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**INTERAÇÕES ENTRE PARASITOIDES DAS FAMÍLIAS BRACONIDAE E
FIGITIDAE (INSECTA: HYMENOPTERA) E SEUS HOSPEDEIROS (DIPTERA:
TEPHRITIDAE)**

**PORTO ALEGRE
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Tese apresentada ao Programa de Pós-Graduação em Biologia Animal, Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como requisito parcial à obtenção do título de Doutora em Biologia Animal.

Área de concentração: Biologia e Comportamento Animal

Orientadora: Profa. Dra. Simone Mundstock Jahnke

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TEPHRITIDAE)**

Aprovada em ____ de _____ de ____.

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O segredo da existência
humana reside não só em viver,
mas também em saber para que
se vive.

Fiodor Dostoievski

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RESUMO

Parasitoides das famílias Braconidae e Figitidae são importantes agentes de regulação populacional em sistemas naturais e agrícolas com espécies utilizadas em programas de controle biológico das moscas-das-frutas (Diptera: Tephritidae). Muitos aspectos das relações dos parasitoides com seus coespecíficos e seus hospedeiros, no entanto, ainda não são conhecidos. Assim, este trabalho avaliou a influência dos hospedeiros de origem, *Anastrepha fraterculus* (Wiedemann, 1830) (AF) e *Ceratitis capitata* (Wiedemann, 1824) (CC) no parasitismo de *Aganaspis pelleranoi* (Brèthes, 1924) (AP) e *Doryctobracon areolatus* (Szépligeti, 1911) (DA), parasitoides nativos; mensurou a preferência e capacidade de parasitismo de *Diachasmimorpha longicaudata* (Ashmead, 1905) (DL), exótico, em larvas de diferentes ínstaes de *A. fraterculus* e *C. capitata*; e, investigou a competição intra e interespecífica entre os parasitoides, *D. longicaudata* e *A. pelleranoi* no hospedeiro *A. fraterculus*. Os bioensaios foram realizados com insetos provindos de criações de laboratório, em ambientes com condições controladas, em arenas. Para avaliar a influência do hospedeiro de origem, foram realizados testes de dupla escolha, oferecendo-se as larvas das duas espécies de moscas aos parasitoides com diferentes origens. A preferência por ínstar de *D. longicaudata* foi avaliada através de dois testes de múltipla escolha, o primeiro foi realizado oferecendo-se um ínstar por vez, com escolha entre os hospedeiros, e o segundo foi realizado oferecendo os três ínstaes de uma única espécie hospedeira. A competição foi avaliada em diferentes regimes de exposição dos hospedeiros para as duas espécies (DL e AP), sendo oferecidas a somente uma espécie em uma única ocasião, a um coespecífico em duas ocasiões, ou às espécies competidoras, em duas ocasiões, alternando-se a ordem de oferecimento. O parasitoide *A. pelleranoi* teve preferência pelos hospedeiros de origem, mas alterou a preferência após passar uma geração pelo hospedeiro alternativo e *D. areolatus* demonstrou preferência somente ao hospedeiro AF; a razão sexual foi desviada para machos em *A. pelleranoi* em ambos hospedeiros e para *D. areolatus* com origem em CC. Em relação ao ínstar preferencial, no teste de escolha simples constatou-se em *D. longicaudata* maior número de

parasitoides emergidos e de pupários parasitados em larvas de primeiro e segundo ínstar do hospedeiro AF e, para CC, não houve diferença nos ínstares testados. No teste de múltipla escolha, as médias de pupários parasitados e parasitoides emergidos foram maiores em larvas de segundo ínstar para AF, e para CC, foi maior em larvas de segundo e terceiro ínstar. Na competição entre *D. longicaudata* e *A. pelleranoi*, as médias de pupários parasitados e parasitoides emergidos foram maiores nos tratamentos em que os hospedeiros foram oferecidos primeiramente à DL, sendo expostos posteriormente a um coespecífico ou a AP. Quando os hospedeiros foram submetidos somente uma vez aos parasitoides, a razão sexual foi desviada para machos, mas quando expostos duas vezes, exceto para a prole de *D. longicaudata* em AP-DL, um maior número de fêmeas foi gerado. O índice de parasitismo foi maior para DL tanto em regime individual de exposição, quanto na competição com AP, independentemente da ordem de oferecimento. Registrou-se correlação positiva entre a média de cicatrizes de oviposição e a média de parasitoides e de fêmeas emergidos. Esta tese evidencia que o hospedeiro de origem é importante para a escolha do hospedeiro, podendo ser alterada em apenas uma geração, o que pode facilitar a criação em laboratório e a utilização no controle biológico. Além disso, *D. longicaudata* não possui um ínstar preferencial, parasitando qualquer ínstar das duas espécies testadas, podendo competir com parasitoides que apresentam especificidade por ínstares iniciais. Considerando a competição entre *D. longicaudata* e *A. pelleranoi* foi evidenciado que pode haver uma supressão da espécie nativa quando ambos parasitam a mesma larva. Para a implementação de programas de controle biológico é importante ter o conhecimento das espécies nativas existentes no ambiente, e se existem barreiras abióticas e/ou bióticas que possam auxiliar na divisão dos nichos, cuidados que podem auxiliar para que não haja desequilíbrio ambiental, com a inclusão de *D. longicaudata* no campo.

Palavras-chave: *Aganaspis pelleranoi*, *Diachasmimorpha longicaudata*, *Doryctobracon areolatus*, parasitoides de tefritídeos.

ABSTRACT

Parasitoids of Braconidae and Figitidae families are important agents of population regulation in natural and agricultural systems with species used in biological control programs of the fruit flies (Diptera: Tephritidae). Many aspects of the interactions of parasitoids with their co-specific and their hosts, however, are not yet known. Thus, this work evaluated the influence of *Anastrepha fraterculus* (Wiedemann, 1830) and *Ceratitis capitata* (Wiedemann, 1824) (CC) on the parasitism of *Aganaspis pelleranoi* (Brèthes, 1924) (AP) and *Doryctobracon areolatus* (Szépligeti, 1911) (DA), native parasitoids; measured the preference and parasitic capacity of the exotic *Diachasmimorpha longicaudata* (Ashmead, 1905) (DL) on larvae of different instars of *An. fraterculus* and *C. capitata*; and, investigated the intra and interspecific competition between the parasitoids, *D. longicaudata* and *A. pelleranoi* on the host *A. fraterculus*. The bioassays were carried out with insects reared in the laboratory with controlled environmental conditions, in arenas. To evaluate the influence of the host of origin, double-choice tests were performed, offering the species of fly to parasitoids with different origins. The choice of *D. longicaudata* was evaluated using two multiple-choice tests, the first one being offered one instar at a time, with a choice between hosts, and the second was performed by offering the three instars of a single host species. The competition was evaluated in different host exposure regimes for the two species (DL and AP), being offered to only one species on a single occasion, to a same species on two occasions, or to the competing species, on two occasions, changing the order of offering. The parasitoid *A. pelleranoi* showed a preference for original host, but altered the preference after passing a generation by the alternative host and *D. areolatus* showed preference only to the host AF; the sex ratio was diverted to males in *A. pelleranoi* in both hosts and to *D. areolatus* originated in CC. In relation to the preferential instar of *D. longicaudata* in the simple choice test, a higher number of emerged parasitoids and pupae parasitized were found in first and second instar larvae of the AF host, and for CC, there was no difference in the tested instars. In the multiple

choice test, the mean number of parasitized pupae and emerged parasitoids were higher in second instar larvae for AF, and for CC, it was higher in second and third instar larvae. In the competition between *D. longicaudata* and *A. pelleranoi*, the mean numbers of parasitized pupae and emerged parasitoids were higher in the treatments in which the hosts were first offered to the DL and later to a co-specific or the AP. When the hosts were exposed only once to the parasitoids, the sex ratio was diverted to males, but when exposed twice, except for the *D. longicaudata* offspring in AP-DL, there was a larger number of females. The parasitism index was higher for DL both in the individual exposure regime and in the competition with AP, regardless of the order of the offer. There was a positive correlation between the mean number of oviposition scars and the mean number of parasitoids and emerged females. This dissertation evidences that the host of origin is important for the choice of the new host, being able to be altered in only one generation, which can facilitate the rearing in laboratory and the use in biological control programs. In addition, *D. longicaudata* does not have a preferential instar, parasitizing any of the two species tested, being able to compete with parasitoids that show specificity for initial instars. Considering the competition between *D. longicaudata* and *A. pelleranoi* it was evidenced that there could be a suppression of the native species when both parasitized the same larvae. For the implementation of biological control programs, it is important to have knowledge of the native species existing in the environment, and if there are abiotic and / or biotic barriers that can help in the division of niches, care that can help to avoid environmental imbalance, with inclusion of *D. longicaudata* in the field.

Keywords: *Aganaspis pelleranoi*, *Diachasmimorpha longicaudata*, *Doryctobracon areolatus*, tephritid parasitoids.

1 INTRODUÇÃO

1.1 Parasitoides

Os parasitoides são organismos cujas larvas se nutrem exclusivamente de outros indivíduos levando-os à morte, enquanto os adultos são de vida livre (GODFRAY, 1994). Apenas um hospedeiro é necessário para que completem seu desenvolvimento e, por vezes, alguns parasitoides se desenvolvem gregariamente sobre um mesmo indivíduo (GODFRAY, 1994). Embora o conceito de parasitoide possa variar um pouco entre os autores (EGGLETON & BELSHAW, 1992), seu modo de vida pode ser entendido como intermediário entre predador e parasita (GODFRAY, 1994). Dentre os insetos, os parasitoides correspondem a 10% das espécies (GODFRAY, 2007). A classificação mais elevada ainda é instável, mas um regime atual reconhece 11 superfamílias e 48 famílias (ZUPARKO, 2008). O parasitismo ocorre nos insetos holometábolos, o que pode sugerir um hábito de vida mais recente, enquanto espécies predadoras são encontradas em quase todas as ordens de insetos. Uma espécie parasitoide pode utilizar um grande número de espécies hospedeiras de diversas ordens (EGGLETON & BELSHAW, 1992) e esta diversidade depende do seu caráter mais generalista ou especialista.

As ordens com maior número de espécies parasitoides são Hymenoptera, Diptera, Lepidoptera, Neuroptera e Coleoptera (KALYANASUNDARAM & KAMALA, 2016). Hymenoptera inclui 280 a 320 mil espécies e destas, PENNACCHIO & STRAND (2006) estimaram que 75% são parasitoides. Acredita-se que Hymenoptera seja o único

grupo monofilético dentre as três principais ordens de parasitoides, e que surgiu a partir de ancestrais micófagos (EGGLETON & BELSHAW, 1992). Esse é também o grupo de parasitoides mais estudados, principalmente por desempenharem papel fundamental em muitos ecossistemas em termos de biodiversidade, impacto ecológico e importância econômica, como seu uso no controle biológico (VINSON, 1998; HAWKINS *et al.*, 1999; LASALLE, 2003), inclusive no Brasil (PARRA *et al.*, 2002). Na região Neotropical já foram descritas em torno de 24 mil espécies de Hymenoptera, porém acredita-se que existam mais de 80 mil (FERNÁNDEZ & SHARKEY, 2006). No Brasil, foram registradas 84 famílias de Hymenoptera, sendo que 64 delas contemplam vespas parasitoides (RAFAEL *et al.*, 2012).

A localização espacial de hospedeiros e de outros recursos do ambiente, como a disponibilidade de nutrientes, influenciam as estratégias reprodutivas e a distribuição de adultos dos parasitoides no habitat (GODFRAY, 1994) e, portanto, atuam sobre a resposta funcional. Durante o forrageamento (busca por hospedeiros) o comportamento realizado pelo parasitoide envolve algumas escolhas e decisões, baseadas na identificação dos estímulos para encontrar e reconhecer seus hospedeiros (JERVIS & KIDD, 1996; EBEN *et al.*, 2000; SILVA *et al.*, 2007; SEGURA *et al.*, 2016). Diversas hipóteses têm sido sugeridas acerca do comportamento de forrageamento, demonstrando que, frequentemente, existe uma intrincada relação tritrófica entre os insetos fitófagos, as plantas hospedeiras e os parasitoides (EBEN *et al.*, 2000; SILVA *et al.*, 2007; SEGURA *et al.*, 2016). Esse processo pode ocorrer em vários estágios, sendo comumente dividido em quatro passos: (1) localização do habitat do hospedeiro, (2) localização do hospedeiro, (3) reconhecimento, e (4) escolha do hospedeiro mais adequado para oviposição (FLANDERS, 1953). Os parasitoides definem uma estratégia de seguir primeiro as pistas de fácil detecção, mas que não levam diretamente ao hospedeiro, para

depois acompanhar sinais mais específicos, que são mais confiáveis, porém difíceis de detectar a longa distância (teoria da detecção/confiabilidade) (VET *et al.*, 1991).

A utilização dessa estratégia está vinculada ao uso de pistas químicas, que podem ser obtidas através da memória ou aprendizado (SEGURA *et al.*, 2007; TOGNON *et al.*, 2013). Além disso, a preferência dos parasitoides por hospedeiros específicos está relacionada ao seu comportamento de busca inato (VET *et al.*, 1995); no entanto, a exposição sucessiva a um determinado hospedeiro pode alterar a preferência, o que indica aprendizagem (VINSON, 1998). Em sentido amplo, aprendizagem é uma mudança de comportamento resultante de uma experiência, aumentando a confiabilidade no reconhecimento das trilhas de localização geradas pelo hospedeiro no espaço e no tempo, o que aumenta a eficiência do forrageamento (VINSON, 1998; CUNNINGHAM *et al.*, 1999; MASRY *et al.*, 2018b).

Para parasitoides, um hospedeiro individual compreende toda a sua fonte de alimento larval e pode ser de grande influência na performance do adulto. Como o desenvolvimento depende de recursos limitados (hospedeiro), a preferência em adultos e o desempenho larval devem ser correlacionados para maximizar a aptidão (HARVEY *et al.*, 2012; 2013). Outros fatores relacionados ao hospedeiro, incluindo seu tamanho, podem influenciar a adequação dos parasitoides, incluindo o número de descendentes, desenvolvimento, longevidade e a proporção entre os sexos (MESSING *et al.*, 1993; LÓPEZ *et al.*, 2009; GONÇALVES *et al.*, 2013).

Fatores bióticos e abióticos também podem interferir na relação hospedeiro-parasitoide (SIVINSKI *et al.*, 2000). Quando, por exemplo, existe uma sazonalidade marcante no clima, isso afeta o desenvolvimento e o ciclo vital dos hospedeiros e consequentemente dos parasitoides, que precisam estar sincronizados para garantir o sucesso no parasitismo (HANSON & GAULD, 1995). Por isso, fatores ambientais como a

temperatura e a umidade podem afetar vespas parasitoides de forma direta, como no desenvolvimento (CARTON & CLARET, 1982) ou indireta, alterando a adequação do hospedeiro ou a sincronização dos ciclos vitais (HANSON & GAULD, 1995; FLEURY *et al.*, 2004). Vale ressaltar que algumas espécies de parasitoides da região Neotropical possuem diapausa, que pode ser mediada por fatores ambientais, reguladores hormonais e moleculares (CARVALHO 2005; ALUJA *et al.*, 1998; OVRUSKI *et al.*, 2007; 2016), sendo necessária para que os insetos consigam superar períodos desfavoráveis (OVRUSKI *et al.*, 2016).

Durante a vida os parasitoides podem experimentar interações complexas com predadores, entomopatógenos e hiperparasitoides e outros parasitoides (BOIVIN & BRODEUR, 2006). A competição entre indivíduos da mesma espécie é conhecida como competição intraespecífica e pode ocorrer quando vários indivíduos da mesma espécie exploram ou podem explorar as mesmas fontes de recursos, às vezes ao mesmo tempo (COUCHOUX & VAN NOUHUYS, 2014). A interação entre espécies de parasitoides (competição interespecífica) ocorre entre aquelas que desenvolveram estratégias ecologicamente semelhantes (BOIVIN & BRODEUR, 2006); e nestes organismos a competição interespecífica pode desempenhar um papel importante na coexistência de espécies, na ocupação e modelagem de estruturas comunitárias (GODFRAY, 1994). Efeitos competitivos interespecíficos podem ocorrer entre parasitoides adultos (concorrência extrínseca), ou seja, interações entre fêmeas que procuram ou exploram os mesmos hospedeiros, bem como entre parasitoides imaturos (concorrência intrínseca), competição que ocorre entre as larvas que se desenvolvem no mesmo hospedeiro (DE MORAES *et al.*, 1999; WANG *et al.*, 2008). O resultado da competição extrínseca pode ser influenciado por diferenças na capacidade de dispersão do parasitoide, de reprodução, de encontro e de sincronização fenológica com o hospedeiro

(DE MORAES *et al.*, 1999; WANG *et al.*, 2008; CUSUMANO *et al.*, 2011; 2012). A competição intrínseca pode ser afetada por diferenças nas taxas de desenvolvimento dos parasitoides, no número de ovos, fase de desenvolvimento do hospedeiro, na ordem na qual a oviposição ocorre e no intervalo de tempo entre as posturas (MACKAUER, 1990; WANG & MESSING, 2002). Este tipo de competição ocorre dentro de um sistema fechado e tende a ser assimétrica e letal em parasitoides solitários, pois cada indivíduo requer posse completa do hospedeiro (WANG *et al.*, 2008).

Uma espécie de parasitoide geralmente ganha a competição intrínseca contra outra através de combate físico ou da supressão fisiológica (MACKAUER, 1990). Muitas vespas endoparasitoides possuem mandíbulas desenvolvidas ou apêndices caudais, típicos durante o primeiro ínstar, utilizados para atacar fisicamente outras larvas (LAWRENCE, 1988; MURILLO *et al.*, 2016). Já a supressão fisiológica pode resultar da liberação de substâncias no momento da oviposição, pelo ovo ou larva, inibindo o desenvolvimento de competidores (MACKAUER, 1986; SILVERS & NAPPI, 1986; VINSON & HEGAZI, 1998). Esta também pode envolver uma forma sutil de competição por recursos ou asfixia por meio de uma mudança na hemolinfa do hospedeiro, tornando inadequado o desenvolvimento de competidores mais jovens (VINSON & HEGAZI, 1998). Algumas espécies de parasitoides empregam ambos os mecanismos (SILVERS & NAPPI, 1986).

Outro fator importante é a capacidade que as fêmeas de muitas famílias de parasitoides possuem de discriminar entre hospedeiros parasitados ou não (RUSCHIONI *et al.*, 2015), em sistemas naturais, várias espécies de parasitoides geralmente atacam o mesmo hospedeiro, produzindo multiparasitismo e competição entre os imaturos (CUSUMANO *et al.*, 2011; 2012). Quando a mesma ou mais de uma espécie de parasitoide ataca um mesmo hospedeiro, pode ocorrer o auto-superparasitismo e o

superparasitismo (a fêmea coloca um ovo em um hospedeiro já parasitado) (MONTROYA *et al.*, 2000b; 2003; GONZÁLEZ *et al.*, 2014). Este fenômeno pode ser considerado natural, mesmo em espécies que demonstram capacidade inata de discriminar hospedeiros já parasitados, podendo ser considerada uma estratégia adaptativa (MONTROYA *et al.*, 2003; 2013). No superparasitismo e auto-superparasitismo pode ocorrer competição física ou fisiológica entre as larvas que compartilham o mesmo hospedeiro (MONTROYA *et al.*, 2000b). Esse comportamento pode gerar um maior número de fêmeas, sem afetar parâmetros demográficos da prole, incluindo longevidade e fecundidade (GONZÁLEZ *et al.*, 2007; MONTROYA *et al.*, 2011).

