that pheromone release decreased after 63 days of exposition. The EAG tests showed that insects perception to pheromone on nanofibers are similar over all the exposition periods. In 2014 the largest catches were in traps baited with 0.01% pheromone. The following year, the same treatment only attracted more insects in the first five weeks, and the commercial septa caught more males from the sixth to tenth week.

Introduction

Worldwide agricultural system is faced with a number of long-term challenges, including climate change, increasing competition for energy, land and water, urbanization and environmental problems such as chemical run-off, i.e., pesticides and fertilizers (Ditta, 2012). Nanotechnology can play a fundamental role in contributing to a more efficient and sustainable agricultural and food production system, with

57   opportunities to increase farm productivity, alleviate environmental issues and reduce

58   costs spent with resources (Mousavi & Rezaei, 2011). This includes techniques that will

59   preserve land and water by increasing crop yield while using lower inputs, as well as,

60   techniques to protect environment quality (Chen & Yada, 2011; Ditta, 2012).

61   Nanomaterials can be used as supports or molds, built with the most modern

62   technology, which permit programmed release of substances and organisms, such as

63   antibiotics, viruses, cells, bacteria and proteins (Dualibi et al., 2004; Boudriot et al.,

64   2005). This new tolls and its conception and handling processes are a new field that also

65   represents a meaningful promise for the agricultural scenario in what concerns to the

66   formulation of nanometric systems as carriers for chemically synthesized pesticides

67   (Campos et al., 2014; Kah & Hofmann, 2014; Kah et al., 2013) and pheromones

68   (Hellmann et al., 2009; Bhagat et al., 2013). Pheromones are considered a highly

69   specific and eco-friendly agent for management and control of agricultural pests (Vilela

70   and Mafra-Neto 2001). However, its deployment requires slow-release and protection

71   from decomposition under ambient conditions (Ghormade et al., 2011; Gonzalez et al.,

72   2014). Hereof, nanotechnology can be applied for agrochemicals by using nanoscale

73   carriers; they have controlled release mechanisms which allow the active ingredient to

74   be taken up slowly, thus improving its effectiveness, while reducing the amount applied.

75   These materials can be made from many products as gels, emulsions and polymers

76   (Niemeyer & Doz, 2001; Oskam, 2006; Puoci et al., 2008). Hellmann et al. (2009)

77   conducted the pioneer work with eletrospinning technique to produce nanofibers with

78   polyamide and cellulose acetate polymers containing a synthetic sexual pheromone of

79   *Lobesia botrana* (Denis & Schiffermuller, 1775) (Lepidoptera, Tortricidae). They

80   incorporated pheromones into nanofibers (about 30 wt.%) and achieved an almost linear

81   release over several weeks. The authors proposed that fiber webs could be distributed

82   across the fields (quite similar to spider webs) in order to allow a uniform release of

83   pheromones for mating disruption. Recently, Hummel et al. (2014) showed that organic

84   polymer nanofibers made from Ecoflex® can also act in mate disruption of *L. botrana*

85   for, at least, seven weeks under field condition.

86   Bhagat et al. (2013) proposed the immobilization of *Bactrocera dorsalis* pheromones

87   within a nanogel (without using any potentially toxic chemicals such as crosslinkers or

88   antioxidants). The evaporation of pheromones in nanogel was significantly reduced

89   compared to the evaporation of pure active ingredient (AI), extending their

90   effectiveness for up to 33 weeks in *Psidium guajava* Linn orchard, while the pure AI

91   lasted for only three weeks.

92   Studies concerning to formulation of oriental fruit moth (OFM) *Grapholita molesta*

93   (Busk, 1916) (Lepidoptera, Tortricidae) pheromone, began in United States (Rothschild,

94   1975; Cardé & Baker, 1979) and Australia (Vickers et al., 1985), followed by

95   researches, that initially tested polymer and polyethylene disperser containing 400-500

96   mg of active compound, distributed in a 500 dispersers per hectare density (Audemard

97   et al., 1989). Lately, other forms of dispersal have been developed for capturing OFM

98   especially pastes, polymer sachets and microencapsulated formulations (ISCA, 2016).

99   In Brazil, OFM pheromone release devices are produced for monitoring strategies as

100   Iscalure®Grafolita septum, formulated with (*E*)-8-dodecenyl acetate, (*Z*)-8-dodecenyl

101   acetate and (*Z*)-8-dodecenol (0.1336%) (ISCA, 2015) and Bio Grapholita®, with (*Z*)-8-

102   dodecenyl acetate, (*E*)-8-dodecenyl acetate, (*Z*)-8-dodecenol, Dodecanol (0.013%)

103   (Biocontrole, 2015). The recommendation, according to manufacturers, is to replace

104   septum each 21 days (ISCA, 2015) or four weeks (Biocontrole, 2015). The

105   recommended control for a peach-tree is from 20 to 30 males/traps/week (Salles, 1984;

106   Nunes et al., 2003; Arioli et al., 2013). Pheromone formulation in nanofibers can be an

107 alternative in relation to those commercial septa, mainly because it might present

108 greater persistence with lower quantity of AI, however it must be evaluated

109 thoughtfully. The goals of this work were: a) to measure the liberation rates of (*Z*)-8-

110 dodecenyl acetate (major pheromone component) from polycaprolactone (PCL) and

111 polyethyleneglycol (PEG) nanofibers (1:1); b) to evaluate the eletrophysiological

112 responses (EAG) of OFM males to nanofibers and rubber septa (ISCAlure®grafolita)

113 with pheromone, and c) to register the efficiency of PCL/PEG nanofibers and

114 commercial septum containing specific pheromone in the capture of *G. molesta* males in

115 commercial peach and plum orchards.

Material and methods

117 **Nanofibers confection through electrospinning technique:** Polymeric nanofibers

118 were produced at Tecnano Research and Services Ltda., using a custom made

119 electrospinning machine with a 60 kV HV supply. The syringe pump consisted of 5 mL

120 syringe and a rotary aluminum collector was adjusted to about 60 rpm. A voltage of

121 1.81 kV cm^{-1} was applied between the needle and the earthed collector (16.5 cm). In this

122 process, polymer dispersions were prepared dissolved in specific solvents THF

123 (tetrahydrofuran) and $CHCl_3$ (chloroform) (3:1), with and without the synthetic sex

124 pheromone of *G. molesta* (P9000 - 90 Bedoukian OFM Technical Pheromone)

125 composed by (*Z*)-8, (*E*)-8 -docecenyl acetate and (*Z*)-8-dodecenol, in concentrations of

126 0.001%, 0.01, 0.1% and 1%. The polymers (PCL/PEG) were acquired from Sigma

127 Aldrich (St. Louis, MO, USA) and processed in a solution (1:1).

128 **Nanofibers analysis:** PCL/PEG nanofibers spheres were individually introduced in a

129 vial, and pheromone extracted in hexane (10 mL) at room temperature for 24 h. Extracts

130 analyses were performed in a Shimadzu 2010 gas chromatography equipped with a

131 mass selective detector QP2010, and Optima-5MS (30 m x 0.25 mm x 0.25 μm). Oven

132   initial temperature was set at 80 ^{o}C. Then, it was heated at 10 ^{o}C min^{-1} up to 300 ^{o}C and

133   kept for 34 min. A volume of 1 µL was manually injected in split mode (10:1). Using

134   He as carrier gas at flow rate of 1 mL min^{-1}. First, standard and extracts mass spectra

135   were acquired in scan mode (50 to 500 m/z interval, 70 eV EI). (Z)-8-dodecenyl acetate

136   (majority pheromone compound) peak was identified by its retention time (12.85 min)

137   and mass spectra (NIST/EPA/NIH). In order to improve selectivity, extracts were also

138   analyzed in SIM mode, single ion monitoring (m/z 67. Injector, interface and detector

139   temperatures were 250°C. A cut time of 11 min was used. We use nanofibers kept under

140   controlled conditions (25 ± 2 ºC; 60 ± 10% RH) in two concentrations (0.01 and

141   0.001% pheromone) and both exposed for 21, 42, 63 and 84 days. It was performed six

142   replicates for each treatment. The mean peak area was analyzed by Kruskal-Wallis and

143   compared with Dunn (α = 0.05).

144   **Insects:** *Grapholita molesta* adults came from a culture kept under controlled

145   conditions (photoperiod of 16 hours; 25 ± 2 ºC; 60 ± 10% RH; 1.5 klx) at Universidade

146   Federal do Rio Grande do Sul, Brazil. The insects were fed with an artificial diet during

147   the larval period (Ivaldi-Sender, 1974) and adults with water-honey solution (15%

148   honey and 5% methylparaben). All tests were performed with three to five days old

149   virgin males.

