

Université de Montréal

**Effets de divers facteurs biotiques et abiotiques sur le comportement de
recherche d'un partenaire sexuel chez *Aphidius ervi* (Hymenoptera : Aphidiidae)**

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Mémoire présenté à la Faculté des études supérieures

en vue de l'obtention du grade de

Maître ès Sciences (M. Sc.)

Mars 2006

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Université de Montréal
Faculté des études supérieures

Ce mémoire intitulé :

**Effets de divers facteurs biotiques et abiotiques sur le comportement de
recherche d'un partenaire sexuel chez *Aphidius ervi* (Hymenoptera : Aphidiidae)**

Présenté par :

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Président rapporteur : Jacques Brodeur

Directeur de recherche : Jeremy N. McNeil

Membre du jury : Conrad Cloutier

Résumé

L'accouplement chez le parasitoïde du puceron, *Aphidius ervi*, est stimulé par des phéromones sexuelles. Les femelles vierges produisent une phéromone à longue distance qui provoque un vol dirigé ainsi qu'une phéromone à courte distance qui élicite une parade nuptiale de la part des mâles. La phéromone à courte distance est produite au niveau de l'abdomen des femelles.

Il existe une périodicité de la production de la phéromone par les femelles et de la réceptivité des mâles à celle-ci, avec un plus grand nombre d'accouplements ayant lieu tôt le matin. L'augmentation de l'âge diminue la production de la phéromone par les femelles ainsi que sa réceptivité par les mâles. De plus, les femelles cessent de produire la phéromone quelques heures après l'accouplement. Mais comme la plupart des femelles *Aphidius* ne s'accouplent qu'une seule fois, il n'est pas surprenant qu'il y ait un déclin de la production en phéromone (pheromonostatis). Les rares cas où un deuxième accouplement a été observé étaient probablement une conséquence d'un accouplement ayant échoué ou du transfert d'un éjaculat de moindre qualité par le mâle. Un accouplement antérieur n'a pas d'effet sur la capacité des mâles à détecter la phéromone femelle.

Le comportement de recherche d'un partenaire sexuel par les mâles est influencé par la vitesse du vent. En tunnel de vol, moins de mâles ont initié un vol et ont atteint la source de phéromone à des vitesses de vent plus élevées que 50 cm/s, et le vol était inhibé à des vitesses de vent de >70 cm/s. Par contre, à ces vitesses de vent, le comportement ambulateur des mâles était soutenu, suggérant que marcher

vers une source de phéromone pourrait être une composante importante du comportement de recherche d'un partenaire sexuel chez *A. ervi*.

Les variations de la pression atmosphérique ont un effet sur divers aspects du comportement de recherche d'un partenaire sexuel d'*A. ervi*. Il y avait un effet marginalement significatif entre la proportion du nombre de mâles prenant envol et le changement absolu de la pression atmosphérique (indépendamment de la direction) dans les 24 hr, mais pas les 12 hr précédant l'expérience. Il y avait aussi une relation positive entre le nombre de mâles qui ont atteint la source et la pression atmosphérique au moment où les bioessais avaient lieu, mais pas avec le nombre de mâles qui ont pris leur envol. À ce jour, il est encore inconnu si les différences observées sont dues à l'émission de la phéromone par les femelles, sa réceptivité par les mâles, ou les deux.

L'importance des changements de la biologie reproductive due aux variations des conditions abiotiques est discutée dans le contexte de l'efficacité d'*A. ervi* en tant qu'agent de contrôle biologique. L'usage potentiel de la phéromone sexuelle pour évaluer les populations de parasitoïdes est aussi discuté.

Mots-Clés :

Aphidius ervi, parasitoïde, phéromone, accouplement, vent, pression atmosphérique.

Abstract

Mating in the aphid parasitoid, *Aphidius ervi*, is mediated by female sex pheromones. Virgin females produce a long distance pheromone that stimulates upwind flight in males and a short distance one that elicits courtship behaviour. At least in the case of the short distance pheromone, production occurs in the abdomen.