Os parasitoides himenópteros são considerados ótimos organismos modelos para diversos estudos de alocação de recursos para diferentes medidas de adaptação, como tamanho corporal e tempo de desenvolvimento (HARVEY, 2005; HARVEY *et al.*, 2012; 2013). No ambiente, os hospedeiros são encontrados em diferentes estágios e densidades, fatores que podem refletir na escolha dos parasitoides (NÚÑEZ-CAMPERO *et al.*, 2016). Além disso, sabe-se que diversas espécies de parasitoides atacam em diferentes fases da vida do hospedeiro (ex. ovo, larva) (KALYANASUNDARAM & KAMALA, 2016). A especialização em um determinado estágio, como o larval que alguns parasitoides de insetos possuem, pode estar intimamente ligada ao tamanho, idade e espécie do hospedeiro, fatores que podem vir a afetar a prole e o *fitness* dos parasitoides, sendo considerados fatores bióticos críticos (MATTIACCI & DICKE, 1995).

O componente mais comum da qualidade do hospedeiro é o seu tamanho (HARVEY *et al.*, 2012; 2013), em geral, os hospedeiros maiores possuem mais recursos e são considerados qualitativamente superiores (MATTIACCI & DICKE, 1995; OVRUSKI *et al.*, 2011).

As interações entre planta-hospedeiro-parasitoide (EBEN *et al.*, 2000; SILVA *et al.*, 2007; EITAM *et al.*, 2003; SEGURA *et al.*, 2016; MASRY *et al.*, 2018a; 2018b), hospedeiro-parasitoide (VAN NIEUWENHOVE & OVRUSKI, 2011; GONÇALVES *et al.*, 2013; 2014; OLIVEIRA *et al.*, 2014; MONTOYA *et al.*, 2011; 2018) e parasitoide-parasitoide (MONTOYA *et al.*, 2000b; 2003; 2011; 2012; 2013; 2017; 2018) são de extrema importância, pois acrescentam conhecimentos básicos e avançados sobre esses organismos que se tornam cada vez mais importantes como agentes no controle biológico. Estes estudos são um pré-requisito necessário para a seleção de inimigos naturais e para a avaliação do desempenho destes após a liberação (LUCK, 1990). Além disso, programas de controle biológico devem considerar as interações intraguildas de parasitoides, uma vez que estas podem alterar o sucesso reprodutivo das espécies e afetar a mortalidade do hospedeiro (FOLLETT *et al.*, 2000). Alguns autores argumentam que quanto mais espécies são introduzidas, maior é o efeito de reduzir a densidade das pragas (STILING & CORNELISSEN, 2005; MIRANDA *et al.*, 2015), enquanto outros sugerem que a liberação de múltiplas espécies pode prejudicar o controle biológico (MURDOCH *et al.*, 1998; DENOTH *et al.*, 2002).

Os parasitoides atuam como reguladores naturais das populações dos seus hospedeiros e, indiretamente, das plantas utilizadas como fontes de alimento (AROUCA, 2009). Este efeito regulador ocorre devido à grande diversidade de adaptações fisiológicas e comportamentais, como resultados da associação fitófago-parasitoide-planta (SOLBRIG, 1991). Desta forma, são essenciais para a manutenção da dinâmica ecológica e para a diversidade de outros organismos (LASALLE & GAULD, 1993; GRISSEL, 1999). Além disso, são indicadores de biodiversidade, pois são fáceis de coletar, atingem um largo espectro de hospedeiros herbívoros e indicam as condições das comunidades vegetais (LOCKWOOD *et al.* 1996).

1.2 Moscas-das-frutas (Diptera: Tephritidae)

As moscas-das-frutas pertencem à ordem Diptera, subordem Brachycera, família Tephritidae (MCALPINE, 1989). Tephritidae apresenta grande diversidade taxonômica e distribui-se no mundo em todas as regiões temperadas e tropicais, com exceção das áreas árticas e desérticas, onde a vida vegetal é escassa (ZUCCHI, 2000). São conhecidas 4.448 espécies e subespécies que são agrupadas em 484 gêneros, mas esse número pode ser maior, já que muitas permanecem sem descrição (NORRBOM, 2006).

As moscas-das-frutas são consideradas um dos maiores grupos de insetos fitófagos com importância econômica mundial, com expressivo impacto sobre a produção e exportação de frutas frescas (DIAS *et al.*, 2018; DOS SANTOS & GUIMARÃES, 2018). A maioria das espécies-praga da família Tephritidae, que atacam frutos no mundo, pertencem aos gêneros *Anastrepha* Schiner, 1868, *Bactrocera* Macquart, 1835, *Ceratitis* Macleay, 1829, *Dacus* Fabricius, 1805 e *Rhagoletis* Loew, 1862 (DÍAZ-FLEISCHER & ALUJA, 1999; NORRBOM, 2006; URAMOTO, 2007). Os gêneros representados por espécies economicamente importantes são classificados na subfamília Trypetinae, tribo Toxotrypanini (*Anastrepha*) e tribo Carpomyini (*Rhagoletis*), na subfamília Dacinae, tribos Ceratitidini (*Ceratitis*) e Dacini (*Bactrocera* e *Dacus*) (NORRBOM, 2006; URAMOTO, 2007).

As principais espécies de mosca-das-frutas de importância econômica e quarentenária no Brasil, pertencem a quatro gêneros: *Anastrepha*, *Bactrocera*, *Ceratitis* e *Rhagoletis* (ZUCCHI, 2000). *Anastrepha fraterculus* (Wiedemann, 1830) (mosca-das-frutas-Sul-Americana), seguida por *Ceratitis capitata* (Wiedemann, 1824) (mosca-das-frutas-do-Mediterrâneo) constituem um dos principais problemas fitossanitários da fruticultura brasileira (DA COSTA *et al.*, 2017; DOS SANTOS & GUIMARÃES, 2018). Esses

insetos-praga possuem ampla distribuição geográfica e estão associados, além de frutíferas cultivadas, a várias espécies de plantas hospedeiras espontâneas (DA COSTA *et al.*, 2017; DOS SANTOS & GUIMARÃES, 2018).

Os danos causados por moscas-das-frutas são gerados principalmente pelas larvas e fêmeas, unicamente nos frutos (MONTES, 2006). A fêmea através oviposição e, mesmo sem depositar os ovos, pode causar danos irreversíveis no epicarpo de alguns frutos, deixando cicatrizes (deformações) (MONTES, 2006; PARANHOS, 2008). O dano principal, entretanto, é produzido pelas larvas, que se alimentam do mesocarpo e pelos agentes patogênicos que atuam em consequência da lesão nos tecidos dos frutos (MONTES, 2006). Esses danos reduzem a produtividade e a qualidade dos frutos, deixando-os impróprios tanto para consumo “in natura”, como para a industrialização (PARANHOS, 2008). As perdas provocadas pelas moscas-das-frutas chegaram a 120 milhões de dólares por ano para a fruticultura brasileira e a mais de dois bilhões de dólares no mundo (DE SOUZA, 2016).

As práticas mais comuns para o controle destas pragas ainda são a utilização de inseticidas e/ou de iscas tóxicas, no entanto, há uma busca constante por alternativas, que acarretou no aumento do uso do controle biológico (DOS SANTOS & GUIMARÃES, 2018). Assim, esse método tem sido incorporado como alternativa válida dentro dos programas de manejo integrado de moscas-das-frutas (OVRUSKI *et al.*, 2000; WALDER, 2002; MALAVASI *et al.*, 2007)

Dentre os diferentes organismos que efetuam o controle biológico de moscas-das-frutas (vírus, bactérias, fungos, nematoides e insetos parasitoides ou predadores), os parasitoides da família Braconidae são os mais efetivos, com utilização prática em vários países (VAN DEN BOSCH *et al.*, 1973). Alguns exemplos bem-sucedidos do uso de parasitoides são relatados por NEWELL & HARAMOTO (1968) e WONG *et al.* (1983) no

Havaí, por BARANOWSKI *et al.* (1993) e SIVINSKI (1991) na Flórida e por LÓPEZ *et al.* (1999) e SIVINSKI *et al.* (2000), no México. Espécies da família Figitidae também já vêm sendo utilizadas como agentes de controle biológico com um grande potencial de controle (NASCA, 1973; ALUJA *et al.*, 2013; GONÇALVES, 2013; 2014).

1.3 Braconidae

O maior número de espécies de parasitoides de tefritídeos pertence à família Braconidae (WHARTON, 1997), a qual engloba cerca de 17.000 descritas e 42.653 estimadas, distribuídas pelas diversas regiões do mundo (JONES *et al.*, 2009). São endoparasitoides coinobiontes, principalmente de Coleoptera, Lepidoptera e de Diptera (principalmente de Tephritidae), as fêmeas ovipositam nas larvas de seus hospedeiros, que permanecem vivas até a fase de pupa, para o completo desenvolvimento do parasitoide (WHARTON *et al.*, 1997).

Não parecem ter preferência por regiões temperadas ou tropicais, ocorrendo nos mais variados ambientes (WAHL & SHARKEY, 1993). São insetos pequenos e ativos, a maioria com coloração preta, castanha ou alaranjada, possuem grau variável de especificidade e normalmente são associados a apenas um hospedeiro (JONES *et al.*, 2009; MARINHO *et al.*, 2018).

Em diversos países da América, o uso de parasitoides pertencentes à Braconidae tem sido intensificado devido às perdas na produção e comercialização de frutos pelos tefritídeos pragas (OVRUSKI *et al.*, 2000; GONZÁLEZ *et al.*, 2007). Da mesma forma, nas últimas duas décadas houve significativo aumento no número de trabalhos sobre braconídeos parasitoides de moscas-das-frutas, relacionados com identificação, distribuição e associação com tefritídeos e plantas hospedeiras em vários estados

brasileiros (SALLES, 1996; COSTA *et al.*, 2007; JAHNKE *et al.* 2014; OLIVEIRA *et al.*, 2014; JÚNIOR *et al.*, 2017).

Entre as espécies de Opiinae, 10 estão associadas com moscas-das-frutas que provocam danos econômicos (MARINHO *et al.*, 2018). O parasitoide exótico *Diachasmimorpha longicaudata* (Ashmead, 1905) é uma das espécies mais importantes e mais utilizada, a nível mundial, em liberações massivas para controlar moscas-das-frutas, estando adaptada a várias espécies de tefritídeos de importância econômica (SIVINSKI *et al.*, 1996; OVRUSKI *et al.*, 2000; MONTOYA *et al.*, 2000b; CARVALHO & NASCIMENTO, 2002; SCHLISERMAN *et al.*, 2003; CANCINO & MONTOYA, 2004), tem origem na região Indo-Australiana, onde parasita, pelo menos, 14 espécies do gênero *Bactrocera* (WHARTON & GILSTRAP 1983).

Diachasmimorpha longicaudata foi importado dos Estados Unidos da América em 1994 e introduzido no Brasil pela Embrapa Mandioca e Fruticultura Tropical, com o objetivo de estudar o comportamento e a eficácia deste parasitoide no controle de moscas-das-frutas, para implementação de um programa de controle biológico no nordeste brasileiro (CARVALHO & NASCIMENTO, 2002). A escolha de *D. longicaudata* para o desenvolvimento deste programa, deveu-se à facilidade de criação e multiplicação em laboratório e a pouca especificidade com o hospedeiro, podendo parasitar *C. capitata* e várias espécies de *Anastrepha* (CARVALHO & NASCIMENTO 2002). Possui eficiência de parasitismo superior a 50% e pode suprimir até 70% das populações de moscas-das-frutas no ambiente, além de ser uma das poucas espécies que parasita hospedeiros que se encontram no nível do solo (SIVINSKI *et al.*, 1996; MONTOYA *et al.*, 2000a, GARCÍA-MEDEL *et al.*, 2007).

A espécie costuma parasitar larvas de segundo e terceiro ínstar de tefritídeos (SIVINSKI *et al.*, 2001; SIME *et al.*, 2006) e sabe-se que este parasitoide pode reconhecer

se os hospedeiros estão ou não parasitados, comportamento que pode ser vantajoso para o sucesso da espécie (MONTOYA *et al.*, 2000b; 2003). Além disso, as larvas de *D. longicaudata* possuem características morfológicas como, mandíbula e cápsula cefálica, bem desenvolvidas, atributos que podem torná-lo mais competitivo (PALADINO *et al.*, 2010; MURILLO *et al.*, 2016). Outra estratégia é a diapausa, podendo ocorrer em baixas temperaturas e umidade no substrato de pupação (CARVALHO, 2005; PALADINO *et al.*, 2010). Trabalhos como de GONZÁLEZ *et al.* (2007) e MONTOYA *et al.* (2011; 2012), demonstraram que o superparasitismo moderado (2-6 cicatrizes de punctura por pupa) pode ser uma vantagem para *D. longicaudata*, gerando mais descendentes e uma prole de fêmeas maior.

Outra espécie de braconídeo que se destaca é *Doryctobracon areolatus* (Szépligeti, 1911), nativo da região Neotropical, ocorre em vários estados brasileiros e em algumas regiões neotropicais de países como, Estados Unidos da América e Argentina, parasitando larvas de Tephritidae (GARCÍA-MEDEL *et al.*, 2007; UCHÔA, 2012). Esse parasitoide exibe diapausa, o que permite expandir seu alcance para regiões com pouca diversidade de plantas (ALUJA *et al.*, 1998; EITAM *et al.*, 2004; OVRUSKI *et al.*, 2004). *Doryctobracon areolatus* tem preferência por parasitar larvas de final de segundo e início de terceiro ínstar (CARVALHO *et al.*, 2000).

É comumente encontrada no campo (CANAL DAZA & ZUCCHI, 2000), possui abundância naturalmente maior em comparação com outras espécies de parasitoides neotropicais (SCHLISERMAN *et al.*, 2016). Devido à frequência, abundância e à capacidade de parasitar larvas de moscas-das-frutas em frutos nativos e exóticos, esta espécie é bastante promissora para compor programas de controle biológico de tefritídeos em agroecossistemas (NUNES *et al.*, 2011; UCHÔA, 2012).

1.4 Figitidae

A família Figitidae é constituída por cerca de 3.000 espécies distribuídas em aproximadamente 255 gêneros (RONQUIST, 1995; GUIMARÃES *et al.*, 1999; BUFFINGTON *et al.*, 2007). Esses parasitoides apresentam uma ampla distribuição geográfica e são considerados importantes inimigos naturais de indivíduos de diferentes espécies de várias famílias de Diptera (GUIMARÃES *et al.*, 1999). Segundo GUIMARÃES *et al.* (1999), devido a essa associação desempenham função importante como inimigos naturais das moscas-das-frutas. A subfamília Eucoilinae é a maior de Figitidae e os estudos sobre este grupo, demonstram o potencial desses parasitoides em programas de controle de moscas-das-frutas (GUIMARÃES *et al.*, 1999).

No Brasil, são conhecidos aproximadamente 29 gêneros e 55 espécies de Eucoilinae, dentre as quais 11 espécies de seis gêneros são parasitoides de dípteros da superfamília Tephritoidea (GUIMARÃES *et al.*, 1999). Devido ao número reduzido de amostras de eucoilíneos examinadas no Brasil, torna-se prematuro o estabelecimento preciso da distribuição das espécies, porém é bastante provável que estes parasitoides estejam presentes em todos os locais de ocorrência dos tefritídeos (MALAVASI & ZUCCHI, 2000).

O gênero *Aganaspis* Lin, 1987 engloba seis espécies, que estão distribuídas na região Neotropical e no Indo Pacífico (DA SILVA, 2011). Destacando-se a espécie *Aganaspis pelleranoi* (Brèthes, 1924), nativa da Região Neotropical, que ocorre do México até a Argentina, atacando uma ampla variedade de hospedeiros pertencentes às famílias Tephritidae e Lonchaeidae em frutos nativos e exóticos (OVRUSKI, 1994b; GUIMARÃES *et al.*, 1999; OVRUSKI *et al.*, 2000; SIVINSKI & ALUJA, 2003). É uma das espécies mais abundante no campo, depois de *D. areolatus* (GONÇALVES *et al.*, 2014; SCHLISERMAN *et al.*, 2016), sendo as fêmeas capazes de forragear e atacar seus

hospedeiros dentro de frutos caídos, penetrando através de buracos ou rachaduras na superfície e infiltrando-se na polpa para encontrar a larva hospedeira (SIVISNKI *et al.*, 2001).

Parasitam preferencialmente larvas de terceiro ínstar de tefritídeos (OVRUSKI, 1994a; GONÇALVES *et al.*, 2013) e são capazes de reconhecer se o hospedeiro já foi ou não parasitado (RUSCHIONI *et al.*, 2015). Essa espécie também possui diapausa, que pode ocorrer em locais onde a temperatura e umidade são mais baixas, e com pouca incidência de luz (CARVALHO, 2005; OVRUSKI *et al.*, 2016). Além disso, é considerada uma espécie promissora para compor programas de controle biológico de moscas-das-frutas em agroecossistemas (NASCA, 1973; NÚÑEZ-CAMPERO *et al.*, 2014; GONÇALVES *et al.*, 2016; SCHLISERMAN *et al.*, 2016).

1.5 Objetivos

1.5.1 Objetivo Geral

- Investigar as interações entre diferentes espécies de himenópteros parasitoides e dois tefritídeos hospedeiros.

1.5.2 Objetivos Específicos

- Avaliar a influência dos hospedeiros de origem, *Anastrepha fraterculus* e *Ceratitis capitata*, na preferência e no sucesso de parasitismo de *Aganaspis pelleranoi* e *Doryctobracon areolatus*.

- Mensurar a preferência e a capacidade de parasitismo de *Diachasmimorpha longicaudata* em larvas de diferentes ínstares de *Anastrepha fraterculus* e *Ceratitis capitata*.

- Investigar a competição intra e interespecífica entre *Diachasmimorpha longicaudata* e *Aganaspis pelleranoi*, em larvas do hospedeiro *Anastrepha fraterculus*.

2 RESULTADOS GERAIS

ARTIGO I: Influence of the original host in the preference of *Aganaspis pelleranoi* and *Doryctobracon areolatus*, parasitoids of Tephritidae larvae.

⇒ Foi observado que os parasitoides *A. pelleranoi* e *D. areolatus* quando originados de *A. fraterculus* (AF) tiveram preferência por parasitar a mesma espécie hospedeira, AF. *Aganaspis pelleranoi* com origem em *Ceratitis capitata* (CC) também evidenciou preferência pelo hospedeiro no qual se originou.

⇒ A razão sexual para *A. pelleranoi* foi desviada para machos, variando de 0,11 a 0,42 dependendo da origem e do hospedeiro. Para *D. areolatus* chegou 0,50 em indivíduos originados em *C. capitata*.

⇒ De uma maneira geral, *A. fraterculus* se mostrou como um hospedeiro melhor, pois neste, para ambos os parasitoides, o número médio de insetos emergidos e de pupários parasitados foi maior.

⇒ Para *D. areolatus* o comportamento inato, relacionado ao hospedeiro original, parece ter maior influência na escolha por parasitismo do que o hospedeiro de origem do indivíduo, pelo menos em uma geração.

⇒ *Aganaspis pelleranoi* alterou sua preferência após somente uma geração desenvolvida em larvas de outra espécie, indicando aprendizagem associada ao reconhecimento do hospedeiro de origem.

ARTIGO II: Does *Diachasmimorpha longicaudata* (Ashmead, 1905) (Hymenoptera: Braconidae) have a preferential instar to parasitize Tephritidae (Diptera)?

⇒ No teste de escolha simples, *D. longicaudata* preferiu larvas de primeiro e segundo ínstar de *A. fraterculus* gerando maior número de pupários parasitados e parasitoides emergidos. Para *C. capitata* não houve diferença entre os ínstars testados.

⇒ A razão sexual foi desviada para machos para ambos os hospedeiros.

⇒ No teste de múltipla escolha, a média de pupários parasitados e de parasitoides emergidos foram maiores em larvas de segundo ínstar para AF e, para CC, a média foi maior em larvas de segundo e terceiro ínstar.

⇒ A razão sexual foi desviada para fêmeas nos tratamentos com larvas de terceiro ínstar para ambos os hospedeiros.

⇒ *Diachasmimorpha longicaudata* apresenta preferência pelo segundo e terceiro ínstar, mas é capaz de parasitar e ter sucesso em todos os ínstars de ambas às espécies de moscas.

ARTIGO III: Intra and interspecific competition between *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) and *Aganaspis pelleranoi* (Brèthes) (Hymenoptera: Figitidae).