150   **Electroantennogram recordings (EAGs):** EAGs were acquired using an IDAC-4 data

151   acquisition controller system, and EAG 2000 software (Syntech, Kirchzarten,

152   Germany). Virgin *G. molesta* males were chilled at 4 °C for at least 20 min before one

153   antenna was excised and positioned onto an antenna holder using a small quantity of

154   Spectra 360 conductive gel (Parker Laboratories, Orange, NJ, USA), that was attached

155   to a Syntech EAG probe (Type PRG-2, internal gain 10×). We tested new and exposed

156   (21, 42, 63 and 84 days) at the same controlled conditions mentioned, nanofibers

157   spheres (1 ± 0.2 g of PCL/PEG) containing pheromone (0.01 and 0.001%) and no

158   attractant (control), as well as, the commercial septa. Treatments were individually

159   placed in an adapted pipette, made with Falcon tuber (50 mL). Stimulus puffs were

160   generated with a Syntech CS-02 stimulus controller with pulse duration of 1 s and flow

161   of 2.5 mL/0.5s. Electroantennogram responses were measured as the maximum

162   amplitude of depolarization elicited by the stimulus applied. Each antenna received a

163   series of puffs delivered once every minute and treatments were randomly applied.

164   Fifteen repetitions of each treatment were performed. Normalized mean EAGs

165   responses were compared through variance analysis (ANOVA, Tukey), using Bioestat$^{®}$

166   5.0 software (Ayres et al., 2012) with 95% of reliability.

167   **Field Experiments:** bioassays were conducted on peach and plum orchards (10 ha)

168   located in Porto Alegre/RS (30° 08' 22.77" and 51° 11' 59.15") during 2014 and 2015

169   seasons crops. Delta traps (ISCA Tecnologias, Ijuí, RS, BR) were baited with individual

170   nanofibers (1 ± 0.2 g) or septum placed inside *voile* sashes, separated by ca. 50 m and

171   rotated clockwise every week. Trees were not sprayed with insecticides during the trial

172   period.

173   In the first bioassay (2014) we evaluated nanofibers containing pheromone in

174   concentrations of 0.01%, 0.1%, 1%. Traps with two *G. molesta* virgin female (3 days

175   old) were considered a positive control. They were placed in a cylindrical transparent

176   plastic pot (3 cm x Ø 1.5 cm), perforated, with an opening covered with voile, entitled

177   "bob". The insects were replaced in each evaluation and fed with a cotton impregnated

178   with water-honey solution (15% honey and 5% methylparaben). In 2015, we decided to

179   test a dose 10 times smaller (0.001%), as well as, to compare these nanofibers with

180   commercial septa (0.13%). We evaluate nanofibers without (control) and with

181   pheromone (0.001% and 0.01%) as well as commercial septum, which was not replaced

182 during all the experiment. The number of captured insects was registered each seven

183 days, for 10 weeks, in both season crops. Four randomized blocks designs were used to

184 compare the proportions of cumulative trap captures per treatment. Data were analyzed

185 by (Kruskal-Wallis, Student test)using Bioestat$^®$ 5.0 software (Ayres et al., 2012) with

186 95% of reliability.

187 **Results**

188 **Laboratory bioassays**

189 *Nanofibers analysis:*

190 Chromatographic analyses reveal that the pheromone release were only observed

191 between nanofibers with 0.001% pheromone not exposed and 42 days after exposure.

192 84 days (H= 20.7852; P<0.05) (Figure 1A). Although, for the nanofibers treatments

193 with 0.01% no differences were recorded between not exposed nanofibers, 21 and 42

194 days after exposure, however pheromone release decreased after 63 days of exposition

195 (H=22.6614; P<0.05) (Figure 1B).

196 *Electroantennogram recordings:* EAGs responses of males antenna to all exposed

197 periods and unexposed pheromone loaded nanofibers at 0.001% was not different

198 between 0 to 84 exposition days (P=0.2122; F=1.4944). The same was observed at

199 0.01% (P=0.172; F=1.6435) and with the commercial product (P=0.347; F=1.149).

200 However EAG responses at 0.001% pheromone were significantly lower, comparing to

201 0.01%, (P<0.05; F=19.079) after 84 days ageing. Control always trigged lower males

202 antennae responses in all treatments (P<0.05) (Figure 2).

203 **Field trials**

204 In the first field experiments (2014) the mean number of males caught in Delta traps

205 baited with virgin females during ten weeks of exposure was 21.85 per trap, statistically

206 equal to those with nanofibers baited with 0.01% pheromone (H=51.4521; P=0.0967).

207   We caught 17.1 males in traps baited with 0.1% pheromone, similar number (16.73

208   males) in traps with 0.01% (H =51.4521; P= 0.5926) and a lower amount (3.32 males)

209   in those with 1% (H = 51.4521; P < 0.00001) (Figure 3). Along weeks it was possible to

210   observe that the number of male catches in trap with 0.01, 1% pheromone nanofibers

211   and virgin females followed an homogeneous behavior. On the other hand, traps baited

212   with 0.1% achieved a pick at the fifth week with a reduction from the sixth week,

213   following all other treatments (Figure 4).

214   In the second year (2015), considering all ten weeks, significantly more males were

215   caught in traps with commercial septa (20.9) (H= 44.5493; P<0.001). However splitting

216   the bioassay in two periods, we observed that a higher number of males (7.6) were

217   captured in traps baited with 0.01% pheromone nanofibers, after five weeks of trial (H =

218   25.7015; P< 0.00001). Furthermore, there was no significant difference in the number

219   of male caught in traps baited with 0.001% (3.1 males) and commercial septa (3.9

220   males) (H = 25.7015; P=1) in the same period. Moth catches in control traps were

221   statistically lower (0.45) than any other treatments (H = 25.7015; P= 0.0402) (Figure

222   5A). From the sixth to the tenth week, traps baited with rubber septa captured

223   significantly more males (38.85) (H = 45.5102; P < 0.0001). During this period, no

224   differences in moth captures were observed among nanofibers (H = 45.512; P = 0.5136)

225   (Fig. 5B).

226   **Discussion**

227   In the present study, the pheromone release rate from PCL/PEG polymers, EAG

228   responses of OFM to nanofibers and commercial septa, as well as, its use in field

229   condition was evaluated. Nanofibers extracts chromatographic analyses showed that,

230   apparently, pheromone mostly is released from non exposed nanofibers to 42 days after

231   exposition, following a sharp decreasing from 63 to 84 days. This result is probably

232 related to the polymers natural degradation along time. Apparently hexane has low

233 solvent effect in PCL systems (Albini et al*.,* 2014). Forim et al. (2013) reported that

234 azadirachtin release in nanoparticles made of PCL was due to relaxation or erosion of

235 polymeric chains, providing thus excellent formulation stability. PCL polymers are

236 biodegradable aliphatic polyester and have been employed as slow-release agents in

237 agriculture for a long time (Sinclair 1973). It degrades into natural materials, harmless

238 to the environment, while slowly releases the encapsulated herbicides or pesticides, due

239 to its mechanical stability and to its display for prolonged degradation (Woodruff &

240 Hutmacher, 2010). Polyethylene glycol (PEG) is also a biocompatible water soluble

241 polyether used for surface modification to obtain co-polymers (Radhakrishnan et. al,

242 2014). Polyethylene glycol nanoparticles were used as a carrier for essential garlic oil

243 (*Allium sativum*) against *T. castaneum* (Tenebrionidae), resulting in a slow and

244 persistently active components release (Yang et al., 2009). Hellman et al. (2009), in a

245 pioneer work, have already showed that it was possible to incorporate pheromones into

246 polyamide and cellulose acetate nanofibers (about 30 wt.%), achieving an almost linear

247 release over several weeks. In this work, we use both polymers (PCL/PEG) as a single

248 blend, aiming to find a product that exhibit a combination of good properties, as

249 photochemical and thermal stability, suitable for applications in pheromone delivery.

250 Electroatennogram bioassays also evidenced that PCL/PEG nanofibers might be a good

251 delivery system in the pheromone release process, once it lasted (0.01% pheromone)

252 for, at least, 84 days, being able to stimulate *G. molesta* antennae in the same intensity

253 independently on time exposure. Bisotto-de-Oliveira et al. (2015) also observed that

254 PCL polymer nanofibers containing 0.02, 0.2 and 1% *G. molesta* pheromone elicited

255 similar electrophysiological responses, even after the third week of exposure. Therefore

256 we can employ that insect antennae were able to elicit the electrical response, over time,

257 when stimulated in EAG apparatus. It is probably due to the antenna chemical

258 perception, i.e, if the stimulus was strong enough, even after a long time treatment

259 exposition, enough receptor proteins were bond, thus threshold was probably reached

260 and action potential remained constant (Roelofs , 1984). However it does not mean that

261 pheromone perception observed in EAG will trigger a motor behavior in the field.