There is a diel periodicity in both the emission of, and receptivity to, the pheromone with most mating taking place in the early morning. Age influences both pheromone production by females and responsiveness in males. Furthermore, females stop pheromone production within hours of mating, but as *Aphidius* species females are generally monandrous this post-mating decline (pheromonstatis) is not surprising. The rare cases of remating are probably the result of an unsuccessful mating or due to a low quality ejaculate transferred by the male. Males having copulated prior to coming into contact with another female are not affected in their ability to respond to the female pheromone.

Male mate searching behaviour is influenced by wind velocity. Fewer males took flight and reached the pheromone source at wind speeds above 50 cm/s, and flight was inhibited at simulated wind speeds > 70 cm/s. However, at these higher wind speeds, males still exhibited ambulatory behaviour suggesting that walking to a pheromone source may be a major component of the mate searching behaviour in *A. ervi* under windy conditions.

Variations in atmospheric pressure (AP) also affected different aspects of mate searching. There correlation between the proportion of males taking flight and the absolute change in atmospheric pressure (regardless of direction) over the 24 h,

but not the 12 h preceding the experiment was marginally significant. However, these changes in AP had no effect on the proportion of males taking flight that reached the source. There was also a positive relationship between the number of males reaching the source and the prevailing atmospheric pressure at the time the bioassay was conducted, but not with the proportion of males taking flight. At this time it is unknown if the differences observed are due to female pheromone emission, male receptivity, or both.

The importance of changes in reproductive biology due to variations in abiotic conditions is discussed within the context of *A. ervi*'s potential use as a biological control agent. The potential uses of the parasitoid sex pheromone to assess parasitoid populations, and as an infochemical for aphid control, are also discussed.

Key Words:

Aphidius ervi, aphid parasitoid, pheromone, mating, wind velocity, atmospheric pressure, mate searching behaviour.

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Liste des sigles et des abréviations

AM : Avant-midi

cm : Centimètre

cm² : Centimètre au carré

cm/s : Centimètre par seconde

D : day

diam : Diamètre

Dr. : Docteur

et al : *et alia*

FE : Female equivalent

h: Hours

hr: Heures

m : Mètres

mb : Millibars

min : Minutes

n= : Nombre d'expériences réalisées

PM: Après-midi

RH : Relative humidity

sec : Secondes

VS: versus

W : watts

°C : Degré Celsius

% : Pourcentage

μ l : Microlitres

< : Moins que

> : Plus que

~ : Environ

\pm : plus ou moins

Remerciements

Je dois remercier mon directeur de thèse, le Dr. Jeremy McNeil, pour m'avoir donné l'opportunité de réaliser mes études de maîtrise sous sa direction, ainsi que pour les opportunités de participer à plusieurs congrès scientifiques.

Je tiens aussi à remercier les membres de mon comité d'encadrement, le Dr. Harper et le Dr. Legendre, pour toutes les suggestions qu'ils ont apportées à mon protocole. Je remercie tout particulièrement le Dr. Legendre dont la disponibilité et l'aide attentive m'ont permis de surmonter mes problèmes de statistiques. Pour leurs critiques judicieuses et leurs conseils fort appréciés, je remercie aussi mon comité d'évaluation, le Dr. Cloutier et le Dr. Brodeur.

Finalement, j'aimerais exprimer ma gratitude envers mes ami(e)s, les membres de ma famille, mon copain Mathieu Chouteau ainsi que sa famille pour leur support et encouragements, sans lesquels tout ceci n'aurait pas été possible. À vous tous, un très grand merci! À mes parents que je ne pourrais jamais assez remercier, je leurs dédie ce manuscrit.