⇒ O maior número de pupários parasitados ocorreu nos tratamentos em que o hospedeiro foi exposto duas vezes aos parasitoides nos tratamentos DL-DL, DL-AP e

AP-DL; e quando exposto somente para *Diachasmimorpha longicaudata*, no tratamento DL.

⇒ A razão sexual foi desviada para machos quando os hospedeiros foram expostos uma vez aos parasitoides, mas quando expostos duas vezes independente do tratamento, exceto DL-AP (parasitoide *Diachasmimorpha longicaudata*), foi gerada prole com mais fêmeas.

⇒ O índice de parasitismo foi de 41,2% para AP (hospedeiro exposto por quatro horas para *A. pelleranoi*); 53,7% para DL (hospedeiro exposto por 40 minutos para *D. longicaudata*), 43,5% para AP-AP (hospedeiro exposto para *A. pelleranoi* por quatro horas e em seguida para um coespecífico por mais quatro horas), 72,1% para DL-DL (hospedeiro exposto para *D. longicaudata* por 40 minutos e em seguida para um coespecífico por mais 40 minutos), para o tratamento AP-DL foram de 12,6 % (AP) e de 46% (DL) e para o tratamento DL-AP foi de 60,3% (DL) e 7,9% (AP).

⇒ Houve correlação positiva entre a média de cicatrizes de oviposição e a média de parasitoides e fêmeas emergidos, exceto para os tratamentos AP-DL e DL-AP (AP).

⇒ A espécie *D. longicaudata* mostra-se superior na competição em qualquer regime de oferecimento dos hospedeiros.

3 REFERÊNCIAS^(*)

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ARTIGO I: Influence of the original host in the preference of *Aganaspis pelleranoi* and *Doryctobracon areolatus*, parasitoids of Tephritidae larvae

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1 **Influence of the original host in the preference of *Aganaspis pelleranoi* and**
2 ***Doryctobracon areolatus*, parasitoids of Tephritidae larvae**

3
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7
8 **Abstract**

9 The study aimed to evaluate the host preference and success of parasitoids from
10 different host origins. The Tephritidae parasitoids *Aganaspis pelleranoi* (AP) and
11 *Doryctobracon areolatus* (DA), native to the Neotropical region, were evaluated.
12 Experiments were performed under laboratory conditions, in dual-choice tests, in which
13 two oviposition units, each containing 25 larvae of either *Anastrepha fraterculus* (AF)
14 or *Ceratitis capitata* (CC), were offered simultaneously to parasitoids that had emerged
15 from pupae of both species. The average number of pupae, emerged parasitoids,
16 parasitized pupae, and sex ratio of the offspring were evaluated. The average number of
17 parasitoids emerged for *A. pelleranoi* that originated from *A. fraterculus* (AP-AF) was
18 significantly higher in the host *A. fraterculus* compared with *C. capitata*. The same
19 occurred for parasitoids originated from *C. capitata* (AP-CC), parasitizing larvae of the
20 host specie *C. capitata*. The emergence rate of *D. areolatus* was higher in parasitoids
21 that originated in *A. fraterculus*, in the same host species. For *A. pelleranoi* with origin
22 in *A. fraterculus*, a higher average of parasitized pupae was observed for the host of the
23 same species. *Doryctobracon areolatus* regardless of the original host, parasitized a
24 larger number of *A. fraterculus* pupae. *Aganaspis pelleranoi* had a male-biased sex
25 ratio, ranging from 0.11 to 0.42 depending on the origin and the host. The sex ratio for

26 *D. areolatus* was 50%, only in parasitoids originated from *C. capitata* (DA-CC) and
27 having host larvae from the same species. The results for *A. pelleranoi* (AP-AF and AP-
28 CC) and *D. areolatus* (DA-AF) indicate that original host origin of female might alter
29 host preference. In addition, *C. capitata* was a less suitable host for rearing these species
30 of parasitoids.

31

32 **Keywords** *Anastrepha fraterculus*, *Ceratitis capitata*, Neotropical parasitoids,
33 preference for host.

34

35 **Introduction**

36 Parasitoid preference for specific hosts is related to innate search behaviour (Vet
37 *et al.*, 1995); however, successive exposure to a particular host can alter the preference,
38 which indicates learning (Vinson, 1998). In the broad sense, learning is a change in
39 behavior resulting from an experience, increasing the reliability in the recognition of the
40 location trails generated by the host in space and time, which increases the efficiency of
41 the foraging (Vinson, 1998; Cunningham *et al.*, 1999; Masry *et al.*, 2018b). For
42 parasitoids, an individual host comprises its entire source of larval food and can greatly
43 influence on the adult's performance. Because development depend on limited
44 resources (host), adult preference and larval performance must be correlated to
45 maximize fitness (Harvey *et al.*, 2012; Harvey, 2015). Other host-related factors
46 including its size can influence the fitness of the parasitoids including the number of
47 offspring, development, longevity, and sex ratio (Messing *et al.*, 1993; López *et al.*,
48 2009; Gonçalves *et al.*, 2013).

49 The influence of the original host on the performance of parasitoids associated
50 with fruit flies has received little study. Ero *et al.* (2010), for example, evaluated the

51 preference of *Diachasmimorpha kraussii* (Fullaway) (Hymenoptera: Braconidae) by
52 four species of the genus *Bactrocera* Macquart (Diptera: Tephritidae), but the parasitoid
53 showed no preference in both the choice test and the non-choice test. Ero *et al.* (2011)
54 studying the same parasitoid, evaluated the preference on five commercial fruit species
55 and two species of fruit flies (*Bactrocera jarvisi* (Tryon) and *B. tryoni* (Froggatt)). The
56 parasitoid responded to all infested fruits, regardless of the species of fruit fly, although
57 it did not show preference. Its offspring preferred guava (*Psidium guajava* Linnaeus),
58 peach (*Prunus persica* Linnaeus), and orange (*Citrus sinensis* Linnaeus). Ovruski *et al.*
59 (2011) evaluated the preference of the parasitoid *Diachasmimorpha longicaudata*
60 (Ashmead), originating from the Indo-Australian region, reared on *Anastrepha*
61 *fraterculus* (Wiedemann) (AF) and *Ceratitidis capitata* (Wiedemann) (CC) (Diptera:
62 Tephritidae), for the host of the same origin. The authors found no difference between
63 the two hosts in the no-choice test, but in the dual-choice test, there was higher
64 parasitism in the *A. fraterculus* larvae. Canale & Benelli (2012) evaluated if *Psytalia*
65 *concolor* (Szépligeti) created for several generations in *C. capitata* could affect the
66 location and parasitism when used against *Bactrocera oleae* (Rossi). The study did not
67 show a significant difference in oviposition behavior and host acceptance for parasitoids
68 without previous experience, but showed that the previous experience in a given host
69 can influence the choice of the female, prioritizing the host already known. Giunti *et al.*
70 (2016) evaluated if the olfactory trails of the original host could affect the preference of
71 the parasitoid *P. concolor* and if recognition of a new host could be learned during the
72 larval stages and in the initial adult stage. The authors demonstrated that there was a
73 preference for the original host in which the parasitoid developed but that females could
74 learn. *Diachasmimorpha kraussii* also showed a significant preference for fruits infested
75 by larvae of a host species, *B. tryoni* compared to fruits infested by non-host larvae,

76 *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) (Masry *et al.*, 2018a).
77 Masry *et al.* (2018b) working with the same parasitoid, parasitizing *B. tryoni* larvae in
78 nectarine (*Prunus persica* var. *nucipersica* Linnaeus) and tomato (*Solanum*
79 *lycopersicum*, var. Gourmet premium Linnaeus) fruits in an associative learning
80 experiment, in sequential studies of olfactometer, closed field and open field. The virgin
81 females showed preference for nectarines, not increased the choice after that had
82 previous training. However with the same tests, the authors observed that after
83 experience with the tomato, there was learning and the females began to recognize the
84 fruit, increasing the choice. The knowledge of these aspects is extremely important
85 when searching for a biological control agent to control fruit flies. For this *Aganaspis*
86 *pelleranoi* (Brethes) (Hymenoptera: Figitidae) (AP) and *Doryctobracon areolatus*
87 (Szepliget) (Hymenoptera: Braconidae) (DA) have a naturally higher abundance
88 compared with others Neotropical parasitoids species on the field. In addition, they are
89 parasitoids on fruit fly larvae in native and exotic fruits, which increases their chances
90 of success in parasitism (Schliserman *et al.*, 2016). They are found mainly parasitizing
91 *Anastrepha* Schiner (native to the American continent) and *Ceratitis* Macleay (from
92 Tropical Africa) (Uchôa, 2012). Both genera of fruit flies include species that cause
93 high economic damage to fruit farming, such as the *A. fraterculus* (South American fruit
94 fly) and *C. capitata* (Mediterranean fruit fly) (da Costa *et al.*, 2017; dos Santos &
95 Guimarães, 2018). Therefore, they are considered promising species for fruit fly
96 biological control programs (Nunes *et al.*, 2011; Uchôa, 2012; Gonçalves *et al.*, 2016).

97 Many aspects of the life cycle of *A. pelleranoi* have already been studied, such
98 as the description of immature stages on *A. fraterculus* and *C. capitata* (Ovruski, 1994),
99 and their mating behaviour (Ovruski & Aluja, 2002). In this species, the females exhibit
100 a significantly more diverse behavioral repertoire than other species of figitids (Aluja *et*

101 *al.*, 2009). The effect of different temperatures on egg-adult development and biological
102 parameters such as longevity and adult fertility were also evaluated (Gonçalves *et al.*,
103 2014). When reared on *A. fraterculus*, the offspring and female proportion were higher,
104 the egg-adult cycle shorter, and the survival rate higher, than when reared on *C. capitata*
105 (exotic host) (Gonçalves *et al.*, 2013).

106 The parasitoids use a wide range of host-related stimuli to find hosts, usually
107 chemical stimuli such as microhabitat, host plant, indecisive stimuli associated with host
108 presence and host-own stimuli (Godfray, 1994). *Doryctobracon areolatus* also uses
109 these chemical cues to find its host, as described by Eitam *et al.* (2003), including
110 markers of host fruits and fly larvae. The egg-adult development period, sex ratio,
111 longevity of males and females, pupal survival rate, and parasitism rates against *A.*
112 *fraterculus* have been also evaluated (Nunes *et al.*, 2011). The interspecific competition
113 has been recorded between this species and the braconid *Utetes anastrephae* (Viereck),
114 but without competitive exclusion (Aluja *et al.*, 2013). Furthermore, the natural
115 parasitism of this species in *A. fraterculus* has been registered in different fruit trees
116 (Costa *et al.*, 2007; Jahnke *et al.*, 2014; Júnior *et al.*, 2017).

117 Understanding parasitism preference is important to select a biological control
118 agent. However, the influence of their original host on the performance and preference
119 of parasitoids associated to the fruit fly has been insufficiently studied. Thus, the aim of
120 this study is to evaluate the host preference and success of the parasitoids *A. pelleranoi*
121 and *D. areolatus* in larvae of *A. fraterculus* and *C. capitata* as affected by their original
122 host.

123

124 **Materials and Methods**

125 Study site

126 The study was conducted in the Laboratory of Biology, Ecology, and Biologic
127 Control of Insects (Bioecolab), at the Federal University of Rio Grande do Sul, under
128 controlled conditions (26 ± 1 °C, $60 \pm 10\%$ RH), and a photoperiod of 14:10 (L:D).

129

130 Host rearing

131 Rearing of *A. fraterculus* (AF) and *C. capitata* (CC) was based on the
132 methodology described by Terán (1977), with adaptations. The adults of fruit flies were
133 kept in wooden cages ($45 \times 30 \times 30$ cm), covered on the sides with voile tissue and
134 front opening for manipulation (sleeve), receiving distilled water and a solid diet *ad*
135 *libitum*, which consisted of crystal sugar, hydrolyzed protein, soybean extract (3:1:1),
136 and vitamin complex, in proportion to two tablets macerated for each 250 g of diet
137 (adapted from Jaldo *et al.*, 2001). The egg-laying substrate used for *C. capitata* was a
138 yellow plastic tube (250 ml), with orifices (FAO/IAEA/USDA, 2003), and for *A.*
139 *fraterculus* the substrate was a bag as described by Meirelles *et al.* (2016). The eggs
140 were collected daily and transferred to a polystyrol tray ($23.5 \times 18 \times 1$ cm) containing
141 artificial diet (carrot, beer yeast, corn flour, sugar and distilled water) (modified from
142 Terán, 1997). After seven days, these were placed inside larger plastic trays ($51 \times 30 \times$
143 9.5 cm), with sterilized sand and covered by voile, where they remained for
144 approximately seven days for pupation. After this, the sand was sifted and the collected
145 pupae placed in plastic pots ($6.6 \times 6.6 \times 6$ cm) until emergence, under controlled
146 conditions (26 ± 1 °C, $60 \pm 10\%$ RH), and a photoperiod of 14:10 (L:D).

147

148 Parasitoids rearing

149 To rear *A. pelleranoi*, araçá fruit [*Psidium cattleianum* Sabine) (Myrtaceae)]
150 infested with *A. fraterculus* was collected from native fruit orchards at Fundação

151 Estadual de Pesquisa Agropecuária, in Taquari, RS, Brazil (29° 48' 00" S 51° 51' 35"
152 O). In the laboratory, the fruit were placed in plastic trays (51 × 30 × 9.5) on a layer of
153 sterilized sand and covered by voile. The sand was sifted after 15 days and the collected
154 pupae kept until the emergence of fruit flies or parasitoids at plastic pots (6.6 × 6.6 × 6
155 cm). Adult parasitoids were placed in wood cages (19.5 × 16.5 × 25.5 cm) and received
156 water by capillary and honey diluted in water (7:3), offered in Petri dishes with cotton
157 wicks. The third-instar larvae of the hosts *C. capitata* or *A. fraterculus* (approximately 6
158 and 9 days old, respectively) were offered daily to the parasitoids (5-15 days of life)
159 (van Nieuwenhove & Ovruski, 2011; Gonçalves *et al.*, 2014; Oliveira *et al.*, 2014). The
160 larvae were placed in oviposition units made of a plastic plate (4 cm of diameter), with a
161 border of 0.3 mm, wrapped in white voile. After six hours of exposure, the larvae were
162 placed on artificial diet in a polystyrol tray (15.5 × 15.5 × 1 cm) placed on a plastic tray
163 (41 × 28 × 7 cm) with sterilized sand covered by voile, where they remained for
164 approximately seven days for pupation. After this, the sand was sifted and the collected
165 pupae placed in plastic pots (6.6 × 6.6 × 6 cm) until emergence, under controlled
166 conditions (26 ± 1 °C, 60 ± 10% RH), and a photoperiod of 14:10 (L:D).

167 The rearing of *D. areolatus* was established with *A. fraterculus* parasitized pupae
168 provided by Embrapa Clima Temperado, Pelotas, RS, Brazil (31° 46' 19" S 52° 20' 34"
169 O). The colony was maintained under the same conditions cited for *A. pelleranoi*, in the
170 *A. fraterculus* and *C. capitata* hosts. Except the instar of the larvae offered in the
171 parasitism units for the maintenance of the breeding, following Eitam *et al.* (2003),
172 were second instar (3 days of life for *C. capitata* and 4 days of life for *A. fraterculus*),
173 and the parasitism units were exposed for eight hours (van Nieuwenhove & Ovruski,
174 2011; Gonçalves *et al.*, 2013).

175

176 Experimental design

177 The experiment was conducted in wooden cages ($15 \times 15.5 \times 20$ cm) covered by
178 voile. Each cage contained five couples of *A. pelleranoi* (AP) or *D. areolatus* (DA),
179 eight days old. The parasitoids received water and food as described previously. Within
180 each cage, oviposition units were arranged with 25 larvae of *A. fraterculus* and 25
181 larvae of *C. capitata* (from third instar larvae to *A. pelleranoi* and second instar larvae
182 to *D. areolatus*). To assess if there were preference, the following treatments were
183 adopted with the hosts AF and CC: parasitoid *A. pelleranoi* with origin from *A.*
184 *fraterculus* (AP-AF); *A. pelleranoi* with origin from *C. capitata* (AP-CC); parasitoid *D.*
185 *areolatus* with origin from *A. fraterculus* (DA-AF); and, *D. areolatus* with origin from
186 *C. capitata* (DA-CC). The experiment was conducted with 40 replicates for AP-AF and
187 AP-CC; and for DA-AF. For DA-CC 35 replicates were made (the smallest number of
188 insect replications used here is due to the small laboratory emergency of the parental
189 generation). The larvae were offered in separate oviposition units, made of a plastic
190 plate (2.7 cm in diameter), with a border of 0.2 cm, wrapped in white voile. To dispose
191 the parasitism units inside the cages, small glass jars ($2.3 \times 2.3 \times 3.8$ cm) were used as
192 carriers. The units were exposed for six hours for *A. pelleranoi* and eight for *D.*
193 *areolatus*, then returned to diet in a polystyrol tray ($15.5 \times 15.5 \times 1$ cm) placed on a
194 plastic tray ($35 \times 17.5 \times 10$ cm) with sterilized sand covered by voile, where they
195 remained for approximately five days (second instar larvae) and seven days (third instar
196 larvae) for pupation. Next, the sand was sifted and the pupae placed in plastic pots (6.6
197 $\times 6.6 \times 6$ cm) until the emergence of fruit flies or parasitoids, under the same conditions
198 as described previously.

199 In the control treatment, second and third-instar larvae were placed in
200 oviposition units, and positioned in the cages for the same period of time described

201 previously, but without parasitoids. Concomitant with the treatments, this procedure
202 was replicated during five consecutive days to verify larvae mortality, without the
203 parasitoid action.

204 We recorded the number of pupae formed, emerged parasitoids, parasitized
205 pupae (emerged parasitoids from the puparia + dissected puparia with parasitoids
206 presence) and the sex ratio of the parasitoids.

207

208 Data analysis

209 The mean values of pupae, emerged parasitoids, and parasitized pupae were
210 tested for normality by Shapiro-Wilk test. Subjected to analysis of variance, the means
211 being compared by Kruskal-Wallis, followed by a Dunn HSD post-hoc at the 5%
212 significance level, by the software BioEstat 5.0 (Ayres *et al.*, 2007). The sex ratio was
213 determined by the following equation: $sr = \text{number of females} / \text{number of females} +$
214 number of males . The χ^2 test of heterogeneity was applied to compare the sr between
215 the species. The apparent parasitism (ap) was calculated by the equation: $ap = \text{number}$
216 $\text{of emerged parasitoids} / \text{total number of emerged insects} \times 100$; and the real parasitism
217 (rp), by: $rp = \text{total number of parasitoids emerged and dissected} / \text{total number of insects}$
218 $\times 100$.

219

220 **Results**

221 *Aganaspis pelleranoi* (AP) (table 1) – Original host influenced the number of
222 emerged parasitoids, parasitized pupae, and the sex ratio at the AP-AF treatment. This
223 effect was also observed in the number of emerged parasitoids in AP-CC. The apparent
224 parasitism was 62.9% in AF hosts and 43% in CC hosts offered to parasitoids
225 originating from AF. In parasitoids originated from CC, the apparent parasitism was

226 6.7% and 9.3 % in hosts from AF and CC, respectively. The real parasitism was 64.2%
227 in AF hosts and 43.9% in CC hosts, both offered to parasitoids originated from AF. For
228 AF hosts offered to parasitoids originated from CC, the index was 15.6% and the CC
229 host, 13.5%.