262 Indeed, in commercial peach and plum orchards a very few captures were registered

263 after the sixth week nanofibers exposition, i.e., approximately, 42 days.

264 In the first field trial the best results with nanofibers were obtained with lower doses

265 nanofibers (0.1 and 0.01%). Otherwise, 0.01% traps were already able to attract more

266 insects from the first three weeks comparing to 0.1 %. On the other hand, over all weeks

267 experiment, 1% nanofibers captured only 3.32 males. A small number of *G. molesta*

268 captures with PCL polymer pheromone (10%) were also registered in commercial peach

269 orchards (Bisotto-de-Oliveira et al*.,* 2015). Although, as we observed, high EAG

270 responses were noticed by the authors in experiments with the same concentration.

271 Those results indicate that on higher pheromone concentrations, traps may cause male

272 disruption instead of attraction. Regarding that the amount of 4% pheromonal blend is

273 used in SPLAT® (Specialized Pheromone & Lure Application Technology) for mating

274 disruption of this specie (ISCA, 2016). The results in 2015 showed, for up to five

275 weeks, more males caught at 0.01%. Nevertheless, from 6 to 10 weeks of trial, a

276 different profile of insects capture has been observed, i.e., commercial septum captured

277 more males than all nanofibers bates. It must be highlighted that the recommendation

278 use for the commercial device is to substitute the septum after four weeks in the field

279 (ISCA, 2016). Contradictorily, our experiment provided evidences that the commercial

280 device might underestimate the insect's presence in the first six weeks in field, being an

281 effective attractant after nanofibers baits deactivation. These results observed with

282 Iscalure®Grafo in 2015 are supported by our results in 2014, where we used similar

283 pheromone concentration (0.1%) in nanofibers, resulting in a capture increase after the

284 fourth week. The present findings could be potentially useful for preparing new nano

285 formulations as pheromone, kairomones and even attract-and-kill devices. Although the

286 promising researches in this area, commercial products based on nanotechnology are at

287 a very early stage of development. This technology calls for a future investigations with

288 an interdisciplinary group. Thus, further studies should be done to discover competitive

289 nano products in the marked in terms of performance and costs.

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398 **FIGURE CAPTIONS**

399

400 Figure 1. Average peak area of (*Z*)-8-dodecenyl acetate (majority *Grapholita molesta*

401 pheromone compound) at A) 0.001 and B) 0.01 %, observed in chromatographic

402 analysis of nanofibers made from polycaprolactone (PCL) and polyethyleneglycol

403 (PEG) (1:1) nanofibers, not exposed and after exposure for 21, 42, 63 and 84 days, kept

404 under controlled conditions. Means followed by different letters on the points indicate

405 significantly difference (Student test, P<0.05; n=6).

406

407 Figure 2. Mean (+SE) normalized electroantenographic (EAG) responses of *Grapholita*

408 *molesta* males antennae to pheromone-containing polycaprolactone (PCL) and

409 polyethyleneglycol (PEG) nanofibers (1:1) (0.001 and 0.01 %) and commercial septum

410 (ISCAlure®Grafo) after different exposure periods. Different lower case letters on the

411 bars indicate significant difference between treatments in each exposure periods and

412 same uppercase indicate no differences over the exposition periods of each treatment

413 (Tukey test; P < 0.05, n=15).

414

415 Figure 3. Mean number (±SE) *Grapholita molesta* males captured in baited traps with

416 pheromone nanofiber made from polycaprolactone (PCL) and polyethyleneglycol

417 (PEG) nanofibers (1:1) with 0.01, 0.1, 1% of conspecific pheromone and virgin females

418 after ten weeks in commercial peach and plum orchards (2014). Means followed by

419 different letters on the bars indicate significantly difference (Student test, P<0.05).

420

421 Figure 4. Population fluctuation of adult *Grapholita molesta* determined by the capture

422 with a Delta traps baited with polycaprolactone (PCL) and polyethyleneglycol (PEG)

423 nanofibers (1:1) with 0.01, 0.1, 1% of conspecific pheromone and virgin females in ten

424 weeks on commercial peach and plum orchards (2014).

425

426 Figure 5. Mean number (±SE) *Grapholita molesta* males captured in baited traps with

427 pheromone nanofiber made from polycaprolactone (PCL) and polyethyleneglycol

428 (PEG) nanofibers (1:1) (0.001 and 0.01%) of conspecific pheromone, Iscalure®Grafo

429 and control nanofibers from (A) 1 to 5 and (B) 6 to 10 weeks in commercial peach and
430 plum orchards. Means followed by different letters on the bars indicate significantly
431 difference (Student test, $P < 0.05$).

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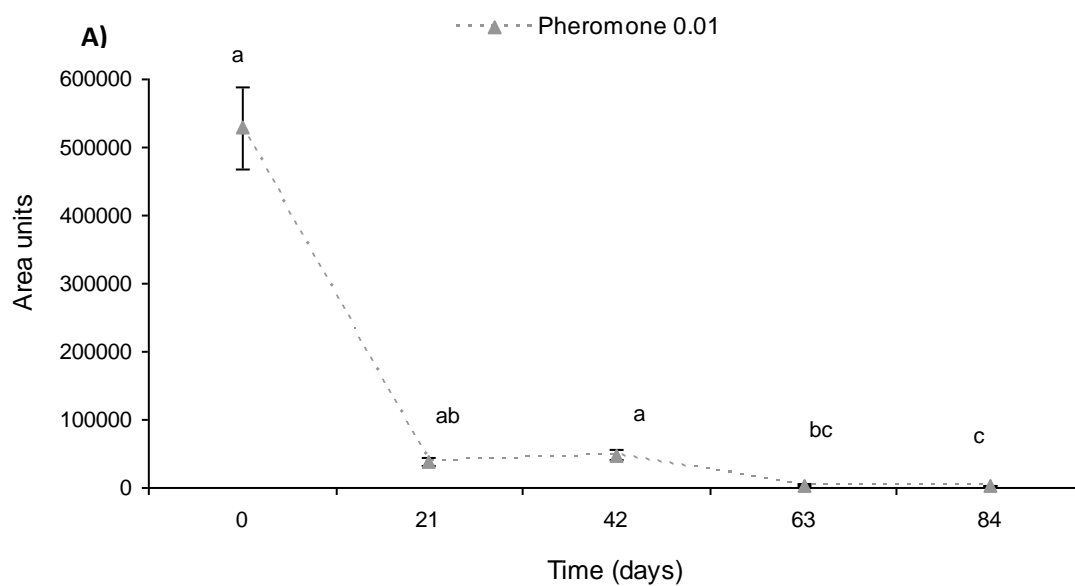
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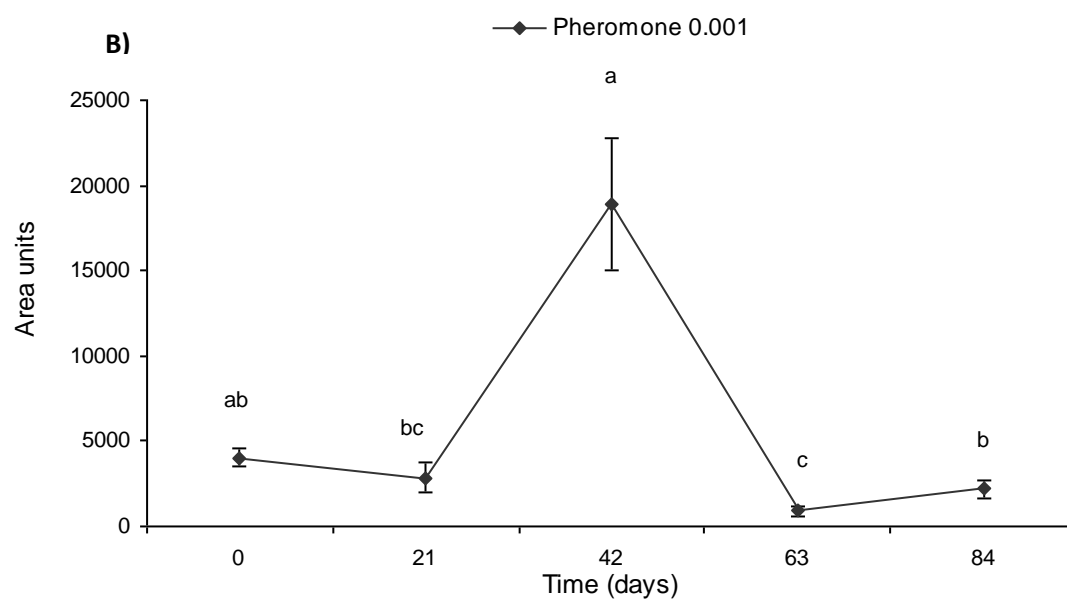
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Figure 3