1. Introduction générale

L'utilisation d'insecticides chimiques dans la lutte contre les insectes nuisibles des milieux agricoles offre certains avantages : ils sont d'action rapide et ils peuvent être appliqués quand et où on veut. Cependant, ils ne sont pas sans désavantages. Ces produits toxiques représentent non seulement un danger écologique par leur faible sélectivité, mais ils sont devenus inefficaces dans plusieurs programmes de lutte en raison de l'évolution de la résistance chez diverses espèces d'insectes (Metcalf, 1980; McNeil et Delisle, 1993). Il est donc nécessaire de promouvoir le développement et l'utilisation de méthodes plus efficaces contre les ravageurs et plus acceptables pour l'environnement. Une option est la lutte biologique à l'aide des ennemis naturels pour diminuer l'impact des herbivores. Il existe trois types d'ennemis naturels qui s'attaquent aux insectes herbivores : les pathogènes, les prédateurs et les parasitoïdes. Il est possible de les diviser en deux grandes catégories : les généralistes et les spécialistes. Les généralistes ont la particularité de s'attaquer à une vaste gamme d'espèces alors que les spécialistes s'attaquent à quelques espèces ou à une seule. Parce que les généralistes peuvent s'attaquer non seulement aux herbivores, mais aussi potentiellement aux autres ennemis naturels (prédation intragilde), il peut y avoir diminution du contrôle exercé par les autres agents de lutte biologique sur les populations d'insectes nuisibles. De plus, en s'attaquant à plusieurs espèces dont des herbivores non ciblés, les généralistes ne répondent pas aussi fortement aux fluctuations de densité d'une seule espèce cible. Pour cette raison, les spécialistes sont habituellement considérés comme étant beaucoup plus étroitement liés à leurs hôtes que les généralistes et exercent donc un plus grand contrôle sur la population de

leurs hôtes (Snyder et Ives, 2001). Chez les parasitoïdes utilisés dans la lutte biologique, appartenant à l'ordre des Strepsiptera, Hymenoptera et Diptera, il y a des spécialistes et des généralistes. De plus on peut les classer comme étant des endoparasitoïdes, dont les œufs sont pondus à l'intérieur de l'hôte, et des ectoparasitoïdes, dont les œufs sont pondus à l'extérieur de l'hôte. Dans le cadre de ma recherche, je me suis intéressée aux parasitoïdes des pucerons, car les pucerons sont d'importants insectes nuisibles et s'attaquent à une longue liste de plantes agricoles (Blackman et Eastop, 2000). Non seulement se nourrissent-ils de sève élaborée, mais ce sont d'importants vecteurs de virus (Pettersson et al., 1994). Il est donc justifié d'étudier la biologie de leurs parasitoïdes qui peuvent être très bénéfiques pour diminuer l'impact des pucerons (Spencer, 1926).

Les parasitoïdes des pucerons appartenant à l'ordre des Hyménoptères se répartissent en deux groupes : 1) Ichneumonoidea, famille Braconidae, sous-famille Aphidiinae; 2) Chalcidoidea, famille Aphelinidae. Les Aphidiinae sont répartis partout dans le monde, mais se retrouvent surtout dans les régions tempérées nordiques et subtropicales. Ils prolifèrent aussi bien dans les milieux naturels que dans les milieux agricoles et ils sont les plus importants parasitoïdes des pucerons (Homoptera : Aphididae) (Spencer, 1926; Brodeur et Rosenheim, 2000). Généralement, chez les Aphidiinae, la femelle pond un seul œuf à l'intérieur de l'hôte. Dans certaines circonstances (i.e. une quantité d'hôtes insuffisante), plus d'un œuf peut être pondu à l'intérieur d'un hôte, mais un seul individu en émergera (Cloutier et Bauduin, 1995). Puisque le développement des parasitoïdes est interne, leur temps de génération est semblable à celui de leur hôte (Snyder et Ives, 2003). Les adultes de ces parasitoïdes sont caractérisés par une longévité relativement courte

mais avec une fécondité élevée permettant aux femelles d'attaquer plusieurs hôtes durant le cours de leur vie (Debach et Rosen, 1991; Mackauer et Völkl, 1993). Les femelles sont prêtes à s'accoupler dès l'émergence, mais la ponte peut avoir lieu avec ou sans accouplement (Spencer, 1926). En effet, les hyménoptères parasitoïdes sont haplodiploïdes, i.e. les œufs fécondés se développent en femelles, et les œufs non fécondés se développent en mâles (arrhénoquie). De plus, les femelles peuvent modifier le sexe ratio de leur progéniture selon la qualité de l'hôte et pondent de préférence des œufs fécondés qui se développeront en femelles dans des hôtes de qualité supérieure (Cloutier et al., 1981; Mackauer et Völkl, 1993).