230 Comparing parasitoids with the same origin (AP-AF) in relation to the two hosts
231 (CC and AF), the mean of emerged parasitoids was higher in the AF host ($H = 4.9150$;
232 $df = 1$; $P = 0.0203$). If exposed to the AP-CC treatment, the mean emergence was higher
233 in the same original host (CC) ($H = 3.2170$; $df = 1$; $P = 0.0397$). Considering the same
234 host in relation to the different origins of the parasitoids, the mean number of emerged
235 parasitoids from AF host pupa was higher in the ones exposed to the AP-AF treatment
236 ($H = 47.4457$; $df = 1$; $P < 0.0001$). Parasitism was higher in CC host offered to the
237 parasitoids of AF-AP treatment ($H = 20.2714$; $df = 1$; $P < 0.0001$). The mean number of
238 parasitized pupae, considering the emerged parasitoids plus the ones inside the dissected
239 puparia, was higher in the host AF ($H = 7.4370$; $df = 1$; $P = 0.0064$) when both hosts
240 were offered to the treatment from AP-AF. There was no difference between the
241 different hosts (AF and CC) offered for the parasitoids from AP-CC treatment ($P >$
242 0.05). *Anastrepha fraterculus* host larvae, when exposed to parasitoids from the same
243 origin, resulted in a higher average number of parasitized pupae than those offered to
244 parasitoids with origin in CC ($H = 35.3600$; $df = 1$; $P < 0.0001$). For CC host when
245 offered to parasitoids of different origins, the highest mean percentage of parasitism for
246 the AP species originated from AF host ($H = 14.3709$; $df = 1$; $P = 0.0002$).

247 The sex ratio (sr) of the offspring obtained in both specie of host larvae,
248 promoted by parasitoids originated from both treatments (AP-AF and AP-CC), was
249 male biased (more than 50% were males). Parasitoids from AP-AF treatment that
250 parasitized AF host larvae, generated a higher number of females ($\chi^2 = 58.3$; $df = 2$; $\alpha =$

251 0.05) compared to the parasitoids from the same origin, parasitizing CC host larvae. The
252 sr of the generated offspring of parasitoids from AP-CC, was superior in AF ($\chi^2 = 47.2$;
253 $df = 2$; $\alpha = 0.05$). The sr of the offspring were superior in both hosts when the
254 parasitoids from the AP-AF treatment, compared to the same host species parasitized by
255 *A. pelleranoi* with CC origin.

256 There was no significant difference in the mean number of pupae formed for
257 both hosts (AF and CC), when offered to parasitoids from the same or distinct origin.
258 The number of formed pupae also did not differ from the control on the different
259 treatments ($P > 0.05$).

260

261 *Doryctobracon areolatus* (DA) (table 2) – When the larvae of AF hosts larvae
262 were offered to DA-AF treatment, the average number of emerged parasitoids and
263 parasitized pupae was higher than CC host. Origin affected sex ratio as well: when the
264 hosts were CC, parasitized by females from DA-CC the sex ratio was higher. The
265 average number of parasitized pupae reflects the real parasitism, which was 27.8% in
266 the AF hosts and 18.6% in the CC hosts, both offered to the parasitoids with origin in
267 AF. For the hosts offered to the parasitoids originated from CC, the ratio was 8.1% in
268 the AF and 4.6% in the CC. The apparent parasitism (only emerged parasitoids) was
269 26.4% in the AF hosts and 16.5% in the CC, offered to parasitoids originated from AF.
270 Those originated from CC achieved 4.0% of parasitism, in the AF host and 2.5% in the
271 CC host.

272 For parasitoids from DA-AF treatment in relation to the different hosts, the mean
273 number of emerged parasitoids was higher in the host AF than in CC ($H = 6.1401$; $df =$
274 1 ; $P = 0.0144$). There was no difference between the average numbers from the hosts
275 AF and CC ($H = 0.3079$; $df = 1$; $P = 0.5790$) offered to DA-CC treatment. Considering

276 the AF host in relation to the different origins of parasitoids (DA-AF and DA-CC), the
277 emergence was higher when the parasitoids had the same origin of the host ($H = 9.8123$;
278 $df = 1$; $P = 0.0017$). A higher emergence was verified when the CC hosts were exposed
279 to DA-AF ($H = 5.9704$; $df = 1$; $P = 0.0473$), compared to DA-CC. The mean number of
280 parasitized pupae was higher in the host *A. fraterculus* compared to *C. capitata*,
281 regardless of the parasitoids' origins ($H = 4.1706$; $df = 1$; $P = 0.0421$, AF-DA, and $H =$
282 3.2170 ; $df = 1$; $P = 0.0341$, CC-DA). There was a higher number of parasitized pupae in
283 the host AF when exposed to the treatment DA-AF ($H = 5.2238$; $df = 1$; $P = 0.0223$),
284 compared to DA-CC treatment. The CC host also had a higher number of parasitized,
285 when exposed to the treatment DA-AF ($H = 3.2284$; $df = 1$; $P = 0.0314$), than those
286 originated from DA-CC.

287 The offspring's sex ratio was male biased, except in parasitoids emerged from
288 CC larvae offered to the same origin that generated 50% of females. Parasitoids
289 originated from DA-AF treatment, did not show sr difference, between the distinct hosts
290 (AF and CC). The DA-CC treatment generated more females when parasitizing CC (χ^2
291 $= 47.6$; $df = 2$; $\alpha = 0.05$). When compared to the same hosts offered to the parasitoids
292 with different origins, the ones exposed to DA-CC treatment had a higher sex ratio.

293 No difference was observed in the average number of formed pupae in both
294 hosts (AF and CC), when offered to DA-AF ($P > 0.05$). A difference in the average
295 number of pupae was recorded at the CC host expose to the DA-CC treatment ($H =$
296 12.1290 ; $df = 1$; $P = 0.0005$). The average values of formed pupae obtained in the
297 treatments did not differ from the control in both host species AF and CC ($P > 0.05$).

298

299 **Discussion**

300 The similarity in the number of formed pupae in the treatments in relation to the
301 control was expected, considering that both species are koinobionts (Ovruski, 1994),
302 that is, do not immediately kill or cause injury in the larval development, allowing
303 pupation before causing death. It is known that fruit fly parasitoids from the families
304 Braconidae and Figitidae only emerge in the host's pupal stage (Guimarães & Zucchi,
305 2004; Aluja *et al.*, 2013). The number of emerged parasitoids, related to the apparent
306 parasitism or the female success (Ovruski *et al.*, 2011), of *A. pelleranoi* originating from
307 CC was higher in the hosts from the same species, though overall AF was a far superior
308 host regardless of parasitoid origin. However, for *D. areolatus*, only females with origin
309 in AF had more success in hosts from the same species. Considering the real parasitism,
310 the performance of the parasitoids originated from AF was superior than the CC,
311 regardless of the host larvae.

312 The better performance of *A. pelleranoi* and *D. areolatus* in *A. fraterculus*
313 larvae, when compared to other Tephritidae hosts, was previously mentioned by
314 Gonçalves *et al.* (2013; 2016), in which the authors report that the number of offspring
315 was affected by the host species, *A. fraterculus* being the superior host. The authors
316 discuss that this occurred due to the bigger larval size of *A. fraterculus* compared to *C.*
317 *capitata*. According to Ovruski *et al.* (2004), the parasitoids of the Neotropical region
318 may not be able to parasitize the larvae of the host *C. capitata*, and when it does it
319 harms the development of their offspring. Harvey (2005) and Harvey *et al.* (2012)
320 pointed out that the diet used for the host can affect their development and survival too.
321 However, in our study, both host species were already reared for a long time with the
322 same diet and adapted very well; therefore, we believe that this would not affect our
323 results.

324 The data on *A. pelleranoi* indicate that this species may be influenced by the
325 original host, because the female displayed an oviposition preference for the Tephritidae
326 larvae (AF or CC) in which it had developed. The choice of the female for different
327 larvae can be related to variables such as perception of the fruit volatiles (host habitat)
328 and the hosts (Eben *et al.*, 2000; Silva *et al.*, 2007; Segura *et al.*, 2016). Thus, the use of
329 chemical cues can be the result of memory or learning (Segura *et al.*, 2007; Tognon *et*
330 *al.*, 2013). The learning can occur through the chemical legacy, whereby the parasitoid
331 that emerged from a specific host is able to distinguish the odor of its original host
332 (Tognon *et al.*, 2014). Thus, the learning process occurs in a different way in each
333 species. However, the data from this study was for only one generation, and it is likely
334 that behavioral changes could occur over the next generations.

335 *Anastrepha fraterculus* is the ancestral host of both species and probably there
336 was a coevolution between them, since the host *C. capitata* was recently introduced on
337 the American continent (Ovruski, *et al.*, 2004; Gonçalves *et al.*, 2013), therefore
338 preference was expected by the AF host. Nevertheless, for *A. pelleranoi*, after just one
339 generation in CC, an alteration in the preference was recorded, suggesting that this
340 species may have learning by chemical legacy (Tognon *et al.*, 2014). Canale & Benelli
341 (2012) and Giunti *et al.* (2016) working with *P. concolor*, observed that the parasitoids
342 preferred to lay their eggs and were more successful parasitizing the host where they
343 were reared. This may be due to learning the chemical signals recognition from original
344 host larvae, because according to Hopkins' host-selection principle the larval instar of
345 the parasitoids can learn from their environment and that memory is transported from
346 pre-imaginary stages to the adult (Barron, 2001; Giunti *et al.*, 2016). Masry *et al.*
347 (2018a) working with *D. kraussii* observed the preference of the parasitoid by the host
348 in which it is naturally found. The parasitoids used had experience of oviposition,

349 suggesting that these wasps learn odors specific to host-infested fruit. The authors
350 define learning as a change in behavior after an experiment, since the experiment was
351 not designed to characterize the type of learning.

352 In this study and others, the larvae were offered in oviposition units, without the
353 presence of fruit or other substrates (Carvalho *et al.*, 1998). Nevertheless, the parasitism
354 occurred, indicating that the females are able to recognize the hosts outside natural
355 conditions. This was already observed in the behavior of *Diachasmimorpha tryoni*
356 (Cameron) and *D. longicaudata* with washed or individualized larvae (Duan &
357 Messing, 2000). The latter authors also demonstrated that the species examined their
358 hosts using the ovipositor independent of the substrate type, as well as used larval
359 vibration as a guide. In the natural environment, however, the larvae are located in fruit,
360 which certainly influences the search and choice of the parasitoids. Possibly the
361 parasitoids use chemical clues as a guide, especially those in the fruit peel (Eitam *et al.*,
362 2003). According to Eitam *et al.* (2003), when the peels were removed and the pulps
363 exposed, there was a drop in the ovipositions by *D. areolatus*. Thus, the host plants
364 appear to be an important source of information for parasitoids during their search for
365 the host, and the parasitoids attracted by plants that provide nutritionally better hosts are
366 favored by natural selection (Canale, 2003; Segura *et al.*, 2016). This must be
367 considered when lab rearings are kept using hosts from artificial diets for later release in
368 the field.

369 The sex ratio of the offspring, in which the majority of the cases was male
370 biased, could be an indication that the female considered that host or the environment
371 conditions were not ideal for the parasitism (Godfray & Shimada, 1999). Despite this
372 fact, *A. pelleranoi* produced a higher number of females in the treatments whose host
373 larvae were AF compared to CC. The sex ratios obtained in this study are similar to

374 those obtained by Gonçalves *et al.* (2013), which were 0.42 in the AF host and 0.19 in
375 the CC host. Although that study did not evaluate the original host, they discuss that the
376 higher number of females in the AF host could be related to the size or chemical
377 differences in the hosts.

378 *Diachasmimorpha longicaudata* showed higher proportions of females in larvae
379 from the host AF than in CC, in which size may have favored choice for egg deposition
380 (Ovruski *et al.*, 2011). It is known that parasitoids lay eggs that result in males in
381 smaller hosts and eggs that generate females in the larger ones, selecting the better host
382 for its descendants (Godfray & Shimada, 1999). Another study found interesting results
383 for *D. longicaudata*, females that emerged from medium and large-size hosts had
384 benefits such as longer life expectancy, higher fertility and faster foraging (López *et al.*,
385 2009). For *D. areolatus*, although only in parasitoids originated from CC and having as
386 hosts larvae from the same species, the sex ratio was 50%, opposing the host size idea
387 related to the sex ratio. However, the parasitism and emergence rates were significantly
388 lower in this treatment. Therefore, the smaller sample number of the offspring may be
389 responsible for this percentage.

390 *Aganaspis pelleranoi* and *D. areolatus* are native and widely distributed in the
391 Neotropical region and are common parasitoids of *A. fraterculus* in Brazil (Canal &
392 Zucchi, 2000; Schliserman *et al.*, 2016). Therefore, it is presumed coevolution between
393 the species occurred, which could explain the more effective response to this host
394 compared to the exotic *C. capitata*. Another factor that could influence the parasitoids
395 during the choice experiment is the larval size. Because many studies have
396 demonstrated that the host size can ensure benefits to the offspring (López *et al.*, 2009;
397 Gonçalves *et al.*, 2013; 2016), and as demonstrated in our study, *C. capitata* is not a
398 good host for the rearing of these parasitoids, because it generates fewer offspring and a

399 sexual ratio almost always with fewer females. This offspring originating from larger
400 hosts may be predisposed to have greater reproductive success, as was observed in
401 parasitoids originated from *A. fraterculus*, which demonstrated more success in the
402 parasitism for both hosts (AF and CC).

403

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414

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613 **Table 1** - Average number (\pm SE) of pupae, emerged parasitoids, parasitized pupae^(*),
 614 and sex ratio of *Aganaspis pelleranoi* originating from *Anastrepha fraterculus* (AP-AF)
 615 and *Ceratitis capitata* (AP-CC), in *A. fraterculus* and *C. capitata*. (N = Number of
 616 larvae per replicate).
 617

Host	Origin: <i>Anastrepha fraterculus</i>		Origin: <i>Ceratitis capitata</i>	
	<i>Anastrepha fraterculus</i>	<i>Ceratitis capitata</i>	<i>Anastrepha fraterculus</i>	<i>Ceratitis capitata</i>
	N = 25	N = 25	N = 25	N = 25
Pupae	23.9 \pm 0.21 Aa	23.9 \pm 0.45 Aa	23.5 \pm 0.28 Aa	24.1 \pm 0.26 Aa
Emerged Parasitoids	9.8 \pm 0.90 Aa	7.5 \pm 0.94 Ba	0.9 \pm 0.19 Bb	1.8 \pm 0.44 Ab
Parasitized pupae^(*)	12.2 \pm 1.07 Aa	8.1 \pm 0.96 Ba	2.8 \pm 0.48 Ab	3.0 \pm 0.60 Ab
Sex Ratio	0.42 Aa	0.21 Ba	0.37 Ab	0.11 Bb

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 619 Upper case letters compare parasitoids from the same origin in the different hosts.
 620 Lowercase letters compare parasitoids from different origins to the same host. Using the
 621 Kruskal-Wallis test, followed by Dunn ($P < 0.05$). Sex ratio, tested by χ^2 for
 622 heterogeneity. (*) parasitoids emerged from the puparia + puparia dissected with
 623 parasitoids presence.
 624
 625
 626

627 **Table 2** - Average number (\pm SE) of pupae, emerged parasitoids, parasitized pupae (*),
 628 and sex ratio of *Doryctobracon areolatus* originating from *Anastrepha fraterculus* (DA-
 629 AF) and *Ceratitis capitata* (DA-CC), in *A. fraterculus* and *C. capitata*. (N = Number of
 630 larvae per replicate).

Hosts	Origin: <i>Anastrepha fraterculus</i>		Origin: <i>Ceratitis capitata</i>	
	<i>Anastrepha fraterculus</i>	<i>Ceratitis capitata</i>	<i>Anastrepha fraterculus</i>	<i>Ceratitis capitata</i>
	N = 25	N = 25	N = 25	N = 25
Pupae	18.7 \pm 0.70 Aa	18.0 \pm 0.97 Aa	18.7 \pm 0.76 Aa	12.9 \pm 1.18 Bb
Emerged parasitoids	4.2 \pm 0.89 Aa	2.4 \pm 0.73 Ba	0.4 \pm 0.16 Ab	0.2 \pm 0.09 Ab
Parasitized pupae^(*)	4.8 \pm 0.96 Aa	2.9 \pm 0.79 Ba	1.2 \pm 0.32 Ab	0.4 \pm 0.14 Bb
Sex ratio	0.30 Ab	0.34 Ab	0.38 Ba	0.50 Aa

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632 Upper case letters compare parasitoids from the same origin in the different hosts.

633 Lowercase letters compare parasitoids from different origins to the same host. Using the

634 Kruskal-Wallis test, followed by Dunn ($P < 0.05$). Sex ratio, tested by χ^2 for

635 heterogeneity. (*) parasitoids emerged from the puparia + puparia dissected with

636 parasitoids presence.

ARTIGO II: Does *Diachasmimorpha longicaudata* (Ashmead, 1905)
(Hymenoptera: Braconidae) have a preferential instar to parasitize Tephritidae
(Diptera)? (*)

(*) Manuscrito aceito para publicação em Fevereiro de 2019, pelo periódico Iheringia: Série Zoologia – Normas no Anexo 1.

Does *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) have a preferential instar to parasitize Tephritidae (Diptera)?

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ABSTRACT. *Diachasmimorpha longicaudata* (Ashmead, 1905) is a koinobiont parasitoid of Tephritidae larvae, the third instar larvae of which is considered preferential, but it is able to parasitize other larval stages and compete with native parasitoids. This study investigated the preference and parasitism capacity of *D. longicaudata* in larvae of different instar of *Anastrepha fraterculus* (Wiedemann, 1830) (AF) and *Ceratitis capitata* (Wiedemann, 1824) (CC). The experiments were carried out under laboratory conditions, one instar being offered at a time in parasitism units, with the following choices among the hosts: 25 AF larvae and 25 CC larvae (first, second and third instar were evaluated). The other test was a multiple-choice in relation to the instar, for larvae of the same host species, with three parasitism units being offered, with 15 larvae of each instar. The mean number of formed pupae, emerged parasitoids, parasitized pupae, unviable pupae and sex ratio were evaluated. In the first bioassay, the mean number of emerged parasitoids and parasitized pupae in the AF host were significantly higher in treatments with first and second instar larvae. For CC there was no difference between the instars tested. In the second bioassay, the mean value of emerged parasitoids and parasitized pupae, was higher in second and third instar larvae for CC, and for

AF was in second instar larvae. The sex ratio was biased for males in all treatments in both bioassays. The results show that *D. longicaudata* can parasitize and be successful in all available larval instars, being able to compete with parasitoids of any instar.

KEYWORDS. *Anastrepha fraterculus*, *Ceratitis capitata*, exotic parasitoid, tephritids.

RESUMO. *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) tem um ínstar preferencial para parasitar Tephritidae (Diptera)? *Diachasmimorpha longicaudata* (Ashmead, 1905) é um parasitoide coinobionte de larvas de Tephritidae sendo que o terceiro ínstar larval é tido como o preferencial, mas pode parasitar outros estágios larvais e competir com os parasitoides nativos. Este estudo investigou a preferência e capacidade de parasitismo de *D. longicaudata* em larvas de diferentes ínstaes de *Anastrepha fraterculus* (Wiedemann, 1830) (AF) e *Ceratitis capitata* (Wiedemann, 1824) (CC). Os experimentos foram realizados em condições laboratoriais, sendo oferecido um ínstar por vez em unidades de parasitismo, havendo escolha entre os hospedeiros: 25 larvas de AF e 25 larvas de CC (foram avaliadas larvas de primeiro, segundo e terceiro ínstar). O outro teste foi de múltipla escolha em relação ao ínstar, para larvas da mesma espécie hospedeira, sendo oferecidas três unidades de parasitismo, com 15 larvas de cada ínstar. Avaliou-se o número médio de pupários formados, parasitoides emergidos, pupários parasitados, pupas inviáveis e razão sexual. No primeiro bioensaio o número médio de parasitoides emergidos e pupários parasitados no hospedeiro AF foram significativamente superiores nos tratamentos com larvas de primeiro e segundo ínstar. Para CC não houve diferença entre os ínstaes testados. No segundo bioensaio, o valor médio de parasitoides emergidos e de pupas parasitadas foi maior nas larvas de segundo e terceiro ínstar para CC, e para AF nas larvas de segundo ínstar. A razão sexual foi desviada para machos em todos os tratamentos, nos dois bioensaios. Os resultados demostram que *D.*

longicaudata pode parasitar e ter sucesso em qualquer ínstar larval disponível, podendo competir com parasitoides de qualquer ínstar.

PALAVRAS-CHAVE. *Anastrepha fraterculus*, *Ceratitis capitata*, tefritídeos, parasitoide exótico.