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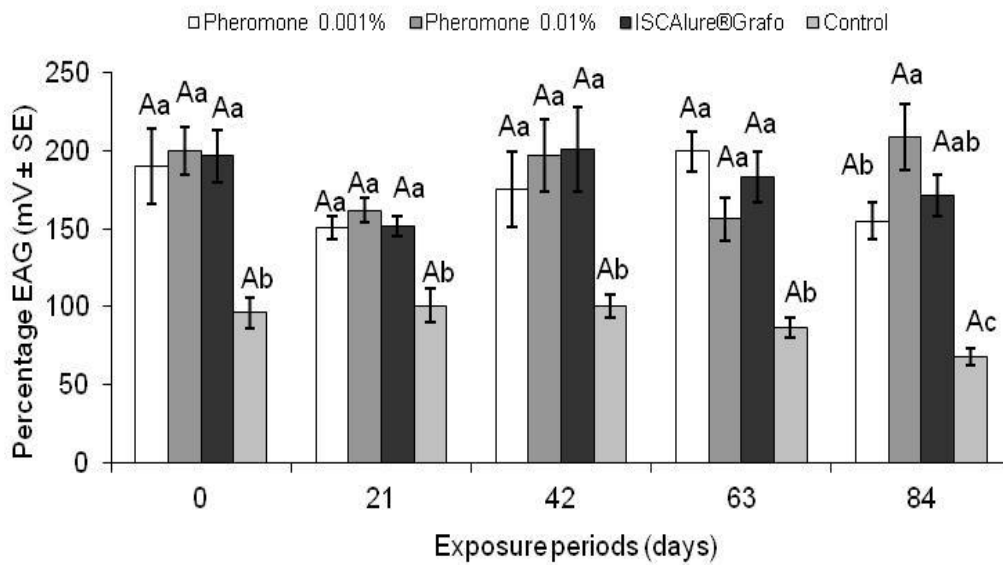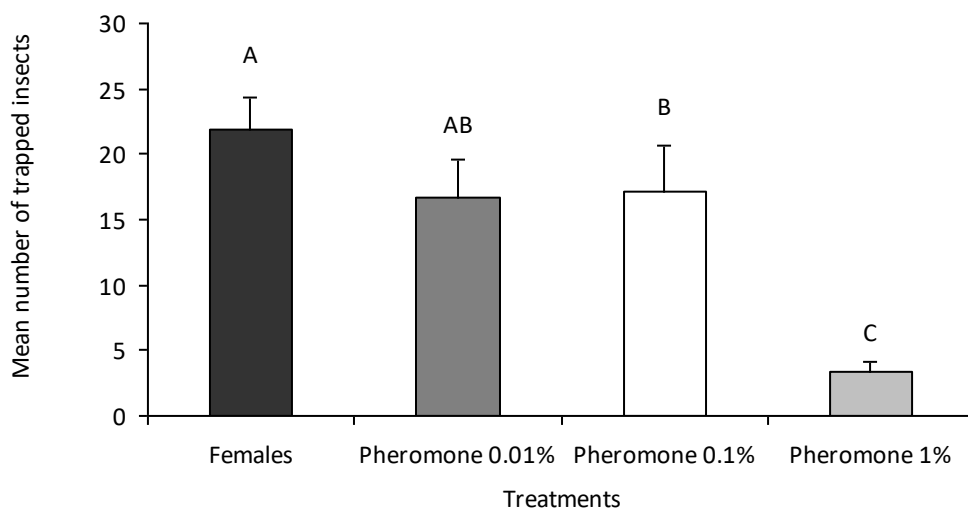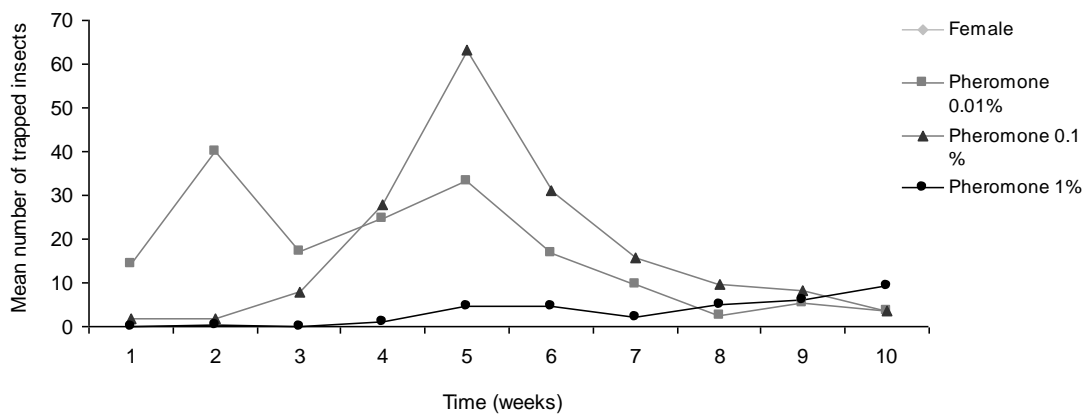462

Figure 4
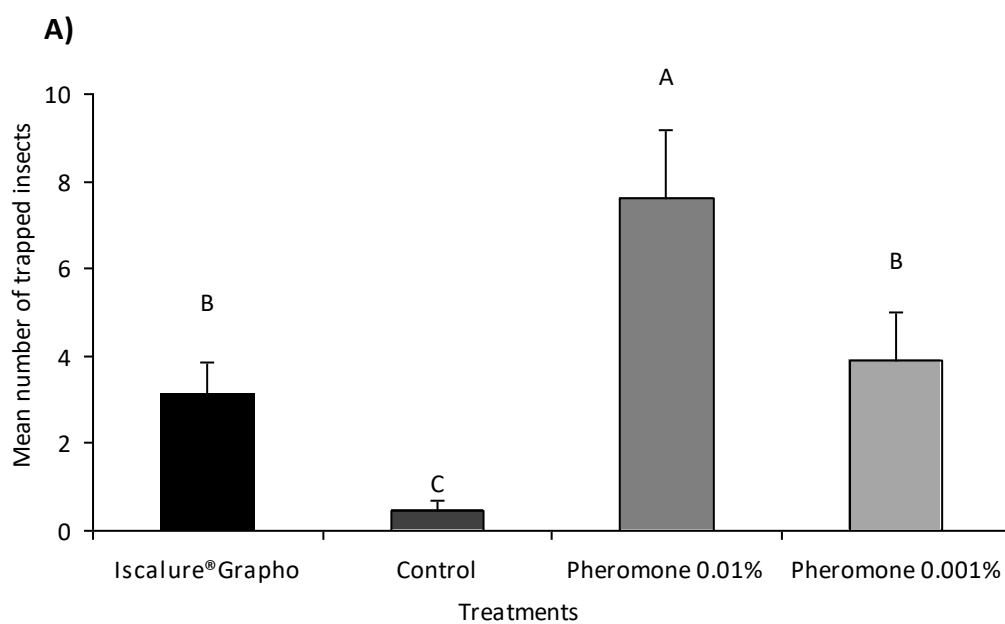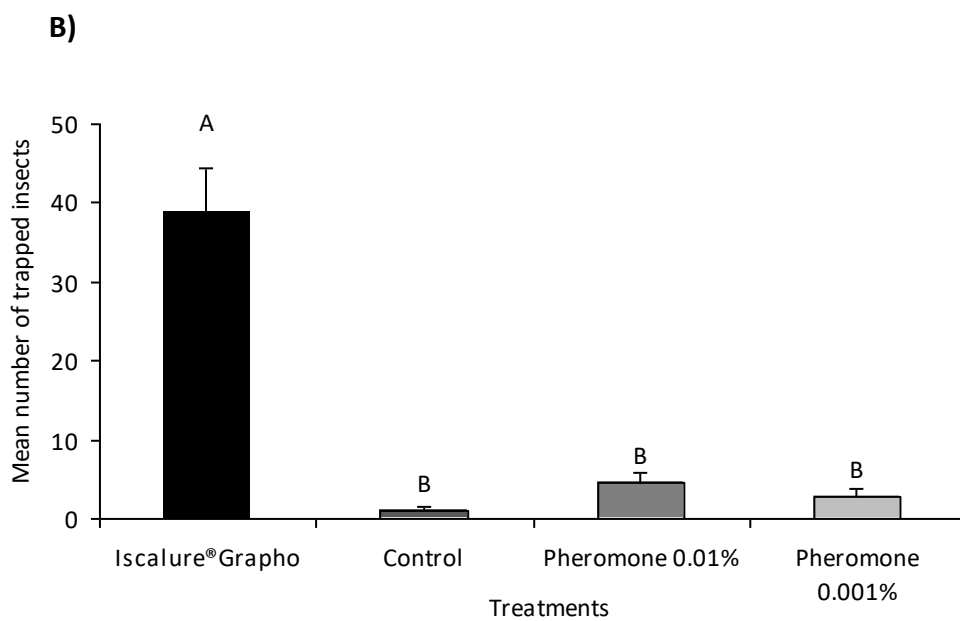
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467   **Figure 5**