Les parasitoïdes semblent donc posséder plusieurs attributs favorables en tant qu'agents de contrôle biologique (Brodeur et Rosenheim, 2000; Snyder et Ives, 2003). Plusieurs introductions intentionnelles de parasitoïdes ont été un succès dans le contrôle biologique de ravageurs agricoles, démontrant clairement leur capacité à contrôler les densités de leurs hôtes (Greathead, 1986; Hassel, 1986; Debach et Rosen, 1991; Godfray et Cook, 1997). En revanche, les parasitoïdes ne sont pas toujours efficaces, et ce pour diverses raisons. Par exemple, *Aphidius nigripes* (Ashmead) est le parasitoïde s'attaquant au puceron de la pomme de terre *Macrosiphum euphorbie* (Thomas) le plus abondant en Amérique du Nord, mais il n'est pourtant pas considéré comme ayant un impact significatif pour le contrôle de la densité de son hôte en agriculture (Shands et al., 1965). Plusieurs hypothèses existent pour expliquer l'inefficacité de certains parasitoïdes, tels que *A. nigripes*, à contrôler la densité de population de leurs hôtes, dont l'hyperparasitisme (Brodeur et McNeil, 1994b) et les prédateurs généralistes (Lucas et al., 1998; Brodeur et Rosenheim, 2000; Snyder et Ives, 2001, 2003). Spencer (1926) avait suggéré que *A. nigripes* ne

pouvait pas survivre aux conditions hivernales et que les populations résulteraient d'immigrants provenant de région plus au sud. Ceci mènerait à un asynchronisme saisonnier entre *A. nigripes* et son hôte. Par contre, Brodeur et McNeil (1989) ont démontré que *A. nigripes* a la capacité d'entrer en diapause et qu'il est présent dans l'habitat en début de saison en même temps que son hôte (Brodeur et McNeil, 1994a).

Mackauer et Völkl (1993), quant à eux, ont postulé que l'inefficacité des parasitoïdes primaires du puceron serait due à certains aspects de leur comportement de recherche d'un hôte. Afin de mieux comprendre l'échec de ces agents de lutte biologique, plusieurs études ont porté sur des aspects du comportement de recherche des femelles, tels que les divers stades auxquels l'hôte est parasité, car ils peuvent varier en qualité et peuvent donc avoir des conséquences importantes sur le succès du parasitisme (Cloutier et al., 1981; Sequeira et Mackauer, 1994). Le comportement de recherche a donc surtout été sujet d'études au niveau des femelles, car ces dernières sont en mesure d'influencer directement la densité de leurs hôtes. Il a été démontré que les infochimiques régissent les comportements de recherche d'un hôte chez les femelles. De nombreuses études ont démontré l'existence de substances chimiques émises par la plante hôte lorsque attaquée par l'herbivore (synomones) qui sont attirantes pour les entomophages (Du et al., 1998; Guerrieri et al., 2002). De plus, il a été démontré que le miellat, l'excrément des pucerons (Bouchard et Cloutier, 1984; Bouchard et Cloutier, 1985; Budenberg, 1990; Budenberg et al., 1992) et la phéromone sexuelle du puceron (Keller, 1990; Hardie et al., 1991, 1994) sont tous deux utilisés en tant que kairomones. Il a été suggéré que l'application de ces infochimiques dans l'objectif de modifier les comportements de recherche serait une possibilité envisageable pour augmenter l'impact des parasitoïdes sur les ravageurs

(Greathead, 1986; Lewis et Martin, 1990; McNeil et Brodeur, 1995; Suckling et al., 2002).