Diachasmimorpha longicaudata (Ashmead, 1905) is a solitary, koinobiont, endoparasitoid from the Indo-Australian region, where it parasitizes at least 14 species of *Bactrocera* Macquart, 1835 (Diptera: Tephritidae) (WHARTON & GILSTRAP, 1983). It is widely used as a biological control agent worldwide for parasitizing species of tephritids (MONTROYA *et al.*, 2000; DEVESCOVI *et al.*, 2017). It can be easily reared in laboratory conditions and it has a low specificity for hosts, being able to parasitize *Ceratitis capitata* (Wiedemann, 1824) and several species of *Anastrepha* Schiner, 1868 (Diptera: Tephritidae) (CARVALHO & NASCIMENTO, 2002). It shows parasitism ability greater than 50% and can suppress up to 70% of the fruit fly populations in natural environment (SIVINSKI *et al.*, 1996; MONTROYA *et al.*, 2000).

Diachasmimorpha longicaudata usually parasitizes second and third instar of tephritids larvae (SIVINSKI *et al.*, 2001; SIME *et al.*, 2006), although there are records of preference for third instar and pupae (CARVALHO, 2005b; OVRUSKI *et al.*, 2011; MONTROYA *et al.*, 2017). Due to these specificities, the research groups that advocate this species release to biocontrol fruit flies argue that this parasitoid would not compete for oviposition sites with other species, especially the native braconid *Doryctobracon areolatus* (Szépligeti, 1911) (Hymenoptera: Braconidae), which has a preference for larvae in early stages of development (MATRANGOLO *et al.*, 1998; CARVALHO *et al.*, 2000; PARANHOS *et al.*, 2013). Nevertheless, MURILLO *et al.* (2015) verified that *D. areolatus* can also parasitize larvae of up to the third instar, which brings the niches of these species even closer. *Diachasmimorpha longicaudata*

was imported from the United States of America in 1994 and introduced in Brazil by *Embrapa Mandioca e Fruticultura Tropical*, with the aim of studying its behavior and effectiveness to control fruit fly, aiming the implementation of a biological control program, started in Northeast Brazil (CARVALHO & NASCIMENTO, 2002). However, evaluations carried out a few years after their release showed that there were alterations in the presence of native parasitoid species and suggested the existence of interspecific competition in oviposition sites (CARVALHO, 2005a). On the other hand, MEIRELLES *et al.* (2016), after release *D. longicaudata* in Rio Grande do Sul field, did not detect a negative impact on native parasitoid populations. Despite parasitizing preferentially third instar larvae (MONTROYA *et al.*, 2018), we affirm that *D. longicaudata* is able of parasitizing and succeeding in any instar, differing from that generally described. The interaction between multiple species of parasitoids in the environment is not fully understood, and the release of *D. longicaudata* may be controversial. Thus, this work aimed to investigate the preference and parasitism capacity of *D. longicaudata* in larvae of native *Anastrepha fraterculus* (Wiedemann, 1830) and exotic *C. capitata* from different instars.

MATERIAL AND METHODS

Study site. The study was conducted at the Laboratory of Biology, Ecology and Biological Control of Insects (Bioecolab), at the *Universidade Federal do Rio Grande do Sul*, under controlled conditions of 26 ± 1 °C, $60 \pm 10\%$ RH, with 14 hours of photophase.

Host rearing. The adults of *A. fraterculus* and *C. capitata* were kept in wooden cages (45 x 30 x 30 cm), covered on the sides with voile fabric, receiving distilled water and a solid diet on an *ad libitum* basis, which consisted of crystal sugar, hydrolyzed protein, soybean extract (3:1:1) and vitamin complex (Lavitan – A-Z[®]), in the ratio of two macerated tablets

per 250 g of diet (adapted from *Jaldo et al.*, 2001). As an oviposition substrate for *C. capitata*, a 250 ml yellow plastic tube with small perforations (FAO/IAEA/USDA, 2003) was used. For *A. fraterculus*, the substrate used was a blue tissue bag covered with silicone, as described in MEIRELLES *et al.* (2016). The eggs were collected daily and placed on polystyrene trays (23.5 x 18 x 1 cm), with an artificial diet based on organic carrot, beer yeast, corn flour, sugar, distilled water, sodium benzoate (Dinâmica[®]), nipagin (Synth[®]) and citric acid (Synth[®]) (modified from *Terán*, 1977). After seven days, these were placed inside larger plastic trays (51 x 30 x 9.5 cm), with sterile sand and covered by organza, where they remained for approximately seven days for the pupation. Subsequently, the sand was sifted and the pupae obtained were placed in plastic containers (6.6 x 6.6 x 6 cm) until emergence.

Parasitoids rearing. The rearing has started from the parasitized pupae of *A. fraterculus*, from *Embrapa Clima Temperado*, Pelotas, RS, Brazil. The adults were kept in wooden cages (19.5 x 16.5 x 25.5 cm), covered with organza material and fed with honey dissolved in water (7:3), offered in Petri dishes (5 x 5 x 1.5 cm) with cotton, water was provide by capillarity through a strip of Spontex Resist[®] fabric. Third instar *C. capitata* larvae were placed in parasitism units, which consisted of a circular plastic plate (4 cm in diameter), with a 0.3 cm border, formed by a small layer of silicone, wrapped with white organza fabric stuck with a rubber band. After one hour of exposure, the larvae were returned to the artificial diet in polystyrene trays (15.5 x 15.5 x 1 cm) and stored in plastic trays (41 x 28 x 7 cm) on a layer of sand sterilized until the pupae formation. After five days, the sand was sifted, and the pupae were packaged in the same manner as for fly breeding, waiting for parasitoids emergence that were reintroduced to the breeding in new cages.

Parasitism in different instars between two host species. The females preference was evaluated by concomitantly offering 25 larvae of *A. fraterculus* (AF) and 25 larvae of *C. capitata* (CC) to five couples of parasitoids (eight days old). First, second and third instar

larvae of the two host species were evaluated. The larvae were offered daily for five days, completing 60 replicates and totaling 1,500 larvae evaluated by treatment. The couples were kept in wooden cages (15 x 15.5 x 20 cm), covered with organza, offered water and food. The larvae were offered in parasitism units, consisting of a circular plastic plate (2.7 cm in diameter), with a border of 0.2 cm, formed by a small layer of silicone and encased in white voile, trapped with an elastic band, disposed on pots with 3.8 cm in height as support. The units were exposed for eight hours, and the larvae were then returned to the artificial diet in polystyrene trays and placed in plastic containers (35 x 17.5 x 10 cm) on a layer of sand until pupa formation.

In order to evaluate larval mortality without action of parasitoids (control treatment) 25 larvae of *A. fraterculus* and *C. capitata* (total of 50 larvae per cage) were placed in parasitism units and these remained in cages for eight hours without parasitoids presence. Following that, the larvae were kept in the same manner as described for breeding.

Multiple-choice parasitism test with different larval instars of the same host. The preference of *D. longicaudata* females was evaluated in cages as described previously with three parasitism units containing 15 larvae of first, second and third instar (total of 45 larvae per replicate) of one host species – AF or CC – to five couples of parasitoids (eight days old). The larvae were offered daily for five days, totalizing 30 replicates and 1,350 larvae evaluated. The units remained exposed for eight hours, and the larvae were then conditioned as described previously.

To evaluate larval mortality, without action of the parasitoids (control treatment), 15 instar larvae each, totalizing 45 larvae per cage, of *A. fraterculus* or *C. capitata* were placed in parasitism units and kept in the cages for the same time as described above, but without the presence of parasitoids.

For both bioassays, after five days, the sand was sifted and the pupae packed in plastic pots until the emergence of flies or parasitoids. The pupae of which there was no emergence were dissected for check the presence of parasitoids or flies. The mean numbers of formed pupae were recorded, as well as parasitized pupae (emerged parasitoids + pupae dissected with parasitoids), emerged parasitoids, unviable pupae [number of offered larvae - (number of flies emerged + emerged parasitoids)], sex ratio of parasitoids, and parasitism rate.

Statistical analysis. The mean values were analyzed for normality by the Shapiro-Wilk test and submitted to analysis of variance, the means being compared by ANOVA, followed by the Tukey test, with a significance level of 5%.

The sex ratio (Rs) was estimated using the formula: $Rs = \text{number of females} / \text{number of females} + \text{number of males}$. The Chi-square (χ^2) of heterogeneity was used to compare Rs between treatments. The parasitism index was calculated using the formula: $IP = \text{number of emerged parasitoids} / \text{number of pupae formed} \times 100$. The tests were performed using the BioEstat 5.0 software (Ayres *et al.*, 2007).

RESULTS

Multiple-choice parasitism in different instars between two host species

Anastrepha fraterculus. The mean number of parasitized pupae and emerged parasitoids was significantly higher ($F = 30.5686$; $df = 2$; $p < 0.0001$, $F = 35.4343$; $df = 2$; $p < 0.0001$, respectively) in larvae of first and second instars when compared to third instar larvae (Fig. 1) (Tab. I). The parasitism rate was 73.8, 74 and 34% in first, second and third instar larvae, respectively.

The mean value (\pm SE) of pupae formed in control treatment (without presence of parasitoids) was 21.0 ± 0.79 (first instar), 18.6 ± 2.10 (second instar) and 23.2 ± 1.05 (third

instar) and they were not significantly different ($p > 0.05$) from those that had the presence of parasitoids. The mean number of pupae, when parasitoids were present, was higher in the third instar only when compared to the first ($F = 6.1750$; $df = 2$; $p = 0.0030$) (Tab. I). In the presence of parasitoids, the mean number of unviable pupae was higher when the third instar larvae were exposed ($F = 35.7765$; $df = 2$; $p < 0.0001$), compared to the other two treatments (Tab. I). The mean (\pm SE) of unviable pupae in the control was 6.0 ± 1.05 ; 8.6 ± 2.09 and 4.8 ± 1.42 , respectively, for the first, second and third instars, lower when compared to larvae of first and third instars that were exposed to parasitoids ($F = 5.6303$; $df = 1$, $p = 0.0194$, $F = 44.3177$, $df = 1$, $p < 0.0001$, respectively).

The sex ratio of the offspring was higher in larvae that were exposed to parasitism in the second instar ($\chi^2 = 20.6$; $df = 5$; $\alpha = 0.05$). In all treatments, there was a higher number of males (Tab. I).

Ceratitis capitata. The mean number of parasitized pupae and emerged parasitoids was not significantly different between treatments ($p > 0.05$) (Fig. 1) (Tab. I). The parasitism rate was 72.4% in first instar larvae and 71.3% and 71.1% in second and third instar larvae, respectively.

The mean value (\pm SE) of pupae formed in the control was 19.8 ± 2.01 in the first instar, 21.2 ± 0.55 in the second instar and 21.7 ± 0.63 in the third instar, was not significantly different among treatments ($p > 0.05$) from those that had the parasitoids presence. In the treatments with the parasitoids presence, the average pupae formed was higher in third instar larvae ($F = 10.0897$; $df = 2$; $p = 0.0002$) (Tab. I). In the treatments with the presence of parasitoids, there was no difference between the instars in the mean number of unviable pupae ($p > 0.05$) (Tab. I). In control, the mean numbers (\pm SE) were 7.4 ± 1.77 ; 7.4 ± 1.44 and 5.9 ± 1.95 , respectively, for the first, second and third instars. There was no difference between treatments with parasitoids and their respective controls on larvae of the

first and third instars ($p > 0.05$). The third instar had fewer unviable pupae in the control when compared to the treatment with parasitoids ($F = 4.1045$; $df = 1$; $p = 0.0440$).

The sex ratio of the offspring was higher in larvae exposed to parasitism in the second instar ($\chi^2 = 13.4$; $df = 5$; $\alpha = 0.05$). In all treatments, there were a higher number of males (Tab. I).

Host preference. When comparing the same instar between the two host species, the mean number of parasitized pupae ($F = 32.9505$; $df = 1$; $p < 0.0001$) (Tab. I) and emerged parasitoids ($F = 38.7731$; $df = 1$; $p < 0.0001$) was higher in *C. capitata* (CC) in the third instar (Fig. 1). Regarding the mean number of pupae formed, there was no difference between AF and CC in all treatments ($p > 0.05$). The mean number of unviable pupae was significantly higher only in the third instar of CC ($F = 59.1417$; $df = 1$; $p < 0.0001$). The sex ratio was always higher in the host AF (Tab. I), regardless of the instar in which the larvae were exposed ($\chi^2 = 22.7$; $df = 2$; $\alpha = 0.05$ – first instar larvae; $\chi^2 = 23.0$; $df = 2$; $\alpha = 0.05$ – second instar larvae, and $\chi^2 = 24.2$; $df = 2$; $\alpha = 0.05$ – third instar larvae).

Multiple-choice parasitism test with different larval instars of the same host

Anastrepha fraterculus. The mean number of parasitized pupae and emerged parasitoids was significantly higher ($F = 9.3968$; $df = 2$; $p = 0.0004$, $F = 9.3969$; $df = 2$; $p = 0.0004$, respectively) in second instar larvae (Tab. II). The parasitism rate was 66.9%, 86.9% and 60.3% in first, second and third instar larvae, respectively.

The mean value (\pm SE) of pupae formed in the control was 13.2 ± 0.86 ; 14.0 ± 0.31 and 14.8 ± 0.20 for first, second and third instar larvae, respectively. The control was not significantly different from the others treatments with parasitoids presence ($p > 0.05$). There was also no difference in the mean of puparia formed between the instars with the presence of parasitoids ($p > 0.05$) (Tab. II). In the treatments with parasitoids, the mean number of

unviable pupae was higher in those exposed in the third instar ($F = 9.4386$; $df = 2$; $p = 0.0004$) (Tab. II). In the control, the mean value (\pm SE) were of 2.6 ± 0.67 ; 2.0 ± 0.83 and 0.6 ± 0.4 for the first, second and third instars, respectively, being lower than treatments with parasitoids only in the third instar ($F = 23.2425$; $df = 1$; $p = 0.0001$).

The sex ratio of offspring generated was higher in third instar larvae, with more females emerged ($\chi^2 = 47.9$; $df = 5$; $\alpha = 0.05$) (Tab. II).

Ceratitis capitata. The mean number of parasitized pupae and emerged parasitoids ($F = 16.6636$; $df = 2$; $p < 0.0001$; $F = 16.36637$; $df = 2$; $p < 0.0001$, respectively) was higher in the second and third instars (Tab. II). The parasitism rate was 78.2% for first instar larvae and 85.9% and 79.9% for second and third instar larvae, respectively.

The mean values (\pm SE) of pupae formed in the control was 12.6 ± 0.74 ; 14.8 ± 0.20 and 14.6 ± 0.24 for first, second and third instar larvae, respectively. There was no difference between control and treatments parasitoids presence ($p > 0.05$). In the treatments with parasitoids, the second and third instars were the ones with the highest mean number of pupae formed ($F = 41.3569$; $df = 2$; $p < 0.0001$) (Tab. II). The mean number of unviable pupae in tests with parasitoids presence was higher in first instar larvae ($F = 8.2180$; $df = 2$; $p = 0.0008$) (Tab. II). The control had the mean values (\pm SE) of 2.4 ± 0.74 ; 0.2 ± 0.2 and 0.4 ± 0.24 for the first, second and third instars, respectively. All treatments that had the presence of parasitoids had a higher mean number of unviable pupae, when compared to their controls ($F = 6.7424$; $df = 1$; $p = 0.0134$ for first, $F = 5.6186$; $df = 1$; $p = 0.0224$ for second, and $F = 5.0216$; $df = 1$; $p = 0.0301$ for third instar).

The sex ratio of offspring generated was higher in larvae exposed in the third instar, with more females emerged ($\chi^2 = 64.4$; $df = 5$; $\alpha = 0.05$) (Tab. II).

DISCUSSION

The lack of difference in pupae number formed between treatments, even with the presence of parasitoids, is expected, considering that *D. longicaudata* is a koinobiont parasitoid (OVRUSKI *et al.*, 2000), that does not kill the larvae of its hosts immediately, allowing them to finish their development and pupate before causing death. This is known for Braconidae fruit fly parasitoids that emerge only at the pupal stage (OVRUSKI *et al.*, 2000; 2003). The higher mortality in some treatments, when compared to the control in this experiment, may be due to the stress caused to the larvae by parasitism, test punctures or even by superparasitism (OVRUSKI *et al.*, 2011; HARBI *et al.*, 2018). In our study, when only one instar was offered, *D. longicaudata* efficiently parasitized larvae of both the first and second instars of *A. fraterculus*, showing that their response may be conditioned to the environment, differing from other studies that registered their preference for the late larval stages (OVRUSKI *et al.*, 2011; VAN NIEUWENHOVE & OVRUSKI, 2011; MONTOYA *et al.*, 2017). In addition, *D. longicaudata* showed no instar preference in *C. capitata* larvae when exposed only one at a time. On the other hand, when the three instars were offered concomitantly, the highest parasitism was in the second and third instar. In general, parasitoids usually to have a preferential or single instar to parasitize, as seek to specialize in relation to the species they use as hosts and can be specialize in certain stages thereof (MATTIACCI & DIKE, 1995; MONTOYA *et al.*, 2018). In the case of *D. longicaudata*, there are records that it is able to parasitize the second and third instars (SIVINSKI *et al.*, 2001; SIME *et al.*, 2006). Additionally, this species has been shown a broad plasticity, adapting easily to environmental conditions (CARVALHO & NASCIMENTO, 2002).

When the three larval instars of *A. fraterculus* were exposed simultaneously, the second instar was preferred, differing from the studies that suggested the third as preferential

(OVRUSKI *et al.*, 2011; VAN NIEUWENHOVE & OVRUSKI, 2011; MONTOYA *et al.*, 2017). The interaction between *D. longicaudata* and *A. fraterculus* can be considered as a “new association”, as they do not share an intense history of coevolution, a factor that may influence the parasitoid-host relationship (HOKKANEN & PIMENTEL, 1989), and even change the parasitoid’s preferences for the parasite. The fact that *A. fraterculus* larvae are larger than *C. capitata* (MEIRELLES *et al.*, 2013; OLIVEIRA *et al.*, 2014; SÁ *et al.*, 2018) or those of many *Bactrocera* species (MAU & KESSING, 1992; THOMAS *et al.*, 2001; SINGH *et al.*, 2010), their original hosts, may cause the *D. longicaudata* to parasitize also the first instars of the South American fruit fly, recognizing the youngest larvae as appropriate for their development, with sufficient nutritional quality and quantity to meet their needs, opposing previous studies (LÓPEZ *et al.*, 2009; HARVEY *et al.*, 2012).

In the environment, hosts can be found at different stages and densities inside the fruits, which may reflect parasitoid choices (NÚÑEZ-CAMPERO *et al.*, 2016). Thus, there is no ensure that *D. longicaudata* will not compete for the same oviposition niche of the native parasitoids. For parasitoids, a single host comprises its entire source of larval food and can have great influence on the adult’s fitness. In general, larger hosts have more qualitative resources to supply parasitoid fitness (MATTIACCI & DICKE, 1995; OVRUSKI *et al.*, 2011; HARVEY *et al.*, 2012). This apparently did not influence in *D. longicaudata* choice in our study, being effective even in first and second instar larvae. In this case, possibly even smaller larvae can guarantee the quantity and nutritional quality for *D. longicaudata* development, as their hosts were originally species of *Bractocera* (WHARTON & GILSTRAP, 1983), smaller than those tested in this study (SINGH & RAMAMURTHY, 2010).

The sex ratio of *D. longicaudata* offspring grown in both *A. fraterculus* and *C. capitata* was biased for males, indicating that host or environmental conditions may not have been proper for the parasitoid (GODFRAY, 1994). When different instars of the same host

species were offered simultaneously, a larger number of females emerged in second and third instar larvae, respectively. The data found in our study corroborate the records that Tephritidae parasitoids that parasitize larvae in later stages tend to produce a larger number of females (EBEN *et al.*, 2000; OVRUSKI *et al.*, 2011; VAN NIEUWENHOVE & OVRUSKI, 2011). On the other hand, MONTOYA *et al.* (2011, 2012) argue that larval size influences superparasitism, which, in turn, influences the sexual ratio of *D. longicaudata*. When moderate superparasitism occurs (2-6 scars per pupa), there is a trend of female emergence, with no detrimental effects on the demographic parameter to offspring, including longevity and fecundity (GONZÁLEZ *et al.*, 2007; MONTOYA *et al.*, 2011; 2012). It is possible that this occurred in our study on the second bioassay, although we did not record the number of scars left on the larvae, given that it could help to evaluate superparasitism and corroborate this hypothesis.