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5 **6 ARTIGO 2**

6 **Novel nanoscale pheromone dispenser for more accurate evaluation of *Grapholita***

7 ***molesta* (Lepidoptera: Tortricidae) attract-and-kill strategies in laboratory[3].**

8 *Running title***: Novel nanoscale pheromone dispenser**

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18 *Key words:* **Nanofibers, attract-and-kill, Oriental fruit moth, pheromone.**

19 **Abstract –** BACKGROUND: Nanotechnology has recentlyallowed the production of

20 formulations for controlled release of active ingredients. In the present study, the

21 electrospinning technique was used for nanoscale dispensers production to attract-and-

22 kill strategies. Non-woven nanofibers containing insecticide (cypermethrin) and (*E)*-8,

23 (*Z)*-8-dodecenyl acetate and *(Z)*-8-dodecanol (0.87mg.L^{-1}), the main components of

24 *Grapholita molesta* (Lepidoptera:Tortricidae) (Busck) pheromone were evaluated in

25 laboratory experiments. Male electroantenographic (EAG) responses and mortality

26 (tarsal-contact and attract-and-kill behavioral cages) bioassays were performed for

27 ³Artigo elaborado segundo as normas da revista Pest Management Science

1 nanofibers (with and without insecticide) exposed for different periods (21, 42, 63 e 84

2 days) in controlled and non-exposed conditions. RESULTS: There were no significant

3 differences in G. molesta male EAG responses based on the time of exposure within

4 treatments. Nanofibers with only pheromone and pheromone plus insecticide elicited

5 equal EAG responses. Mortality in tarsal-contact bioassays was greater than 87%, after

6 84-day exposure. Regarding attract-and-kill bioassays, mortality ranged from 28.4 to

7 56.6%, though no difference was observed on insect mortalities over time (24, 48 and

8 72 h). CONCLUSION: Incorporation of cypermethrin in nanofibers did not interfere in

9 G. molesta attractiveness. Both aspects of the strategy, the attractant and killing effects,

10 were recorded using innovative nanofibers, and long-term effects suggest a controlled

11 release of pheromone and insecticide.

12 **1 INTRODUCTION**

13 Pesticides play an important role in agriculture, as they help in preventing and reducing

14 crop losses caused by pests[1]. However, less than 1% of the applied pesticides actually

15 reaches the target pests[2,3]. Indiscriminate pesticide usage increases pathogen and insects

16 resistance, reduces soil biodiversity, diminishes nitrogen fixation, contributes to toxic

17 bioaccumulation and negatively affects beneficial organisms[4]. There is an urgent need

18 to reduce the use of these agrochemicals with the utilization of more sustainable pest

19 control methods[5]. As a general approach, nanotechnology is a promising new tool for

20 agriculture pest management[6,7,8], and particularly, for pest control based on

21 pheromones[9,10,11]. Pheromones are chemical substances used for the communication

22 between insects released and perceived in minute concentrations[12]. Insects are very

23 sensitive to small variations of their quantity in the environment[12]. The pheromone

24 dispensers are potential beneficiaries of the controlled release of the active ingredients

25 (AI) resulting from nanotechnology methods[13]. The use of these technologies for

producing pheromone and/ or insecticide dispensers brings further technological

development and accuracy in the evaluation of pheromone release strategies. Previous

studies demonstrated the accuracy of the pheromone release in laboratory [10] where the

authors verify the attractiveness of innovative nanofibers formulations with Trimedlure

(TML) for the male of *Ceratitis capitata* in laboratory and field cage tests, and field

trials[11] whose objective was to produce nanofibers incorporating synthetic sex

pheromones from the oriental fruit moth, *Grapholita molesta* (OFM), using different

polymers. The conclusion of both studies reveals that the nanofiber matrixes prepared

with the electrospinning technique can be used for the manufacture of controlled release

dispensers of active ingredients, such as insect pheromones.

The innovative application of nanotechnology in agriculture, specifically polymeric

nanofiber as vehicles for semiochemical dispenser, was based on a pioneering study by

Hellmann et al. (2009)[9]in Germany. In that study, the authors produced cellulose acetate

nanofibers containing the grape berry moth *Lobesia botrana* (Denis & Schiffermüller,

1775) (Lepidoptera, Tortricidae) synthetic sex pheromone. They examined the

incorporation of this species pheromone within nanofibers to be used in mating

disruption in grapevine orchards. Lindner et al. (2011)[14] also performed laboratory tests

to characterize the nanofibers containing the *L. botrana* pheromone,while Hummel et al.

(2011)[15] performed bioassays in the wind tunnel, and quantified the mass loss of

nanofibers using analytical balances and conducting thermogravimetric analysis (TGA).

The authors used the electrospinning technique which allows the production of regular

thickness nanofibers, about 100 microns, which is not achieved with any other dispenser

technology that is not nanotechnological-based[16]. The electrospinning is an

electrohydrodynamic technique. It makes an electrostatic polymer deposition enabling

the production of a uniform polymer matrix layer. With the cited microscopic thickness,

while the polymer dispersion incorporating AI is released through a syringe pump

equipped with a stepper motor with high accuracy[17]. Insecticide nanoformulations are

supposed to be less harmful to non-target organisms and may potentially decrease the

development of insects resistance[18,19]. This technology can result in lower pesticide

doses, this way reducing losses by leaching, volatilization and degradation, increasing

action time on pests, and reducing the amount of agricultural chemicals [20]. Electrospun

nanofibers incorporating the insecticide thiamethoxam, for example, were efficient at

50% of the recommended dosage (9 d glass house tests, whitefly)[21]. The authors suggest

that a potential advantage of such nanofibers over spheres or capsules lies in their

ability to avoid the release bursts which occur when the AI is not homogeneously

distributed within the polymer matrix[18]. It seems that nanoscale delivery vehicles can be

useful not only for sole pheromone dispensers, but also for attract-and-kill system

incorporating insecticides. Some authors report that attract-and-kill systems that use

insecticides which produce true repellency are undesirable since they might reduce

control efficacy [22]. It is known that repellency to pyrethroids varies among insect

species[23,24], but it has not been widely reported in Lepidoptera[25,26]. However, there is

some evidence pyrethroids may become repellent to some insects only when used above

a behavioral threshold concentration[28,29,30].

Oriental fruit moth (OFM) *Grapholita molesta* (Lepidoptera: Tortricidae) (Busck) is a

cosmopolitan pest distributed in various agricultural regions of the world where fruit

species of Rosaceae are cultivated; this pheromone was used in a previous study of our

group using nanofibers as the dispenser vehicle [11].

The nanofibers are known by their higher surface area to volume ratio, a feature that

could also be valuable to the release of a contact and ingestion insecticide, such as the

pyrethorids. To date, information about the use of nanofibers as vehicles for dispersing

1   insect pheromones is scarce. It's largely based on the results obtained by Hellmann et

2   al. (2009)[9], Hummel et al. (2011)[15], Lindner et al. (2011)[14], and Bisotto-de-Oliveira et

3   al. (2015)[11] which served as parameters in the selection of polymer and pheromone

4   concentration to be investigated. To our knowledge, there are no previous studies

5   concerning the incorporation of attractants and insecticides in only one polymeric base

6   (i.e. nanofiber) using electrospinning technique. In this context, *G. molesta* male

7   electroantenographic responses, male mortality in tarsal-contact bioassays and cages

8   attract-and-kill behavioral experiments of nanoscale dispensers incorporating

9   pheromone and/or cypermethrin (pyrethroid insecticide) during different periods of time

10   were evaluated.