L'accouplement est essentiel pour la survie à long terme d'une population, et de ce fait même, pour un contrôle continu de l'hôte par les parasitoïdes. Les facteurs pouvant inhiber la recherche d'un partenaire sexuel mèneraient à un sexe ratio de la population biaisé vers les mâles et éventuellement à l'extinction locale (Schwörer et al., 1999). Pourtant, très peu d'attention a été portée sur le comportement de recherche d'un partenaire sexuel par les mâles. Plusieurs études sur le comportement d'accouplement des parasitoïdes ont démontré la présence des phéromones sexuelles (Vinson, 1972; Yoshida, 1978; Decker et al., 1993; Shu et Jones, 1993; Syvertsen et al., 1995; Nazzi et al., 1996; Tillman et al., 1999) bien que l'identification chimique soit connue chez très peu d'espèces de parasitoïdes (Eller et al., 1984; Kainoh et al., 1991; Swedenborg and Jones, 1992; Swedenborg et al., 1993; Syvertsen et al., 1995). Chez *A. nigripes*, les mâles ne semblent pas répondre aux infochimiques dont les femelles se servent pour trouver un hôte (Bouchard et Cloutier, 1984) même s'ils pourraient servir d'indices indirects dans la recherche d'un partenaire sexuel potentiel en fournissant des informations sur les sites de ponte. Cependant, il semblerait exister deux phéromones femelles ou divers composantes d'une même phéromone qui élicite différents comportements chez les mâles : une phéromone à longue distance dont les mâles se servent pour voler ou marcher vers la source et une phéromone sexuelle à courte distance qui initie une tentative d'accouplement (Bouchard et Cloutier, 1985; McNeil et Brodeur, 1995).

Quoique la source de la production de la phéromone à courte distance chez les parasitoïdes soit souvent inconnue, l'abdomen semble être l'emplacement

anatomique le plus commun (Yoshida, 1978; McNeil et Brodeur, 1995; Syvertsen et al., 1995; Nazzi et al., 1996; Tillman et al., 1999). La phéromone sexuelle à courte distance déclenche quatre éléments essentiels du comportement d'accouplement du mâle : l'orientation, la vibration des ailes, le toucher à l'aide des antennes pour se positionner par rapport à la femelle et la tentative d'accouplement (Matthews, 1975). La vibration des ailes chez les mâles est une étape cruciale avant la copulation et, en conséquence, elle est utilisée dans les bioessais afin de confirmer la présence de phéromone sexuelle ainsi que sa provenance (McNeil et Brodeur, 1995; Nazzi et al., 1996). La signification du comportement de vibration des ailes n'est pas encore bien comprise, mais on pense que les sons produits jouent un rôle de signal acoustique agissant à courte distance (Field et Keller, 1993; Briceno et al., 2002; Mankin et al., 2004). De plus, des observations faites avec de la poussière de craie révèlent que la vibration des ailes tire l'air de l'avant à l'arrière des mâles, et ce passage d'air sur les antennes permet aux mâles parasitoïdes de s'orienter vers la source d'odeur (Vinson, 1972). Cependant, l'orientation à proximité d'un autre insecte peut aussi être guidée par des indices visuels car, en présence de la phéromone femelle, les mâles peuvent s'orienter et tenter de s'accoupler avec d'autres mâles (Shorey, 1976). Battaglia et al. (2002) ont démontré la présence de glandes au niveau des antennes de *A. ervi* qui sécrètent une phéromone à courte distance libérée lorsque les mâles frottent leurs antennes sur celles des femelles. Il est possible aussi que cette phéromone soit transmise dans l'air vers la femelle par la vibration des ailes. Cette phéromone déclencherait l'acceptation du mâle par la femelle et l'initiation de l'accouplement.