When the hosts *A. fraterculus* and *C. capitata* were exposed simultaneously, we observed that in *A. fraterculus* there was a higher proportion of females. In relation to the emergence of parasitoids and mortality, however, both had similar means, except for third instar larvae of *A. fraterculus*, with a higher mean number of unviable pupae and lower number of emerged parasitoids. Although *C. capitata* has been used for a long time in rearing of *D. longicaudata* in several places of the world, *A. fraterculus* has already been used, showing a good performance as a host (MESSING *et al.*, 1993; VAN NIEUWENHOVE & OVRUSKI, 2011, MEIRELLES *et al.*, 2016; HARBI *et al.*, 2018), and our study confirms this data. This aspect is important in mass rearing since studies such as those by SEGURA *et al.* (2007) and TOGNON *et al.* (2013) have demonstrated that parasitoids that are reared in a given host are easier to recognize through chemical tracks, obtained by memory or learning, which would provide greater efficiency in the control of the target pest (MATTIACCI & DICKE, 1995; EBEN *et al.*, 2000).

Our study demonstrates the plasticity of *D. longicaudata* at the moment of host selection, and that it can be considered a good competitor. It is important that *D. longicaudata* coexist with other parasitoids, not leading their populations to decline. Therefore, before releasing exotic wasps species, it is important to know how they respond (behavior) in the field. Other factors such as biotic and abiotic conditions (SIVINSKI *et al.*, 2000), chemical tracks of plants (EITAM *et al.*, 2003; SILVA *et al.*, 2007; SEGURA *et al.*, 2016) and patch isolation (EITAM *et al.*, 2004) may also interfere in search and parasitism. Considering that not all environments have abiotic and biotic barriers, which may help in the niches division, and that *D. longicaudata* is a competitive species, easily parasitizing any instar, its introduction into new environments should be well evaluated, so as not to cause suppression of other species and a subsequent imbalance in the environment.

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Table 1 – Mean number (\pm SE) of formed pupae, parasitized pupae, unviable pupae and sex ratio of hosts *Anastrepha fraterculus* (AF) and *Ceratitis capitata* (CC), exposed to parasitism by *Diachasmimorpha longicaudata* on first, second and third-instar larvae.

Variables evaluated	Instar					
	First		Second		Third	
	AF	CC	AF	CC	AF	CC
Formed pupae	17.9 \pm 0.80 bA	18.2 \pm 0.65 bA	19.6 \pm 0.44 abA	18.5 \pm 0.56 bA	21.4 \pm 0.78 aA	21.7 \pm 0.63 aA
Parasitized pupae ⁽¹⁾	13.3 \pm 0.68 aA	13.2 \pm 0.70 aA	14.6 \pm 0.60 aA	13.3 \pm 0.61 aA	7.9 \pm 0.65 bB	13.4 \pm 0.71 aA
Unviable pupae ⁽²⁾	10.4 \pm 0.72 bA	9.2 \pm 0.66 aA	9.6 \pm 0.62 bA	10.5 \pm 0.58 aA	17.1 \pm 0.71 aA	9.4 \pm 0.70 aB
Sex ratio	0.24 bA	0.10 bB	0.31 aA	0.21 aB	0.27 bA	0.10 bB

Lowercase letters compare the different treatments with the same host species. Upper case letters compare the same treatments against the two host species. ANOVA test, followed by Tukey ($p < 0.05$). Sex ratio, tested by χ^2 for heterogeneity. ⁽¹⁾ emerged parasitoids + pupae dissected with parasitoids. ⁽²⁾ number of larvae offered – (number of emerged flies + emerged parasitoids).

Table 2 – Mean number (\pm SE) of formed pupae, parasitized pupae, emerged parasitoids, unviable pupae and sex ratio of hosts *Anastrepha fraterculus* and *Ceratitis capitata* exposed to parasitism by *Diachasmimorpha longicaudata* on first, second and third-instar larvae.

<i>Anastrepha fraterculus</i>			
Variables evaluated	Instar		
	First	Second	Third
Formed pupae	11.4 \pm 0.46 a	12.2 \pm 0.57 a	12.1 \pm 0.86 a
Parasitized pupae ⁽¹⁾	7.6 \pm 0.64 b	10.6 \pm 0.55 a	7.3 \pm 0.59 b
Emerged pupae	7.6 \pm 0.64 b	10.6 \pm 0.55 a	7.3 \pm 0.59 b
Unviable pupae ⁽²⁾	5.2 \pm 0.58 b	4.3 \pm 0.54 b	7.7 \pm 0.57 a
Sex ratio	0.27 b	0.32 b	0.70 a
<i>Ceratitis capitata</i>			
Formed pupae	10.2 \pm 0.50 b	13.9 \pm 0.27 a	14.3 \pm 0.15 a
Parasitized pupae ⁽¹⁾	8.0 \pm 0.54 b	11.9 \pm 0.43 a	11.4 \pm 0.57 a
Emerged pupae	8.0 \pm 0.54 b	11.9 \pm 0.43 a	11.4 \pm 0.57 a
Unviable pupae ⁽²⁾	5.7 \pm 0.50 a	2.9 \pm 0.45 c	3.6 \pm 0.57 b
Sex ratio	0.41 b	0.45 b	0.61 a

Lowercase letters compare the different treatments with the same host species. ANOVA test, followed by Tukey ($p < 0.05$). Sex ratio, tested by χ^2 for heterogeneity. ⁽¹⁾ emerged parasitoids + pupae dissected with parasitoids. ⁽²⁾ number of larvae offered – (number of emerged flies + emerged parasitoids).

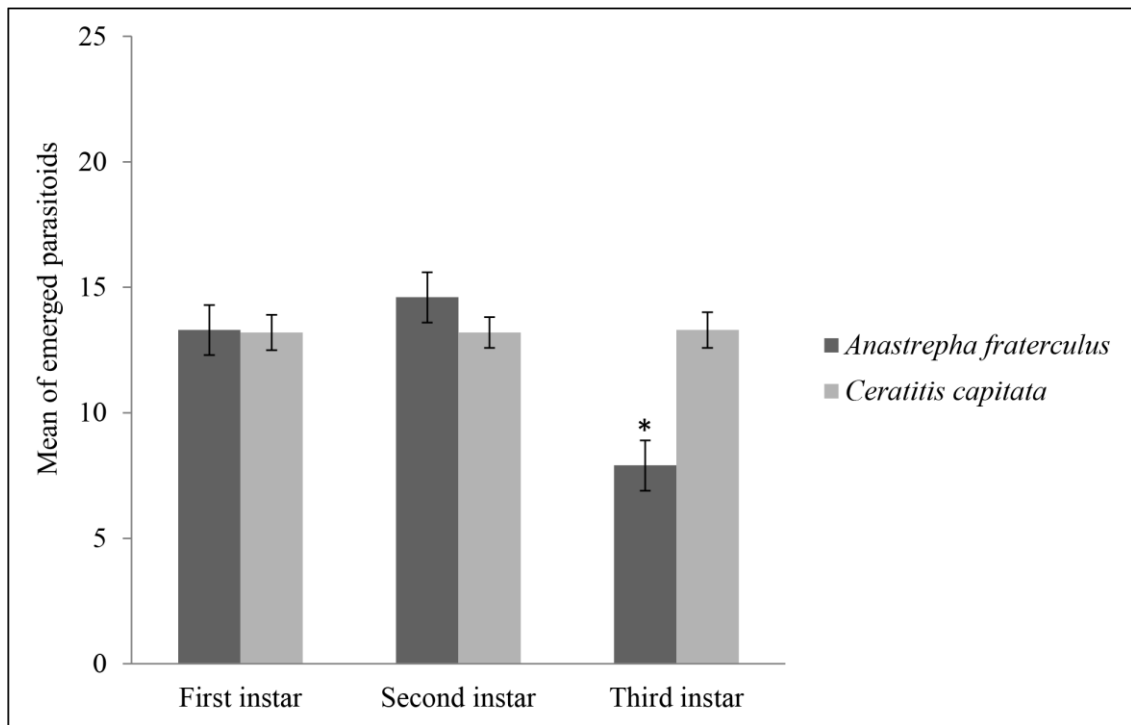


Figure 1 – Mean number of emerged parasitoids in hosts *Anastrepha fraterculus* and *Ceratitis capitata*, exposed to parasitism by *Diachasmimorpha longicaudata* on first, second and third-instar larvae. The bars correspond to the standard error. Bars with asterisk presented significant difference (ANOVA test, followed by the Tukey test, $p < 0.05$) of the other instars for the same host species.

ARTIGO III: Intra and interspecific competition between *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) and *Aganaspis pelleranoi* (Brèthes) (Hymenoptera: Figitidae) (*)

(*) Manuscrito formatado segundo as normas do periódico Biological Control (Anexo 3).

**Intra and interspecific competition between *Diachasmimorpha longicaudata*
(Ashmead) (Hymenoptera: Braconidae) and *Aganaspis pelleranoi* (Brèthes)
(Hymenoptera: Figitidae)**

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Running head: Competition between *D. longicaudata* and *A. pelleranoi*

Abbreviations:

DL - *Diachasmimorpha longicaudata*

AP - *Aganaspis pelleranoi*

AF - *Anastrepha fraterculus*

SR - Sex ratio

PR - Parasitism rate

Abstract

Our study assessed the interspecific competition between the parasitoids *Diachasmimorpha longicaudata* (exotic) (DL) and *Aganaspis pelleranoi* (native) (AP), in larvae of the host *Anastrepha fraterculus* (AF). The bioassays were carried out in the laboratory by offering host larvae to only one parasitoid on a single occasion or on two occasions, or even to two parasitoid species, alternating the offering sequence. Thus, six exposure regimes were completed: AP (host exposed for four hours); DL (host exposed for 40 minutes); AP-AP (host exposed to AP for four hours and then to a co-specific for a further four hours); DL-DL (host exposed to DL for 40 minutes and then to a co-specific for a further 40 minutes); AP-DL (host exposed to AP for four hours and then to DL for 40 minutes); and DL-AP (host exposed to DL for 40 minutes and then exposed to AP for four hours), with 40 replicates each. The controls consisted of larvae in the parasitism units, for the same periods, without parasitoids. The mean numbers of emerged flies, parasitized pupae (emerged parasitoids + pupae dissected with parasitoids), emerged parasitoids, oviposition scars, sexual ratio of parasitoids and parasitism rates were compared between the different exposure regimes. The mean number of emerged flies was higher in controls when compared to treatments, but there was no significant difference between treatments. The mean number of parasitized pupae and emerged parasitoids was higher in the DL-DL and DL-AP treatments. When the hosts were exposed only once to the parasitoids, the sex ratio was diverted to males (AP and DL treatments); but when exposed twice, the treatments spawned offspring with more females, except at the AP-DL (DL). The parasitism rate was 41.2% for AP, 53.7% for DL, 43.5% for AP-AP, and 72.1% for DL-DL, as well as 12.6% (AP) and 46% (DL) for the AP-DL treatment and 60.3% (DL) and 7.9% (AP) for the DL-AP

treatment. There was a positive correlation between the mean number of oviposition scars and the mean number of parasitoids and emerged females at the treatments AP, DL, AP-AP, DL-DL and DL-AP (DL). The results show that there may be competition between the parasitoids, when both parasitize the same host. Irrespective of the order of occurrence of parasitism, *D. longicaudata* suppresses the emergence of *A. pelleranoi*.

Keywords: *Anastrepha fraterculus*, neotropical parasitoid, exotic parasitoid.

1. Introduction

Parasitoids play a key role in many ecosystems in terms of biodiversity, ecological impact and economic importance (Vinson, 1998; Hawkins et al., 1999), and may experience complex interactions with predators, other parasitoids, entomopathogens, and hyperparasites (Boivin and Brodeur, 2006). The competition between individuals of the same species is known to be intraspecific competition and may occur when several individuals of the same species exploit the same sources of resources, sometimes at the same time (Couchoux and van Nouhuys, 2014). The interaction between parasitoid species, called as interspecific competition, occurs among those that have developed ecologically similar strategies (Boivin and Brodeur, 2006), and in these organisms can play an important role in the coexistence of species and in the occupation and modeling of community structures (Godfray, 1994). Interspecific competitive effects may occur between adult (extrinsic competition) and immatures parasitoids (intrinsic competition) (de Moraes et al. 1999; Wang et al., 2008). This kind of competition occurs within a closed system and tends to be

asymmetrical and lethal in solitary parasitoids, as each individual requires complete possession of the host (Wang et al., 2008).

Females from many parasitoid families have the ability to discriminate between parasitized and non-parasitized hosts (Ruschioni et al., 2015), as in natural systems, several species may attack the same host, producing multiparasitism and competition between immature stages (Cusumano et al., 2011, 2012). When the same or more than one species of parasitoid attacks a single host, self-superparasitism and superparasitism can occur, possibly due to the physical or physiological competition between the larvae sharing the same host (Montoya et al., 2000b, 2003; González et al., 2009). This phenomenon can be considered natural, even in species that show an innate ability to discriminate parasitized hosts, may be considered an adaptive strategy, and can lead to a higher sex ratio of females, without affecting the demographic parameters of the offspring, including longevity and fecundity (González et al., 2007; Montoya et al., 2003, 2011, 2013).

Biological control programs should consider the interactions between parasitoid guilds, since competitive effects may alter the reproductive success of the species, thereby affecting host mortality (Follett et al., 2000) and establishment of the parasitoid species in the environment. Understanding how interspecific competition in parasitoids can affect pest suppression may improve biological control. Some authors argue that the more species are introduced, the greater is the effect of reducing pest density (Stiling and Cornelissen, 2005; Miranda et al., 2015), while others suggest that the release of multiple species may impair biological control (Murdoch et al., 1998; Denoth et al., 2002).

Braconidae and Figitidae have important agents in biological control, used for the suppression of fruit flies (Diptera: Tephritidae). The main highlight is

Diachasmimorpha longicaudata (Ashmead) (Hymenoptera: Braconidae), which is widely used as a biological control agent in the world; it is natural from the Indo-Australian region, where it parasitizes at least 14 species of *Bactrocera* Macquart (Wharton and Gilstrap, 1983; Montoya et al., 2000a; Devescovi et al., 2017). This parasitoid is also easy to breed and multiplied in laboratory environments and has little specificity regarding the host, being able to parasitize *Ceratitis capitata* (Wiedemann) (Mediterranean fly) and several species of *Anastrepha* Schiner (Carvalho and Nascimento, 2002). It has a parasitism efficiency exceeding 50% and can suppress up to 70% of the fruit fly populations in the environment, besides being one of the few species that parasitize hosts located at the soil level (Sivinski et al., 1996; Montoya et al., 2000a, García-Medel et al., 2007).

Aganaspis pelleranoi (Brèthes) (Hymenoptera: Figitidae) has a natural abundance superior to other species of Neotropical parasitoids, and it is considered a promising species to compose programs of biological control of fruit flies in agroecosystems (Nasca, 1973; Núñez-Campero et al., 2014; Gonçalves et al., 2016; Schliserman et al., 2016). Furthermore, they have the ability to parasitize fruit fly larvae in native and exotic fruits, increasing their chances of success in parasitism (Guimarães and Zucchi, 2004; Schliserman et al., 2016). They also parasitize larvae of *C. capitata* and several species of the genus *Anastrepha* and the Lonchaeidae family (Ovruski, 1995; Wharton et al., 1998; Guimarães et al., 1999; Ovruski et al., 2000, 2004). Females forage and attack their hosts within fallen or cracked fruits (Sivinski et al., 2000).

Aganaspis pelleranoi and *D. longicaudata* are both solitary endoparasitoids, koinobionts, and the adult emerges from the puparia (Wharton and Gilstrap, 1983). Both are able to recognize whether or not the hosts are parasitized (Montoya et al., 2000b; Montoya et al., 2003; Ruschioni et al., 2015). Biacheri et al. (2018) verified in

olfactometry tests, that adults of *A. pelleranoi* and *D. longicaudata* responded to the volatiles emanating from both *C. capitata* and *A. fraterculus* larvae, although both parasitoids preferred the chemical stimulus originated from the combination of the host larvae and the fruit host. In addition, the two species mainly parasitize third-instar fruit fly larvae (Ovruski 1994a; Sime et al., 2006; Gonçalves et al., 2013), although there are records of *D. longicaudata* parasitizing other instars and pupae (Carvalho 2005a; Ovruski et al., 2011; Montoya et al., 2017).

Studies that address the interaction between *A. pelleranoi* and other species are still scarce, such as the works of Aluja et al. (2009), Núñez-Campero et al. (2014) and Montoya et al. (2017). The competition between *D. longicaudata* and other parasitoids have already been more studied, like the works of Palacio et al. (1991), Baranowski et al. (1993), Ramadan et al. (1994), López et al. (1999), Sivinski et al. (2000), Eitam et al. (2004), Wang et al. (2008), Miranda et al. (2015), Murillo et al. (2016), Montoya et al. (2017, 2018). However, no study records were found regarding the competition between *A. pelleranoi* and *D. longicaudata*. Knowing that *A. pelleranoi* and *D. longicaudata* can parasitize the same larval instar and that the female fruit fly parasitoids recognize whether or not the host is parasitized, the purpose of this work was to evaluate the interspecific competition between *D. longicaudata* and *A. pelleranoi*, in larvae of the host *A. fraterculus*.

2. Material and Methods

2.1. Study site

The study was conducted in the Laboratory of Biology, Ecology, and Biologic Control of Insects (Bioecolab), at the Universidade Federal do Rio Grande do Sul, under controlled conditions (26 ± 1 °C, $60 \pm 10\%$ RH), and a photoperiod of 14:10 (L:D).

2.2. Host rearing

The adults of *Anastrepha fraterculus* were kept in wooden cages (45 x 30 x 30 cm), covered with voile fabric, receiving distilled water and a solid diet given on an ad libitum basis, which consisted of granulated sugar, hydrolyzed protein, soybean extract (3:1:1) and vitamin complex (Lavitan – A-Z[®]), in the ratio of two macerated tablets per 250 g of diet (adapted from Jaldo et al., 2001). The oviposition substrate was a blue tissue bag covered with silicone (30 x 30 cm), having at one end a bottleneck with a cap, through which water was placed (Meirelles et al., 2016). The substrate was rested on the upper part of the cage, covered with voile, to obtain the eggs. The eggs were collected daily and placed in polystyrene trays (23.5 x 18 x 1 cm), with an artificial diet based on organic carrot, beer yeast, corn flour, sugar, and distilled water (modified from Terán, 1977). After seven days, these were placed inside larger plastic trays (51 x 30 x 9.5 cm), with sterile sand and covered by voile, where they stayed for approximately seven days to enable the larvae to leave the diet and soak in the sand. Subsequently, the sand was sifted and the collected pupae placed in plastic pots (6.6 × 6.6 × 6 cm) until emergence, under controlled conditions (26 ± 1 °C, 60 ± 10% RH), and a photoperiod of 14:10 (L:D).

2.3. Parasitoids rearing

To rear *A. pelleranoi*, araçá fruit [*Psidium cattleianum* Sabine) (Myrtaceae)] infested with *A. fraterculus* was collected from native fruit orchards at Fundação Estadual de Pesquisa Agropecuária, in Taquari, RS, Brazil. In the laboratory, the fruits were packed in plastic trays (51 x 30 x 9.5 cm) on a layer of sterilized sand and covered by voile fabric. The sand was sieved after 15 days, and the puparia obtained were

conditioned until the emergence of parasitoids. For the establishment of *D. longicaudata*, parasitized puparia of *A. fraterculus* were obtained from the experimental establishment of Embrapa Clima Temperado, in Pelotas, RS, Brazil. The puparia obtained were stored in a plastic pot and kept under controlled conditions ($26 \pm 1^\circ\text{C}$, $60 \pm 10\%$ RH, with 14 photophase hours) until the emergence of the parasitoids.