11   **2 EXPERIMENTAL METHODS**

12   **2.1 Insects:** oriental fruit moth (OFM) *G. molesta* were reared in a controlled condition

13   chamber (25 ± 2 °C, 60 ± 10% RH; 16 hour photophase and luminance equal to 1.5

14   klx). The insects, during the larval period[30], were fed with an artificial diet, and the

15   adults, with water-honey solution (honey 150 $g.L^{-1}$ and methylparaben 50 $g.L^{-1}$). All

16   tests were performed with three to five-day-old virgin males.

17   **2.2 Chemicals and formulation.** Polymeric nanofibers were produced at Tecnano

18   Research and Services Ltd. (RS-Brazil), using a custom made electrospinning machine

19   with a 30 kV HV supply. In that process, polymer dispersions were dissolved in specific

20   solvents THF (tetrahydrofuran) and $CHCl_3$ (chloroform) (3:1) from Merck (Darmstadt,

21   Germany), with and without the synthetic sex pheromone of *G. molesta* (P9000 - 90

22   Bedoukian OFM Technical Pheromone), and cypermethrin insecticide. The polymeric

23   blend dissolved in the binary solvent mix was homogenized in a magnetic stirrer

24   overnight. The solution was fed into the electrospinning machine with a syringe pump

25   at a rate of 0.05 ml/min using a 5 mL syringe with a blunted needle (BD Precision Glide

1   22G). A 1.81 kV/cm voltage difference was applied between the nozzle and aluminum

2   collector where the nanofibre film was deposited. The polymers poly-ε-caprolactone

3   (PCL; average MW 80,000) and polyethylene glycol (PEG) were obtained from Sigma

4   Aldrich (St. Louis, MO, USA) and processed in a solution (1:1). The tested insecticide

5   was 250 g.L^{-1} cypermethrin (NORTOX® 250 EC, Brazil). The dispenser weight and AI

6   concentrations were used as follows: nanofibers weighting (1 ± 0.2 g) folded to a sphere

7   format: a) with pheromone (0.87mg.L^{-1}), and insecticide (125mg.L^{-1}); b) only with

8   pheromone (0.87g.L^{-1}); c) only with insecticide (125mg.L^{-1}); and d) without both. We

9   experienced fresh nanofibers as well as exposed ones (same rear conditions of insects)

10   for 21, 42, 63 and 84 days.

11   **2.3 Electroantennogram recordings (EAG).** Moths were chilled at 4 °C for 20 min.;

12   then one antenna was excised and positioned onto silver electrodes using Spectra 360

13   conductive gel (Parker Laboratories, Orange, NJ, USA). Antenna holder was attached to

14   a Syntech EAG probe (Type PRG-2, internal gain 10×). Nanofibers spheres were

15   individually placed into an adapted pipette, made with Falcon tubes (50 mL). Stimulus

16   was generated with a Syntech CS-02 controller with 1s pulse duration and 2.5 mL/0.5s

17   flow. EAGs were measured as the maximum amplitude of depolarization elicited by

18   stimulus applied. Each antenna received a series of puffs delivered once at every

19   minute, and treatments (within the same time exposure or without exposure) were

20   randomly applied to each antenna. Bioassays were recorded by using IDAC-4 data

21   acquisition controller system and EAG 2000 software (Syntech, Kirchzarten, Germany).

22   Fifteen repetitions to each treatment were performed. Data were analyzed by ANOVA

23   and compared with Tukey test (α = 0.05), using Bioestat® 5.0 software[31].

24   **2.4 Contact bioassay.** We evaluated OFM mortality after contact with PCL/PEG

25   nanofibers spheres containing the attracticide formulation or only with pheromone

1   (control), exposed and fresh (without exposure). It was also recorded natural mortality,

2   keeping individuals (n = 40) in plastic pots (50 mL) with food and kept in rear

3   conditions without handling or exposure to treatments. Empty syringes (5 mL), with

4   needles and nozzle cut off were used, and they were placed vertically over nanofibers,

5   covering their bottom. Each insect was introduced into the syringe and compressed with

6   the piston (attached with a soft sponge) until tarsal legs touched the nanofiber for five

7   seconds (Figure 1). Males were taken from the system with a manual vacuum,

8   individually transferred into plastic pots (50 mL), fed with a honey solution (150 g.L^{-1})

9   with methylparaben (50 g.L^{-1}) and kept in rear conditions. The number of dead insects

10   observed was 1, 6 and 24 hours after contact test. The average and total number of dead

11   insects was corrected by the Abbott formula[32], analyzed by Kruskal-Wallis and

12   compared by Dunn (α=0.05), using Bioestat® 5.0 software [31].

13   **2.5 Cage test.**   In order to investigate the attract-and-kill effects of attracticide

14   nanofibers on *G. Molesta*, nanofibers, fresh and after exposure were used for the test, as

15   previously described, and nanofibers containing only pheromone, served as control.

16   Twenty male moths were placed in a screen cage (0.6 x 0.4 x 0.4 m) provided with a 15

17   x 15 cm opening door. One nanofiber was fixed, using a double face tape, on the right

18   cage wall, few centimeters below the cage ceiling (Figure 2). The number of dead males

19   recorded was 24, 48 and 72 h after insects release. During bioassays, moths were fed

20   with the solution previously described. After each experiment, all cages were cleaned

21   with water and alcohol 96%. Three repetitions (20 moths)/treatment were performed.

22   The average and total number of dead insects was analyzed by ANOVA and compared

23   by Tukey (α=0.05), using Bioestat® 5.0 software [31].

24   **3 RESULTS**

1   **3.1 EAG.** Responses of males antenna to new and all exposed nanofiber spheres with

2   pheromone ($0.87mg.L^{-1}$) and/or insecticide ($125mg.L^{-1}$), as well as control showed no

3   significant differences over time (Fig. 2.: $P > 0.05$). Both treatments with pheromone

4   triggered higher EAG responses in comparison to control and only insecticide

5   treatments (Figure. 3: $P < 0.05$). Differences in nanofibers loaded with only pheromone

6   were not found in comparison with those using attracticide formulation (P=0.8888;

7   F=0.018). Furthermore, it was not observed differences in antenna perception between

8   control and only insecticide treatments (P=0.7036; F=0.1473) (Fig. 3).

9   **3.2 Contact test.** Male mortality was not statistically different from all nanofiber

10   exposure intervals (0 to 84 days) (P= 0.0969; H= 7.8589) (Table 1). It ranged from

11   62.5% (63 days exposed) to 100% (21-day exposure), 24 hours after the insect contact

12   with spheres containing pheromone and insecticide. No differences were found in male

13   death within each treatment over time (1, 6 and 24 h) ($P > 0.05$) either. Insects handled

14   with syringe method exposed to pheromone formulation (control) and those maintained

15   to observe natural mortalitydid not die or presented knocked-down individuals.

16   **3.3 Cage test:** Attract-and-kill effects observed in males released in cages with exposed

17   and non-exposed nanofibers ranged from 28.34% (24 h observation/63day exposure) to

18   56.67% (72 h observation/84 day exposure). However, no difference was observed in

19   insect mortality within each treatment over time (24, 48, and 72 h) (F= 0.2463; P=

20   1.7843). We also did not notice differences between insects death and nanofiber

21   exposure time (F=0.5937; P=0.6773). No mortality of OFM was registered in cages

22   with nanofibers containing only pheromone.

23   **4. DISCUSSION**

24   Active ingredient physicochemical parameters such as molecular weight, molecular

25   shape, evaporation rate and boiling temperature play a key role in the AI release kinetics

1   from either oil-based or polymeric dispensers . Oil dispenser, such as the traditional

2   paste dispensers, depends on vehicle viscosity, density, melting and temperature, among

3   other parameters[33], whereas in a polymeric dispenser, the free volume within the

4   polymeric material, segmental mobility of polymer chains and crosslink density of

5   polymers are the important issues[34] The polymeric dispenser can be expected to slowly

6   release the AI when compared to the paste dispenser.

7   In Brazil, OFM pheromone release devices are produced for monitoring strategies as

8   Iscalure®Grafolita rubber septum, formulated with (*E*)-8-dodecenyl acetate, (*Z*)-8-

9   dodecenyl acetate and (*Z*)-8-dodecenol (0.1336%)[35] and Bio Grapholita®, with (*Z*)-8-

10 dodecenyl acetate, (*E*)-8-dodecenyl acetate, (*Z*)-8-dodecenol, Dodecanol (0.013%)[36].

11 According to the manufacturers, it is recommended to replace septum each 21 days[35] or

12 four weeks[36].