Marchand (1998) a suggéré l'utilisation de la phéromone sexuelle en combinaison avec des kairomones pour attirer à la fois les mâles et les femelles dans

l'habitat du ravageur et ainsi conserver le sexe ratio de la population du parasitoïde. Mais pour que ces méthodes soient envisageables, une connaissance plus approfondie des facteurs influençant l'émission de la phéromone sexuelle par les femelles, sa réceptivité par les mâles et le comportement de recherche des mâles est nécessaire. Il est donc important de bien comprendre l'influence des divers facteurs biotiques et abiotiques sur l'émission et la réception des phéromones sexuelles chez les parasitoïdes, car de telles informations sont très utiles pour choisir la période et l'endroit convenant le mieux au déploiement des agents de lutte biologique. Par contre, jusqu'à maintenant, la majorité des observations sur les effets des facteurs biotiques (e.g. âge, accouplement, présence de conspécifiques, plantes hôtes) et abiotiques (température, lumière, vent, humidité relative et changement de la pression atmosphérique) sur la production et la détection des phéromones sexuelles a été faite sur les Lépidoptères (voir McNeil 1991 et références citées).

Les parasitoïdes femelles sont habituellement monandres (Gilchrist, 1996) et prêtes à s'accoupler peu de temps après l'émergence (Spencer, 1926). La production de la phéromone sexuelle suit une périodicité journalière, qui semble diminuer avec l'âge des femelles et les femelles accouplées sont peu attirantes pour les mâles (Mackauer, 1969; Matthews, 1975; Yoshida, 1978; McNeil et Brodeur, 1995; Godfray et Cook, 1997; Schwörer et al., 1999). La réceptivité des parasitoïdes mâles pour la phéromone peut varier avec l'âge (McNeil et Brodeur, 1995; Schwörer et al., 1999). Comme chez d'autres insectes, les parasitoïdes mâles peuvent s'accoupler plusieurs fois (Matthews, 1975; van den Assem, 1986; Schwörer et al., 1999). Cependant, aucune étude n'a tenté de déterminer si des accouplements préalables avaient un impact sur leur réceptivité pour la phéromone sexuelle. Il existe plusieurs

études sur les effets des facteurs abiotiques sur la dynamique des populations des parasitoïdes (Weisser et al., 1997), car il arrive souvent qu'un parasitoïde réduisant efficacement la population d'un insecte nuisible dans son lieu d'origine soit inefficace, en raison des différences climatiques, lorsque introduit ailleurs pour réduire l'impact du même ravageur (Juillet, 1964; Askew, 1971; Debach et Rosen, 1991). La température ainsi que l'humidité sont des facteurs très importants pour la survie et le succès reproducteur de nombreux insectes (Johnson, 1969; Cardé et Baker, 1984; Bell, 1991) et certaines températures sont plus favorables au parasitisme (Wang et al., 1997). La pluie a un fort effet mécanique et il a été démontré qu'une fraction significative de la population est détruite lors de fortes précipitations (Fink et Völkl, 1995). De plus, il y a une baisse du taux de parasitisme due aux mouvements des parasitoïdes qui sont entravés par les gouttes d'eau, ainsi qu'une difficulté à identifier l'hôte lorsque ce dernier est recouvert d'une fine pellicule d'eau (Fink et Völkl, 1995). Le vent aussi est un facteur important étant donné la taille des parasitoïdes et le faible seuil des vitesses de vent pouvant inhiber leurs déplacements est fréquent dans les milieux naturels (e.g. Juillet, 1964; Casas, 1989; Keller, 1990; Decker et al., 1993; Jönsson et Anderbrant, 1993; Fink et Völkl, 1995; Messing et al., 1997; Weisser et al., 1997; Marchand et McNeil, 2000). Decker et al. (1993) ont observé que les *A. rhopalosiphi* mâles avancent vers une source de phéromone en une série de vols de courte distance et qu'ils utilisent leurs antennes afin de rester en contact avec la plume de phéromone et de déterminer la direction de leur prochaine envolée. Casas (1989) a observé qu'à certaines vitesses de vent les insectes se posent sur la feuille la plus proche et prennent la position offrant le minimum de résistance et Keller (1990) a mis en évidence que le temps de latence avant l'envol augmente

avec la vitesse du vent chez *Cotesia rubecula* (Braconidae). La marche pourrait donc être un mode de locomotion important dans la recherche d'un partenaire sexuel chez les insectes de petite taille, tel que démontré pour les pucerons mâles (Goldansaz et McNeil, 2006). Ceci a déjà été observé chez des parasitoïdes (Decker et al., 1993; Messing et al., 1997), mais n'a toutefois jamais été examiné expérimentalement en laboratoire.