The adults of both species were placed in wooden cages (19.5 x 16.5 x 25.5 cm), covered with voile fabric, and received water by capillarity and honey dissolved in water (7:3), offered in Petri dishes (5 x 5 x 1.5 cm) with cotton. The parasitoids of third-instar *A. fraterculus* larvae (Ovruski, 1994b; Sime et al., 2006) were offered to the parasitoids. The larvae were placed in parasitism units, which consisted of a circular plastic plate (4 cm in diameter), with a border of 0.3 cm, encased in white voile fabric, fastened with an elastic band. After the exposure, for four hours (for *A. pelleranoi*) (based on Gonçalves et al., 2016) and 40 minutes (for *D. longicaudata*) (based on Suárez et al., 2012), the larvae were returned to the diet in polystyrene trays (15.5 x 15.5 x 1 cm) and placed in plastic trays (41 x 28 x 7 cm) on a layer of sterilized sand and covered with voile fabric until puparium formation. After a period of five days, the sand was sifted, and the puparia were packaged in the same manner as for fly breeding, waiting for the emergence of the parasitoids that were reintroduced to the breeding in new cages.

2.4. Bioassay

The bioassay was performed in arenas constituted of plastic bottles (7 x 10.5 cm diameters), with an upper opening (6 cm in diameter) covered with voile fabric for ventilation. Within each arena was inserted, a female of *A. pelleranoi* or *D. longicaudata*, paired and with previous experience of parasitism, with eight days old.

Females received water and food as described above. For each female, 10 larvae were offered in parasitism units, which consisted of a circular plastic plate (2.7 cm diameters), with a border of 0.2 cm, formed by a small layer of silicone and enveloped with white voile, fastened with an elastic band. To dispose of the parasitism units within the cages, plastic vials (0.9 x 5 x 1.2 cm) were used as carriers.

To assess the effects of competition among species, different exposure regimes of *A. fraterculus* larvae were established: AP – host exposed for four hours to *A. pelleranoi*; DL – host exposed for 40 minutes to *D. longicaudata*; AP-AP – host exposed to *A. pelleranoi* for four hours and then to a co-specific for a further four hours; DL-DL – host exposed to *D. longicaudata* for 40 minutes and then to a co-specific for a further 40 minutes; AP-DL – host exposed to *A. pelleranoi* for four hours and then to *D. longicaudata* for 40 minutes; and DL-AP – host exposed to *D. longicaudata* for 40 minutes and then exposed to *A. pelleranoi* for four hours. The experiment was conducted with 40 replicates for each treatment.

After each exposure, in all treatments, the larvae were observed individually with the aid of a Wild Heerbrugg stereomicroscope, to count the number of oviposition scars. For treatments with more than one offering, the larvae were placed in the parasitism units again, and exposed to the second stage of parasitism, being examined for the second time for scoring. After being offered parasitism in all regimens, the larvae were returned to the diet in plastic units (4.4 x 0.9 cm in diameter) and packed in plastic containers (7 x 6.8 x 5.5 cm) on a layer of sand until puparium formation. After five days, the sand was sifted, and the puparia were packed in plastic pots (6.6 x 6.6 x 6 cm) until the emergence of flies or parasitoids.

To evaluate the mortality of larvae without action of the parasitoids (control), 10 larvae of *A. fraterculus*, totaling 40 replicates, were placed in parasitism units and

positioned in the cages for the same periods of time described above, but without the presence of parasitoids, and are then placed in the same manner as described above.

For all treatments, the puparia of which there was no emergence were dissected for the presence of parasitoids or flies. Mean numbers of emerged flies, parasitized puparia (emerged parasitoids + puparia dissected with parasitoids), emerged parasitoids, oviposition scars and sexual ratio of the parasitoids were recorded.

2.5. Statistical analysis

The mean values of emerged flies, parasitized puparia and emerged parasitoids were tested for normality by Shapiro-Wilk and homoscedasticity by Bartlett test. After being subjected to analysis of variance, the means were compared by the ANOVA HSD post-hoc test, followed by the Tukey test, with a significance level of 5%.

The relationship between the mean number of parasitoids and emerged females, and the mean number of oviposition scars was tested using the Spearman Correlation Coefficient ($\alpha = 0.05$), followed by a polynomial regression.

The sex ratio (SR) was estimated using the formula: $SR = \text{number of females} / \text{number of females} + \text{number of males}$. The parasitism rate was calculated using the formula: $PR = \text{number of emerged parasitoids} / \text{number of puparia formed} \times 100$. Both were compared between treatments using the Chi-square (χ^2) test for heterogeneity. The tests were performed using the BioEstat 5.0 software (Ayres et al., 2007).

3. Results and Discussion

The number of flies emerged in the controls was significantly higher to all treatments (Table 1), indicating that the exposure conditions at different time intervals did not affect larval development and pupation. The natural mortality of the larvae

ranged from 7% to 19.5%, similar to those reported by Bressan-Nascimento (2001) and Jaldo et al. (2007).

The mean number of parasitized pupae was higher in the regimens in which the larvae were twice offered to *D. longicaudata* (DL-DL) or first to *D. longicaudata* and then to *A. pelleranoi* (DL-AP) (Table 1). Conversely, the lowest mean number of parasitized pupae was recorded in the treatments in which the larvae were only offered to *A. pelleranoi*, either in one or two exposures (AP or AP-AP) (Table 1). Gonçalves et al. (2016), in third-instar larvae of *A. fraterculus*, with a single exposure to *A. pelleranoi* and without choice of instar, found mean values similar to those of our study (3.8 ± 0.13). Reflecting this data, the highest parasitism rate was for *D. longicaudata* when the larvae were exposed twice to the parasitoid (DL-DL), reaching 72.1%. When exposed first to *A. pelleranoi* and then to *D. longicaudata* (AP-DL), the rate was 12.7% and 46%, respectively. *Aganaspis pelleranoi* had the lowest parasitism rate, of 7.9%, in the DL-AP treatment. In fact, for *D. longicaudata*, a 43.2% parasitism was recorded in *A. fraterculus* (Ovruski et al., 2011), indicating the potential of this species.

The high parasitism rate of *D. longicaudata* reported in our study for the species individually or in interspecific competition was also reported by Bautista and Harris (1997) in larvae of another fruit fly species, *Bactrocera dorsalis* (Hendel), with the highest offspring percentage (99%) reported when *D. longicaudata* was the first parasitoid to have access to the host, followed by the braconid *Psytalia incisi* (Silvestri). Similarly, *D. longicaudata*, when competing with its congener *Diachasmimorpha tryoni* (Cameron), also won the competition, generating more offspring (Ramadan et al., 1994). This occurring independently of the order in which the *B. dorsalis* larvae were offered. The prevalence of *D. longicaudata* in competitions appears not to be dependent on the host species, as we verified this in *A. fraterculus*,

and the same pattern was observed in the competition between *D. longicaudata* and *D. areolatus* on larvae of *Anastrepha suspensa* (Loew) (Paranhos et al., 2013) and with *D. crawfordi* on other species of *Anastrepha* (Miranda et al., 2015).

Conversely, irrespective of the host species, the order in which the larvae are offered to the parasitoid can alter this pattern, as recorded by Wang et al. (2008), who observed that the greatest number of offspring occurred when *D. longicaudata* was the second parasitoid to have contact with the host, *C. capitata*, its precursor being the egg parasitoid *Fopius ceratitivorus* Wharton. In contrast, when competing with *U. anastrephae*, if the *A. suspensa* larvae were first offered to *D. longicaudata*, the latter wins the competition, but in a reverse order of oviposition, *Utetes anastrephae* (Viereck) generated more offspring (Paranhos et al., 2013). In our study, when competing with *A. pelleranoi*, the order did not influence the outcome (Table 1).

Another factor that may influence competition is the specificity of the parasitoid in relation to the larval instar of the host. In the studies conducted by Ramadan et al. (1994), on larvae of *B. dorsalis*, and Paranhos et al. (2013), on the host *A. suspensa*, the larvae offered to both *D. longicaudata* and its competitors were of the third instar. However, for *D. areolatus* and *P. incisi*, the preferred instars are the first and second, respectively (Murillo et al., 2015; Yang et al., 2018), which may have interfered in the performance of these parasitoids in relation to *D. longicaudata*. In our study, the larval instar offered was the third one, the preference of both parasitoid species for this one being known, irrespective of the host species, which may be *C. capitata*, *Bactrocera oleae* Gmelin or *Anastrepha* spp. (Ovruski, 1994b; Sime et al., 2006), a factor that should not have influenced the parasitoid response in this case.

The competitive superiority of *D. longicaudata* may also be associated with the morphological characteristics of larvae of the parasitoid in the early stages, whose

mandible and cephalic capsule are well developed, these being factors that may make it more competitive (Paladino et al., 2010; Murillo et al., 2016), although this has not been evaluated in this study. It is known that *D. longicaudata* has well-developed mandibles in its first instars (Murillo et al., 2016), and this may have generated an advantage when the host larvae were offered before to it and subsequently to *A. pelleranoi*, as described for others studies (Palacio et al., 1991; Wang et al., 2008). Furthermore, larvae of *D. longicaudata* hatch at around 24 to 48 hours after parasitism, whereas those of *A. pelleranoi*, between 78 and 80 hours (Ovruski, 1994b; Paladino et al., 2010), also promoting an advantage in the initial time of development of the exotic species. In the DL-AP treatment, the parasitism rate of *D. longicaudata* was 60.3%, not differing from the DL treatment, which was 53.7% ($P > 0.05$).

An interesting aspect regarding *A. pelleranoi* was that there was no increase in parasitism when the larvae were exposed twice to the females of this species. The parasitism rate was maintained on both occasions, with 41.6% (AP) and 43.5% (AP-AP), with no significant difference ($P > 0.05$), which may be associated with the recognition of the co-specific parasitoid by hosts that have already suffered from parasitism (Ruschioni et al., 2015). Additionally, the mean number of *A. pelleranoi* emerged was lower when larvae were also offered to *D. longicaudata*, irrespective of order (AP-DL or DL-AP) (Table 1).

Studies that evaluated the competition between *A. pelleranoi* and *D. longicaudata* were not found in the literature, although there are reports that, when released to the field, *D. longicaudata* can alter the abundance of *A. pelleranoi* and other native parasitoids (Montoya et al., 2017). In this sense, Paranhos et al. (2013) warned that the establishment or augmentation of *D. longicaudata* could result in the elimination of native parasitoids. On the other hand, other studies evaluating the

releases of *D. longicaudata* (Carvalho, 2005b; dos Santos et al., 2016; Meirelles et al., 2016) observed that there was no loss of biodiversity of native species present in the orchards. Carvalho (2005b) found that there were alterations in the frequency of species, which may have occurred due to the existence of interspecific competition in the exploitation of oviposition sites.

The results presented and discussed in this study corroborate the fact that in intra- and interspecific interactions *D. longicaudata* is considered as a better competitor (Miranda et al., 2015, Murillo et al., 2016; Montoya et al., 2017), being able to win in an intrinsic competition with native parasitoids (Paranhos et al., 2013). It also has great plasticity, adapting easily to environmental conditions, and can reach higher parasitism levels than other species (Carvalho and Nascimento, 2002; Paranhos et al., 2013; Miranda et al., 2015).

The sex ratio (SR) varied in relation to the treatments (Table 1). When the larvae were only offered once to one of both species, *D. longicaudata* and *A. pelleranoi* had an offspring SR close to 50%. In the treatments in which there were two exposures to the parasitoid females, both species generated more females. The exception occurred in the DL-AP treatment, in which *D. longicaudata* had a higher number of emerged males (Table 1).

For both species, *A. pelleranoi* and *D. longicaudata*, it is known that they generate more females when they parasitize larvae in later and larger instars (Eben et al., 2000; Ovruski et al., 2011; van Nieuwenhove and Ovruski, 2011; Gonçalves et al., 2013, 2016). This occurs because parasitoids select the best host for their offspring and, upon finding it, they tend to breed offspring with more females (Godfray and Shimada, 1999). In general, larger hosts have more resources and are considered qualitatively superior in terms of efficiency to the parasitoid (Mattiacci and Dicke, 1995; Ovruski et

al., 2011). The most common component in host quality is its size. Females tend to produce more females in larger hosts and males in smaller ones (Godfray and Shimada, 1999; Harvey et al., 2013). However, because all hosts in our study had approximately the same size, this factor may not have influenced the results at the treatments AP, DL and DL-AP (DL).

Another result of our study (Table 1) shows that *A. pelleranoi* had fewer emerged parasitoids when compared to *D. longicaudata* but presented a higher sex ratio in treatments with two exposures. In an interspecific competition test between *D. tryoni* and *D. longicaudata*, Ramadan et al. (1994), observed that, similarly to our study, although the second species generated more offspring, it did not have the largest number of females. The authors inferred that this may have occurred because immature females of *D. tryoni* may be better competitors than males, or simply because adults oviposit more fertilized eggs (eggs that would give rise to females) in case of competition. It is possible that this applies to *A. pelleranoi*, with competition among the larvae, although this aspect has not been evaluated in this study.

The mean number of oviposition scars (Table 1) was higher in the DL-DL treatment than in all others, which did not differ, except for AP compared to the AP-DL, again indicating the highest aggressiveness of *D. longicaudata* in relation to the hosts.

There was a positive correlation between the mean number of oviposition scars and the mean number of offspring generated, as well as the mean number of emerged females in the treatments in which the parasitoids only had contact with the hosts or in the intraspecific interaction (Fig. 1 a-d). In cases where there was an interspecific interaction, this correlation was not observed for *A. pelleranoi* offspring, (Fig. 1 e, h), possibly because a small number of parasitoids emerged from this species. For the offspring of *D. longicaudata*, when the hosts were first offered to *A. pelleranoi* (AP-

DL) (Fig. 1 f), there was also no correlation between the increase in scars and emerged individuals, the only indicative of which may have there was interference of the pioneer species. It is interesting to note that, in all cases where, there was a positive correlation for the average number of offspring generated, this also applied to the number of emerged females, corroborating the information of Montoya et al. (2011, 2012), according to which there is a positive correlation between the number of scars (a reliable indication of superparasitism) and the number of females generated.

Information on superparasitism in *A. pelleranoi* is not present in the literature. Therefore, our study brings new information on the subject, showing that in intra- or interspecific competitions, *A. pelleranoi*, despite having a lower average emergence, resulted in more females in the treatment in which the hosts were exposed twice to the parasitoid (AP-AP) or in the competitor treatments, AP-DL and DL-AP.

The correlation between oviposition scars and the emergence of parasitoids and females has also been studied for *D. longicaudata* (Altafini et al., 2013) in different hosts. Several studies have indicated that, in moderate superparasitism (2-6 scars per pupa), there is a tendency for females to emerge (González et al., 2007; Montoya et al., 2011, 2012, 2013), which corroborates our results, in which an average of 2.3 to 5.3 scars per larva was found. These authors also commented that this superparasitism does not imply in detrimental effects on the demographic parameter of the offspring, including longevity and fecundity.

To test whether this behavior occurred naturally in the field, Montoya et al. (2013) collected mango fruits [*Mangifera indica* L. (Anacardiaceae)] and evaluated the puparia from these fruits. The authors showed a positive correlation between the fruit size to the infestation levels of *Anastrepha* spp. and to the number of pupae parasitized and superparasitized by *D. longicaudata*. Superparasitism was also positively correlated

with a biased sex ratio for females, demonstrating that superparasitism is present in natural populations of *D. longicaudata*. Notwithstanding, in our study, it was observed that in competition with *A. pelleranoi*, this pattern was altered (Table 1), as the sexual ratio of *D. longicaudata* in the treatment (DL-AP) was 0.41, indicating that, although the native species generally has a smaller offspring generated in situations of competition, physical competition or a physiological suppression may occur between the parasitoid larvae (Mackauer, 1990) by the females, in relation to the larvae of the competing species.

Due to the similarities in the behavior and preference between *A. pelleranoi* and *D. longicaudata* already described and confirmed in this study, it is important to conduct experiments on the competition of these species in the field, as although some works state that *D. longicaudata* does not compete with native parasitoids (dos Santos et al., 2016; Meirelles et al., 2016), others state the opposite (Paranhos et al., 2013; Montoya et al., 2017), leaving questions regarding this issue. Our results show that *D. longicaudata* competes and diminishes the emergence of *A. pelleranoi* when both species parasitize the same larva. Therefore, there are no guarantees that *D. longicaudata* will not compete with native parasitoids in the field. Moreover, other biotic and abiotic factors should be considered, as they may cause this parasitoid to coexist with *A. pelleranoi*. One of the aspects that may interfere in this field competition is the fact that the ovipositor size of *A. pelleranoi* has a mean size of 0.2 mm (Tormos et al., 2013), smaller than that of *D. longicaudata* (5.27 mm) (Sivinski et al., 2001), which limits the search of the first species by hosts in fruits with a thicker mesocarp. It is known that *A. pelleranoi* exhibits a specific foraging behavior by depositing fruit on cracked or fallen fruits (Sivinski et al., 2000); however, if host larvae have already been

parasitized by *D. longicaudata*, our study shows that the species will not be able to succeed in the competition.

Given that *D. longicaudata* is considered a highly competitive species, with ease of adaptation to new environments and larvae with competitive characteristics, its introduction into new environments should be carefully evaluated, as it may cause not only the suppression of *A. pelleranoi*, but also of other species that are present in the environment.

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Figure Legend

Fig. 1. Correlation between oviposition scars caused by *Diachasmimorpha longicaudata* (DL) or *Aganaspis pelleranoi* (AP) on larvae of *Anastrepha fraterculus* and parasitoids and females emerged in the treatments (a) AP, (b) DL, (c) AP-AP, (d) DL-DL, (e) AP-DL (progeny of *A. pelleranoi*), (f) AP-DL (progeny of *D. longicaudata*), (g) DL-AP (progeny of *D. longicaudata*) and (h) DL-AP (progeny of *A. pelleranoi*). Spearman correlation coefficient ($\alpha = 0.05$).