13 The controlled release of AI could be true for capsules and fibers as well, where the

14 release might be slower, because, due to geometrical issues, the path to the border of the

15 fiber is longer. No decrease in EAG perception was observed in *G. molesta* males with

16 cypermethrin incorporated into the nanofiber pheromone spheres in this study. Hellman

17 et al. (2009)[9] have already showed that it was possible to incorporate pheromones into

18 polyamide and cellulose acetate nanofibers (about 30 wt.%), achieving an almost linear

19 release over several weeks. In this work, it was used both polymers (PCL/PEG) as a

20 single blend, aiming at finding a product exhibiting a combination of good properties,

21 such as photochemical and thermal stability, suitable for applications in pheromone

22 delivery.

23 Electroatennogram bioassays also evidenced that PCL/PEG nanofibers might be a good

24 delivery system in the pheromone release process, once it lasted (0.01% pheromone)

25 for, at least, 84 days, being able to stimulate *G. molesta* antennae at the same intensity

1   independently of time exposure. Also the presence of cypermethrin triggered similar

2   EAG responses to control nanofibers, which represents no impact on EAG responses.

3   More dead males were also found in attract-and-kill experiments (cage tests) in the

4   presence of nanofibers with insecticide than in control. In these experiments, the results

5   provided evidence that cypermethrin did not interfere in *G. molesta* attractiveness. It

6   can be hypothesized that the novel polymeric matrix led to low cypermethrin diffusion

7   and consequent slow evaporation rate, having a release below the adult fruit moth

8   threshold, preventing the dispenser from eliciting true-repellency from the target pest

9   species, as would be expected in the presence of large evaporation rate[27,37]. That fact is

10   supported by similar responses observed in antennae exposed to nanofibers with

11   insecticide and control, as well as nanofibers loaded with OFM pheromone, with or

12   without insecticide. In a similar case, Curkovic & Brunner[38] studied an attracticide

13   formulation against *Choristoneura rosaceana* (Lepidoptera: Tortricidae) and *Pandemis*

14   *pyrusana* (Lepidoptera: Tortricidae) containing insecticide permethrin (6% AI) and they

15   also did not find repellency on those species with the insecticide presence. Although the

16   pyrethroids are known for becoming repellent to some species when used above the

17   threshold concentration[27,28,29]. In our study, the $125mg.L^{-1}$ cypermethrin concentration

18   used was also low to be detected.

19   The available product used in attract-and-kill strategy for OFM in Brazil is the paste

20   dispenser SPLAT CIDA GRAFO BONA® (SCGB) [39]. It is claimed that, after contact

21   with the cited SCGB, the male would receive sub-lethal insecticide doses that harm its

22   reproductive performance[40]. In this study, it was not observed, reproductive effects after

23   insects contacting with the nanofiber spheres. Previous studies with SCGB in tarsal-

24   contact assays over different periods of exposure[41] reported that after 60 days of

25   exposure, male mortality achieved 73% (H = 2,88; P = 0,08) and after 90 days, it was

1   reduced to around 13%. However, in tarsal-contact assay, it was found a mortality rate

2   of 87.5 % even after 84 days of nanofiber exposure , supporting that the studied

3   polymeric nanofibers can present prolonged insecticide activity[42], even with an AI

4   concentration four times lower than that used in SCGB formulation. The mortality of

5   insects after contact with SCGB not exposed to the environment would hardly be

6   observed under field conditions at least for the first few days after application. The

7   products used to hinder the encounter between couples contain a high concentration of

8   pheromone, besides having a release rate around 21.4 µg /h under field conditions[43].

9   This concentration associated with a constant release rate would make it difficult to

10   perceive the trails leading to the product, making it impossible the contact with the

11   insecticide. It is presumed that the nanofiberapproach is advantageous because of the

12   uniform distribution of the AI in the nanofibers, as shown by Hellmann[9]. Cypermethrin

13   was selected for the present study due to ecological issues. On soils, cypermethrin

14   photodegrades fast with a half life of 8–16 days, and it is also subject to microbial

15   degradation under aerobic conditions[44], making it a benign insecticide in the

16   environmental point of view. The results achieved in this paper indicated that

17   cypermethrin incorporated into the nanofibers had a residual value and persistence

18   suggesting a slower kinetics release than in a commercial dispenser, which would lead

19   to spaced application intervals. Furthermore the polymer would prevent volatile AI

20   against UV degradation due to physical blocking action specially when associated with

21   mineral oxides[45], and it could reduce phytotoxicity and ease of handling of toxic

22   materials[46]. The use of such specific attracticides can play a leading role on the pest

23   management . It could be used efficiently for several weeks without harming any other

24   insect specie and humans because of the minimum manipulation of the device and the

25   absence of residue in food [47].

Nanoscale polymeric fibers would reduce significantly pheromone amounts needed to be used to capture insects in field conditions, although with a controlled release of AI and possible cost benefits, large scale tests are in need of evaluating plant protection efficiency. The continuous research on nanotechnology can help produce new pesticides, insecticides and insect repellants. The present study showed that the nanofibers are a beneficial approach for attract-and-kill strategies in laboratory.

5. CONCLUSION

Nanotechnology methods can be used for an accurate evaluation of attract-and-kill strategies at laboratory scale. With the use of nanofibers dispensers, cypermethrin did not interfere in *G. molesta* attractiveness in laboratory study, which was shown by similar antenna responses observed in nanofibers containing insecticide and control. Both aspects of the strategy, the attract-and-kill effects, were evaluated using the innovative nanofibers, and long-term effects found suggest that a controlled release of both the pheromone and the insecticide was achieved.
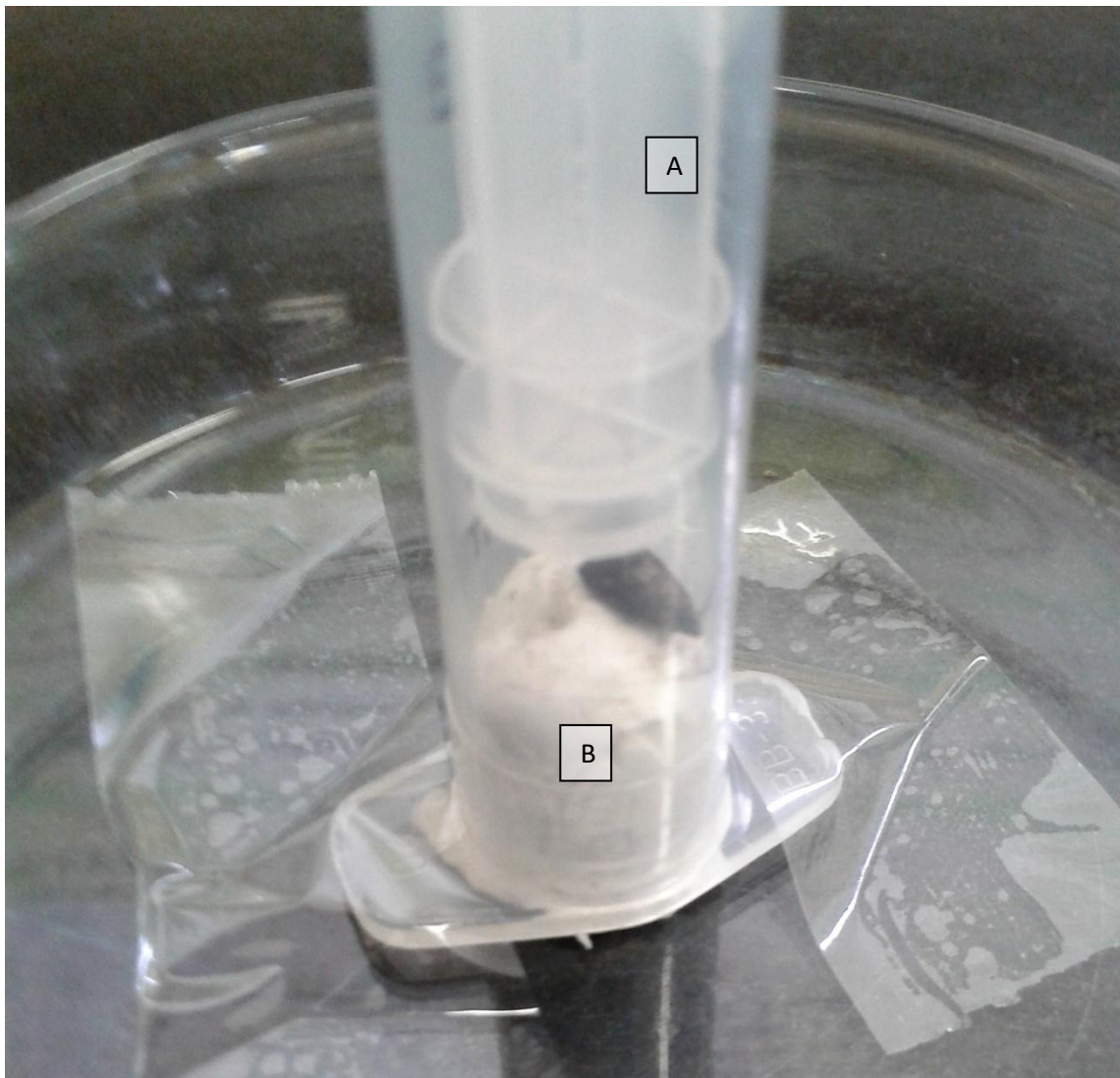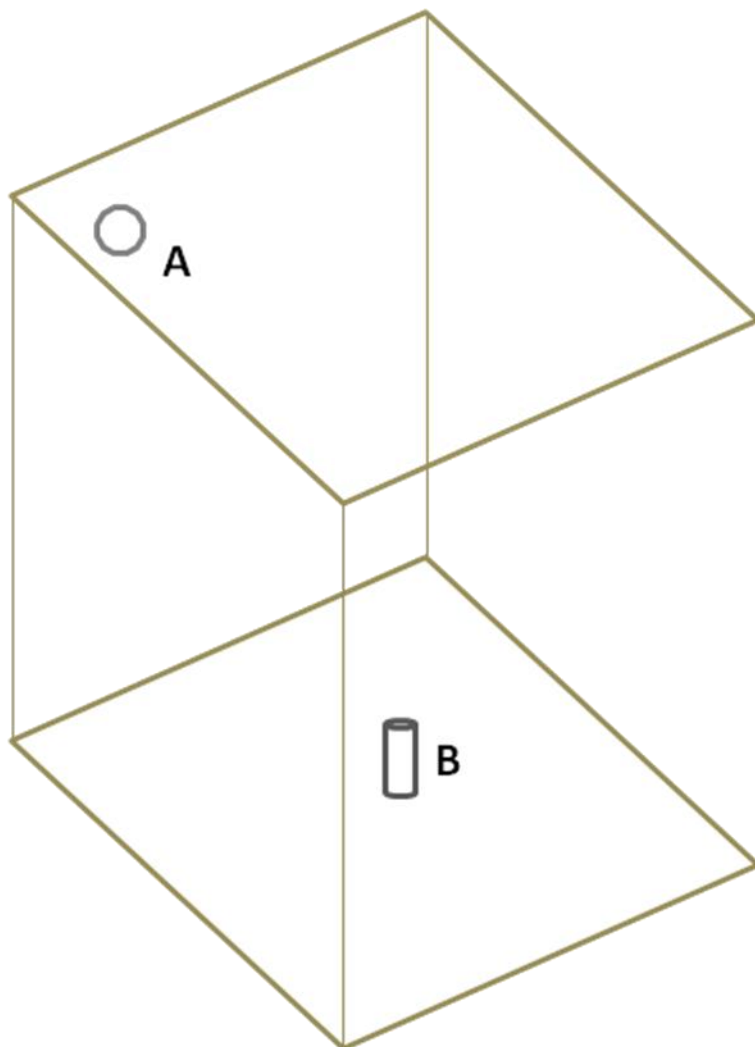
1