En percevant des indices précurseurs, telles les chutes de pression atmosphérique, les insectes peuvent modifier leur comportement reproducteur avant que la pluie ou les vents violents ne diminuent leur espérance de vie ou leur capacité de déplacement. Lanier et Burns (1978) ont observé que les Coléoptères *Scolytus multistriatus* et *Ips pini* (Scolytidae) répondaient moins bien à leur phéromone d'agrégation lorsque la pression atmosphérique ambiante chutait et il est raisonnable que ces insectes ne se déplacent pas lorsque les conditions ne favorisent pas l'atteinte de la source, tels que le vent et la pluie. Steinberg et al. (1992) ont observé une corrélation entre les variations de la pression atmosphérique et le vol des femelles parasitoïdes *Cotesia glomerata* (Braconidae) vers les complexes plante/hôte. Roitberg et al. (1993) ont conclu que les femelles *Leptopilina heterotoma* (Hymenoptera: Eucoilidae) étaient plus actives et qu'elles rencontraient et parasitaient davantage d'hôtes lors d'une baisse de la pression atmosphérique, mais qu'elles étaient moins discriminantes quant à la qualité de leurs hôtes. Tout comme le comportement de recherche d'un hôte (Fink et Völkl, 1995), le comportement de recherche d'un partenaire sexuel par les mâles peut être influencé par les conditions météorologiques (Schwörer et al., 1999). Quoique le comportement de recherche des femelles représente un facteur important dans la détermination du nombre de descendants

produits et soit donc étroitement lié au succès reproducteur (Weisser et al., 1997) pour la dynamique de la population à long terme, la stratégie des mâles pour localiser une partenaire et copuler est tout aussi importante que le succès des femelles à localiser des hôtes (Schwörer et al. 1999). Pourtant peu d'études se sont penchées sur la question (Schwörer et al., 1999; Marchand et McNeil, 2000) et il est important de corroborer les résultats obtenus afin de vérifier si les hypothèses générales tiennent pour d'autres espèces.

Le parasitoïde *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae) est natif de l'Europe et a été introduit en Amérique du Nord vers 1960 comme agent biologique contre le puceron du pois, *Acrythosiphon pisum* (Harris) (González et al., 1978). Il est répandu en tant que parasitoïde du puceron du pois sur la luzerne et le trèfle dans plusieurs régions de l'Amérique du Nord (Michaud et Mackauer, 1994). Depuis, il a aussi été introduit en Amérique du Sud et en Australie (Schwörer et Völkl, 2001). Quelques études ont été faites sur le comportement de recherche des femelles (Du et al., 1998; Schwörer et al., 1999; Guerrieri et al., 2002) et sur l'acceptation des divers stades larvaires des hôtes (Sequeira et Mackauer, 1994). Mais hormis la description du comportement d'accouplement par Mackauer (1969) et de l'étude par Battaglia et al. (2002) sur le rôle des antennes lors de l'accouplement, très peu d'études ont été réalisées sur sa biologie reproductrice.

Problématique :

Cette recherche vise à décrire le comportement d'accouplement de *A. ervi* en mettant l'emphasis sur les facteurs biotiques et abiotiques pouvant influencer la production de phéromones chez les femelles et la réceptivité des mâles à celle-ci.

J'ai choisi d'examiner différents aspects de la biologie reproductrice d'*A. ervi* qui est stimulée par une phéromone sexuelle. Dans un premier temps, cette étude cherche à déterminer (i) l'origine anatomique de la production de la phéromone, (ii) s'il y a une périodicité journalière dans sa production par les femelles ou dans sa détection par les mâles, et (iii) si l'âge et/ou un accouplement antérieur ont un effet sur la production ou sur la détection de la phéromone. Dans un deuxième temps, des tests de capture sur le terrain à l'aide de pièges collants ont été menés afin de corroborer les résultats obtenus en laboratoire.