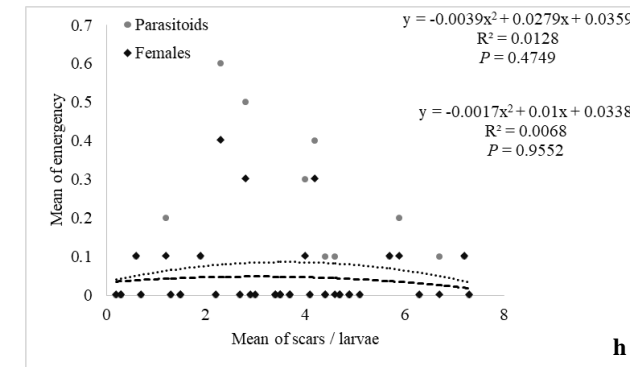
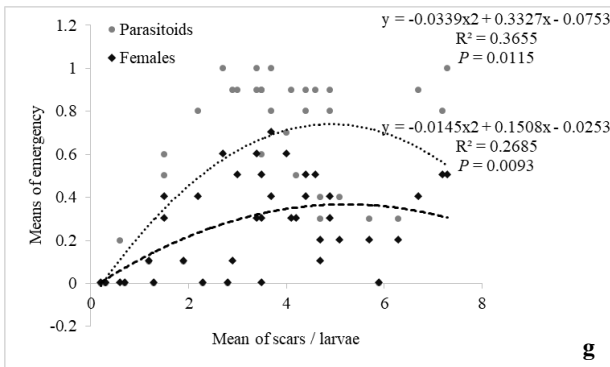
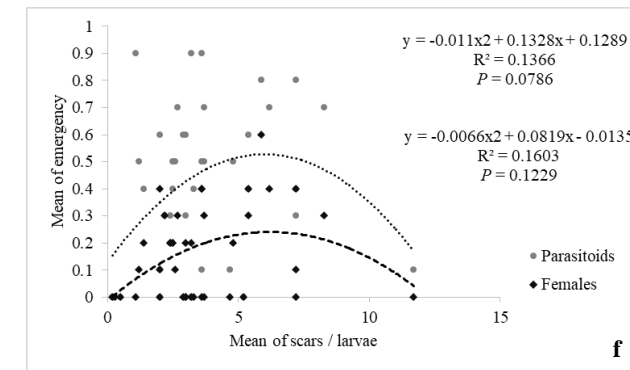
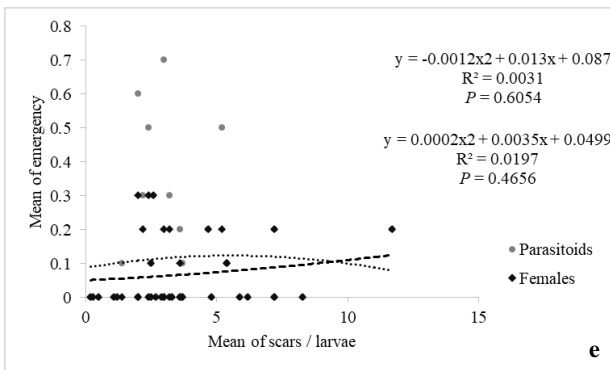
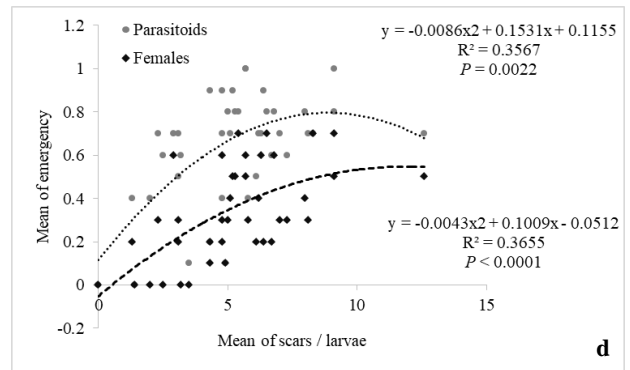
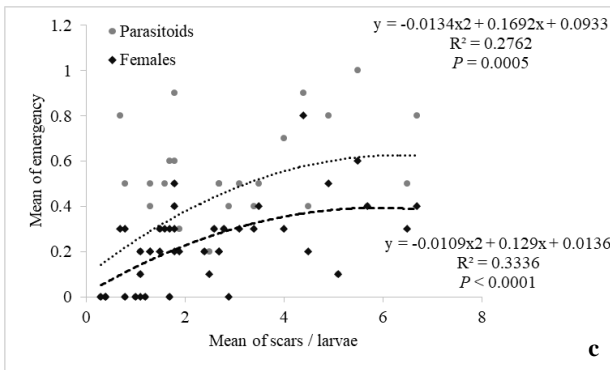
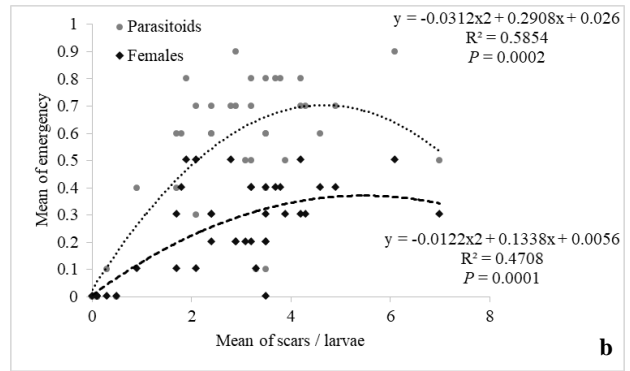
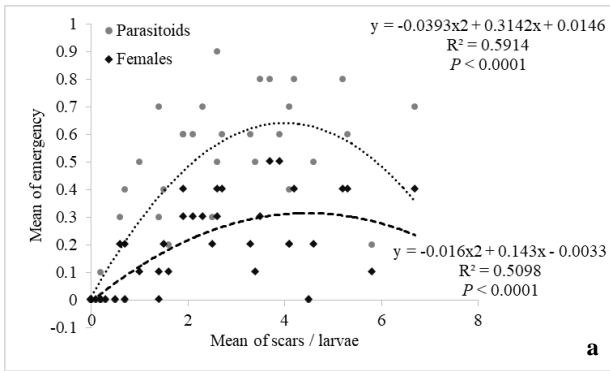


Table 1. Mean number (\pm EP) of emerged flies, parasitized pupae, emerged parasitoids by species and total, oviposition scars and sex ratio of *Aganaspis pelleranoi* (AP) and *Diachasmimorpha longicaudata* (DL) parasitizing larvae of the host *Anastrepha fraterculus* in different intra and interspecific competition arrangements.

Treatments	AP	DL	AP-AP	DL-DL	AP-DL	DL-AP
Flies emerging in control	9.1 \pm 0.14 aA	8.9 \pm 0.15 abA	8.0 \pm 0.19 bA	9.0 \pm 0.14 aA	8.5 \pm 0.17 bA	9.3 \pm 0.12 aA
Flies emerging in treatments	3.9 \pm 0.24 aB	1.9 \pm 0.19 bcB	3.5 \pm 0.23 aB	0.5 \pm 0.10 dB	1.1 \pm 0.16 cdB	2.5 \pm 0.21 bB
Parasitized pupae ⁽¹⁾	4.0 \pm 0.49 d	5.3 \pm 0.50 bc	3.9 \pm 0.23 d	6.3 \pm 0.24 a	5.2 \pm 0.25 c	6.2 \pm 0.24 ab
Emerged <i>A. pelleranoi</i>	4.0 \pm 0.24 a	-	3.9 \pm 0.23 a	-	1.1 \pm 0.15 b	0.7 \pm 0.13 c
Emerged <i>D. longicaudata</i>	-	5.1 \pm 0.25 b	-	6.3 \pm 0.24 a	4.1 \pm 0.24 c	5.5 \pm 0.24 b
Total of emerged parasitoids	4.0 \pm 0.24 b	5.1 \pm 0.25 b	3.9 \pm 0.23 b	6.3 \pm 0.24 a	5.2 \pm 0.25 b	6.2 \pm 0.24 a
Oviposition scars	2.3 \pm 0.29 c	2.7 \pm 0.26 bc	2.5 \pm 0.26 bc	5.3 \pm 0.38 a	3.7 \pm 0.37 b	3.5 \pm 0.30 bc
Sex ratio	0.47 b	0.49 b	0.60 a	0.52 b	0.60 a (AP); 0.55 a (DL)	0.41 c (DL); 0.59 a (AP)

Lowercase letters compare treatments on the same line. Upper case letters compare controls and treatments. ANOVA test, followed by Tukey ($P < 0.05$). Sex ratio, tested by χ^2 for heterogeneity. ⁽¹⁾ parasitoids emerged from the puparia + puparia dissected with parasitoids.

Highlights:

- Our study shows that *D. longicaudata* competes and decreases the emergence of *A. pelleranoi* when both parasitize the same larva.
- An interesting aspect regarding *A. pelleranoi* was that there was no increase in parasitism when the larvae were exposed twice to the females of this species, which may be associated with the recognition of the co-specific parasitoid by hosts that have already undergone parasitism.
- *Aganaspis pelleranoi* had fewer emerged parasitoids compared to *D. longicaudata* but showed a higher sex ratio in treatments with two exposures.

4 CONSIDERAÇÕES FINAIS

As interações entre parasitoides, assim como suas relações com seus hospedeiros são importantes, pois podem afetar o equilíbrio do ambiente em que estas espécies vivem. Os parasitoides, *A. pelleranoi* e *D. areolatus* (nativos) e *D. longicaudata* (exótico) são considerados promissores para o controle biológico das moscas-das-frutas (*A. fraterculus* e *C. capitata*), na América do Sul.

Sendo *D. longicaudata* uma das espécies mais utilizadas no mundo, pouco era conhecido de como esta poderia afetar as espécies nativas. Nossos trabalhos vieram contribuir para o conhecimento entre as interações dos parasitoides entre si e com seus hospedeiros.

Através destes trabalhos podemos observar que o hospedeiro de origem é importante, pois os parasitoides *A. pelleranoi* e *D. areolatus* (somente quando com origem em *A. fraterculus*) demonstraram que possuem preferência pelo hospedeiro no qual se originaram. Além disso, verificamos que a preferência em *A. pelleranoi* pode ser alterada/induzida em apenas uma geração, o que pode facilitar a criação destes organismos em laboratório e favorecer sua utilização em campo para o controle biológico.

Outro resultado importante de nosso estudo foi à constatação que *D. longicaudata* possui grande plasticidade no momento da escolha do hospedeiro em relação ao ínstar deste, podendo ter sucesso em qualquer ínstar larval que estiver disponível no ambiente. Desta forma, a prerrogativa de que ela não competiria com

outras espécies que parasitam diferentes instares, deve ser revista. No trabalho de competição entre *D. longicaudata* e *A. pelleranoi* ficou claro que a espécie exótica é mais agressiva e supera a nativa em condições de laboratório, indicativo de que pode haver uma supressão da espécie autóctone, quando ambos parasitam a mesma larva. Entretanto, em condições de campo, há outros mecanismos de variabilidade temporal e espacial, e diferenças ecológicas que podem permitir a coexistência de várias espécies mesmo competindo por um mesmo recurso.

Diachasmimorpha longicaudata é considerada uma espécie superiormente competitiva e com facilidade de adaptação a novos ambientes. Antes que liberações aconteçam é importante que os programas de controle biológico avaliem quais espécies nativas existem e qual abundância e frequência das mesmas no ambiente. Da mesma forma, é necessário observar a existência ou não de barreiras abióticas e/ou bióticas que possam auxiliar na divisão dos nichos entre os parasitoides nativos e o exótico. Todos esses cuidados são essenciais e podem auxiliar para que não haja desequilíbrio no ambiente após a inclusão de *D. longicaudata*.

5 ANEXOS

ANEXO 1



ISSN 0073-4721 *versão impressa*

ISSN 1678-4766 *versão online*

Escopo e política

O periódico Iheringia, Série Zoologia, editado pelo Museu de Ciências Naturais da Fundação Zoobotânica do Rio Grande do Sul, destina-se a publicar trabalhos completos originais em Zoologia, com ênfase em taxonomia e sistemática, morfologia, história natural e ecologia de comunidades ou populações de espécies da fauna Neotropical recente. Notas científicas não serão aceitas para publicação. Em princípio, não serão aceitas listas faunísticas, sem contribuição taxonômica, ou que não sejam o resultado de estudos de ecologia ou história natural de comunidades, bem como chaves para identificação de grupos de táxons definidos por limites políticos. Para evitar transtornos aos autores, em caso de dúvidas quanto à adequação ao escopo da revista, recomendamos que a Comissão Editorial seja previamente consultada. Também não serão aceitos artigos com enfoque principal em Agronomia, Veterinária, Zootecnia ou outras áreas que envolvam zoologia aplicada. Manuscritos submetidos fora das normas da revista serão devolvidos aos autores antes de serem avaliados pela Comissão Editorial e Corpo de Consultores.

Os artigos aceitos para a publicação se tornam propriedade da revista.

Forma e preparação de manuscritos

1. Submeter o manuscrito eletronicamente através do site:
<http://submission.scielo.br/index.php/isz>.
2. Os manuscritos serão analisados por, no mínimo, dois consultores. A aprovação do trabalho, pela Comissão Editorial, será baseada no conteúdo científico, respaldado pelos pareceres dos consultores e no atendimento às normas. Alterações substanciais poderão

ser solicitadas aos autores, mediante a devolução dos arquivos originais acompanhados das sugestões.

3. O teor científico do trabalho é de responsabilidade dos autores, assim como a correção gramatical.

4. O manuscrito, redigido em português, inglês ou espanhol, deve ser impresso em papel A4, em fonte “Times New Roman” com no máximo 30 páginas numeradas (incluindo as figuras) e o espaçamento duplo entre linhas. Manuscritos maiores poderão ser negociados com a Comissão Editorial.

5. Os trabalhos devem conter os tópicos: título; nomes dos autores (nome e sobrenome por extenso e demais preferencialmente abreviados); endereço completo dos autores, com e-mail para contato; abstract e keywords (máximo 5) em inglês; resumo e palavras-chave (máximo 5) em português ou espanhol; introdução; material e métodos; resultados; discussão; agradecimentos e referências bibliográficas. As palavras-chave não deverão sobrepor com aquelas presentes no título.

6. Não usar notas de rodapé.

7. Para os nomes genéricos e específicos usar itálico e, ao serem citados pela primeira vez no texto, incluir o nome do autor e o ano em que foram descritos. Expressões latinas também devem estar grafadas em itálico.

8. Citar as instituições depositárias dos espécimes que fundamentaram a pesquisa, preferencialmente com tradição e infraestrutura para manter coleções científicas e com políticas de curadoria definidas.

9. Citações de referências bibliográficas no texto devem ser feitas em Versalete (caixa alta reduzida) usando alguma das seguintes formas: BERTCHINGER & THOMÉ (1987), (BRYANT, 1915; BERTCHINGER & THOMÉ, 1987), HOLME *et al.* (1988).

10. Dispor as referências bibliográficas em ordem alfabética e cronológica, com os autores em Versalete (caixa alta reduzida). Apresentar a relação completa de autores

(não abreviar a citação dos autores com “et al.”) e o nome dos periódicos por extenso. Alinhar à margem esquerda com deslocamento de 0,6 cm. Não serão aceitas citações de resumos e trabalhos não publicados.

Exemplos:

BERTCHINGER, R. B. E. & THOMÉ, J. W. 1987. Contribuição à caracterização de *Phyllocaulis soleiformis* (Orbigny, 1835) (Gastropoda, Veronicellidae). **Revista Brasileira de Zoologia** 4(3):215-223.

BRYANT, J. P. 1915. Woody plant-mammals interactions. *In*: ROSENTHAL, G. A. & BEREMBAUM, M. R. eds. **Herbivores: their interactions with secondary plants metabolites**. San Diego, Academic. v.2, p.344-365.

HOLME, N. A.; BARNES, M. H. G.; IWERTSON, C. W. R.; LUTKEN, B. M. & MCINTYRE, A. D. 1988. **Methods for the study of marine mammals**. Oxford, Blackwell Scientific. 527p.

PLATNICK, N. I. 2002. **The world spider catalog, version 3.0. American Museum of Natural History**. Disponível em: <<http://research.amnh.org/entomology/spiders/catalog81-87/index.html>>. Acesso em: 10.05.2002.

11. As ilustrações (desenhos, fotografias, gráficos e mapas) são tratadas como figuras, numeradas com algarismos arábicos sequenciais e dispostas adotando o critério de rigorosa economia de espaço e considerando a área útil da página (16,5 x 24 cm) e da coluna (8 x 24 cm). A Comissão Editorial reserva-se o direito de efetuar alterações na montagem das pranchas ou solicitar nova disposição aos autores. As legendas devem ser autoexplicativas. Ilustrações a cores implicam em custos a cargo dos autores. As figuras devem ser encaminhadas apenas em meio digital de alta qualidade (ver item 16).

12. As tabelas devem permitir um ajuste para uma (8 cm) ou duas colunas (16,5 cm) de largura, ser numeradas com algarismos romanos e apresentar título conciso e autoexplicativo.

13. Figuras e tabelas não devem ser inseridas, somente indicadas no corpo do texto.

14. A listagem do material examinado deve dispor as localidades de Norte a Sul e de Oeste a Leste e as siglas das instituições compostas preferencialmente de até 4 letras, segundo o modelo abaixo:

VENEZUELA, Sucre: San Antonio del Golfe, (Rio Claro, 5o57'N 74o51'W, 430m) 5 ♀, 8.VI.1942, S. Karpinski col. (MNHN 2547). PANAMÁ, Chiriquí: Bugaba (Volcán de Chiriquí), 3 ♂, 3 ♀, 24.VI.1901, Champion col. (BMNH 1091). BRASIL, Goiás: Jataí (Fazenda Aceiro), 3 ♂, 15.XI.1915, C. Bueno col. (MZSP); Paraná: Curitiba, ♀, 10.XII.1925, F. Silveira col. (MNRJ); Rio Grande do Sul: São Francisco de Paula (Fazenda Kraeff, Mata com Araucária, 28o30'S 52o29'W, 915m), 5 ♂, 17.XI.1943, S. Carvalho col. (MCNZ 2147).

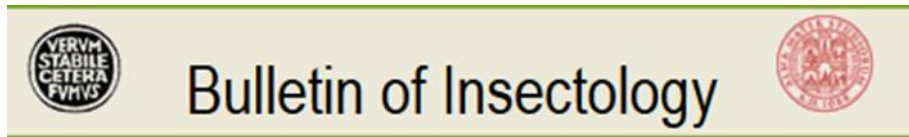
15. Recomenda-se que os autores consultem um artigo recentemente publicado na Iheringia Série Zoologia para verificar os detalhes de formatação.

16. Enviar o arquivo de texto em Microsoft Word (*.doc) ou em formato "Rich Text" (*.rtf). Para as imagens utilizar arquivos Bitmap TIFF (*.tif) e resolução mínima de 300 dpi (fotos) ou 600 dpi (desenhos em linhas). Enviar as imagens nos arquivos digitais independentes (não inseridas em arquivos do MS Word, MS Power Point e outros), nomeados de forma autoexplicativa (e. g. figura01.tif). Gráficos e tabelas devem ser inseridos em arquivos separados (Microsoft Excel para gráficos e Microsoft Word ou Excel para tabelas). Para arquivos vetoriais utilizar formato Corel Draw (*.cdr).

17. Para cada autor será fornecido um exemplar da revista. Os artigos também estarão na página do Scientific Electronic Library Online, SciELO/Brasil, disponível em www.scielo.br/isz.

Não há taxa para submissão e avaliação de artigos.

ANEXO 2



Instructions for authors

- General

The articles are published in English. Manuscripts submitted for publication may not be offered to any other journal for prior or simultaneous publication. The papers will be available on the web site for free (open access).

- Manuscript

Manuscripts should be submitted in MS Word for Windows by e-mail. The sequence should be: title, author(s) (names in full, family names/surnames/last names in capital), author(s) affiliation, abstract, key words, introduction, materials and methods, results (or results and discussion), discussion (or discussion and conclusions), conclusions, acknowledgements, references, author(s)' address(es) (corresponding author's e-mail included), table and figure legends, table(s), figure(s). If necessary, it could be possible to add online supplemental materials.

- Scientific names

Scientific names and authorities must be provided at first mention of each organism in the abstract and again in the text. Names of authorities should not be abbreviated, except with L. for Linnaeus and F. for Fabricius (authority name without diacritical marks).

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Incorrect examples: *Musca domestica* Linnaeus, *Ostrinia nubilalis* (Hübner), *Ostrinia nubilalis* Hbn., *Phaonia fuscata* Fallén; *Sitochroa palealis* (Den. & Schiff.).

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KNERER G., 1993. - Life history diversity in sawflies, pp. 33-59. In: *Sawfly life history adaptations to woody plants* (WAGNER M., RAFFA K. F., Eds).- Academic Press Inc., San Diego, California, USA.

SWAIN T., 1977. - The effect of plant secondary products on insect plant co-evolution, pp. 249-256. In: *Proceedings of XV International Congress of Entomology*, (PACKER J., WHITE D., Eds) Washington D. C., 19-27 August 1976. Entomological Society of America, College Park, MD, USA.

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ANEXO 3



Guide for Authors

Introduction

Biological Control promotes the science and technology of biological control through publication of original research articles and reviews of research and theory. The focus includes new and emerging trends in this field. Biological control is defined as the reduction or mitigation of pests and pest effects through the use of natural enemies. Biotechnologies dealing with the elucidation and use of genes or gene products for the enhancement of biological control agents are also of interest.

The journal encompasses biological control of viral, microbial, nematode, insect, mite, weed, and other invertebrate and vertebrate pests in agricultural, aquatic, forest, natural resource, stored products, and urban environments. Biological control of arthropod pests of human and domestic animals is also included. Ecological, behavioral, molecular, and biotechnological approaches to advancing the understanding of biological control agents are welcome.

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