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Figure 1. Photograph of *Grapholita molesta* male submitted to tarsal-contact bioassays.
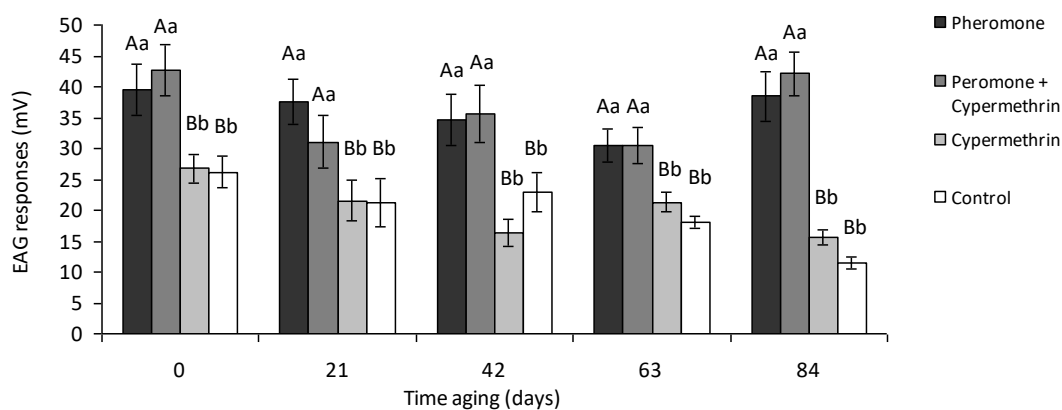A) seringe; B) nanofiber containing treatments.

1

Figure 2. Schematic representation of the attract-and-kill condition bioassays *voile* cage
used to evaluate nanofiber for *Grapholita molesta* . A) nanofibers containing
attractant+insecticide or control (attractant only); B) vial containing food.

1

Figure3. Mean (±SE) electrophysiological responses (EAG) (mV) of *Grapholita molesta* male submitted to polycaprolactone (PCL) and polyethyleneglycol (PEG) nanofibers (1:1) containing synthetic pheromone ($0.87mg.L^{-1}$) and/or cypermethrin ($125mg.L^{-1}$) and without both (control) after different aging periods. Means followed by different letters differ from each other. Uppercase letters represent differences within different exposure periods of each treatment and the lowercase, within time aging (Tukey, $P < 0.05$).

Table1. Average number of dead insects (± SD) and corrected mortality (Mc - Abbott's formula) (%) of *Grapholita molesta* males 1; 6 and 24 hours after contact with the attracticide from polycaprolactone (PCL) and polyethyleneglycol (PEG) (1:1) nanofibers containing synthetic pheromone (0,87mg.L^{-1}) and cypermethrin (125mg.L^{-1}), unexposed or exposed for 21, 42, 63 and 84 days, under controlled conditions. Control = nanofibers only with pheromone. Natural mortality = insects without contact with nanofibers.

Time ageing (pheromone + cypermethrin) (days)	Evaluation periods								
	1h			6h			24h		
	N°		Mc	N°		Mc	N°		Mc
0	4.0±2.94	a*	40	6.75±1.58	A	67.5	7.25±0.57	Aa	72.5
21	8.75±1.5	A	87.5	10±1.5	A	100	10±1.5	Aa	100
42	8.25±0.5	A	82.5	9.75±0,58	A	97.5	9.75±0,58	Aa	97.5
63	4.50±5.2	A	45	4.50±5.2	A	45	6.25±0.96	Aa	62.5
84	6.75±3.8 6	A	67.5	7.25±0.58	A	82.5	8.75±1.0	Aa	87.5
Control	0	-	0	0	-	0	0		0
Natural mortality	0	-	0	0	-	0	0	-	0

*Means followed by different lowercase letters on line and uppercase, on column, differ from each other (Kruskal Wallis test; P<0.05, n=40).

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7 CONSIDERAÇÕES FINAIS

Em geral, nanoformulações poliméricas parecem ter o maior potencial de desenvolvimento e aplicação prática, a julgar pelo número de publicações relacionadas e a sua maior eficácia em comparação com formulações comerciais.

Considerando as aplicações da nanotecnologia na agricultura, pode ser sugerido que a utilização de nanomateriais resultará no desenvolvimento de sistemas eficientes e abordagens potenciais para a gestão de pragas de insetos na agricultura. Literatura disponível sobre este tema traz conclusões de que apenas alguns pesquisadores no mundo estão trabalhando nesta área, e, portanto, há uma necessidade premente de aplicar a nanotecnologia e, portanto, garantir estudos detalhados.

Os resultados oriundos deste estudo indicam que nanofibras podem ser suportes para a dispersão de feromônio em programas de monitoramento de *G.molesta,* sendo a concentração de 0,01% a mais adequada, proporcionando a captura contínua de insetos por até cinco semanas em condições de campo. Quanto ao uso de nanofibras para a técnica de atrai-e-mata, foi possível verificar que os polímeros têm capacidade de retenção ou encapsulamento da cipermetrina, o que pode ser constatado pelas taxas de mortalidade por contato ao longo de todos os períodos testados neste trabalho.

Esta linha de pesquisa tem caráter inovador e com poucos trabalhos publicados nesta área. Contudo pode ser uma alternativa mais sustentável para viabilizar ferramentas de manejo e controle de pragas, buscando a redução do uso de agrotóxicos

e um maior equilíbrio ecológico em agroecossistemas. No entanto, tal como todas as inovações tecnológicas, o uso da nanotecnologia em agroecossistemas deve ser avaliado de forma criteriosa para que esta ferramenta possa realmente proporcionar uma melhor qualidade de vida para agricultores e consumidores.