Quant à l'effet de certains facteurs abiotiques sur le comportement de recherche d'une partenaire sexuelle par les mâles, j'ai (i) étudié l'effet de la vitesse de vent (sous des conditions contrôlées en tunnel de vol) sur les capacités du parasitoïde à voler, et (ii) testé si les vitesses de vent élevées inhibent aussi leur capacité ambulatoire. J'ai aussi (iii) examiné s'il existe une relation entre les variations de pression atmosphérique et le comportement de recherche d'un partenaire sexuel des mâles d'*A. ervi*.

L'importance des résultats obtenus sera discutée dans le contexte de la biologie reproductive et de l'efficacité d'*A. ervi* en tant qu'agent de contrôle biologique.

2. Article 1 : Courtship behaviour in relation to the female sex pheromone in the parasitoid *Aphidius ervi* (Hymenoptera : Aphidiidae)

Article accepté dans Journal of Chemical Ecology

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Abstract- Mating in the aphid parasitoid, *Aphidius ervi*, is mediated by sex pheromones. Virgin females produce pheromones that stimulate both upwind flight and elicit close range courtship behaviour by males. Field studies and laboratory bioassays clearly demonstrated that the time of day and adult age affects both the emission of, and receptivity to, the sex pheromones. In contrast, mating affected female pheromone production but not male responsiveness.

Key Words-*Aphidius ervi*, parasitoid, pheromone, mating

2.1 Introduction

The use of natural enemies for the biological control of insects has become an integral part of many pest management programs and it has been suggested that their efficiency may be increased through the use of host associated chemical cues (McNeil and Brodeur, 1995). Honeydew (Budenberg, 1990; Bouchard and Cloutier, 1984, 1985; Budenberg et al., 1992) and the host aphid sex pheromone (Hardie et al., 1991, 1994; Keller, 1990) are potential kairomones that could increase the impact of aphid parasitoids.

Parasitoid sex pheromones may also have a potential use for monitoring abundance in the field, detecting the start of parasitoid activity early in the season and predicting the incidence of parasitism. In addition, the female sex pheromones might be used to attract males, if the kairomones mentioned above were deployed to attract females into the desired habitat. However, to date there are not a large number of detailed studies on the pheromone-mediated mating of parasitoids and even fewer cases where the sex pheromones have actually been identified (Eller et al., 1984, Kainoh et al., 1991; Swedenborg and Jones, 1992; Swedenborg et al., 1993; Syvertsen et al., 1995).

Previous work on the pheromone-mediated mating of the aphid parasitoid, *Aphidius nigripes*, (Ashmead) (Hymenoptera: Braconidae) reported a diel periodicity in mating. This was modulated by two female pheromones, one that stimulated male upwind flight from a distance and a second, short distance one, present in the cuticle of the female. (McNeil and Brodeur, 1995; Marchand and McNeil, 2000). The short distance stimulated male courtship behavior when the male is in close proximity to

the female but does not elicit upwind flight. Furthermore, there was a significant, and permanent reduction in pheromone production following mating (McNeil and Brodeur, 1995). In order to determine whether the patterns observed hold true for other species in the genus we undertook a study of the pheromone-mediated mating behavior of *Aphidius ervi* Haliday. *Aphidius ervi* is a solitary endoparasitoid that was introduced into North America from Europe in the 1960s as a biocontrol agent for the pea aphid (*Acrythosiphon pisum* (Harris)) (Gonzalez et al., 1978), where it is now also found on alfalfa in certain regions (Michaud and Mackauer, 1994). More recently it has been introduced as a biological control agent into South America and Australia (Schwörer and Völkl, 2001). Some research has been done on the foraging behaviour of *A. ervi* females (Du et al., 1998; Schwörer et al., 1999; Guerrieri et al., 2002), and on the acceptance of various larval stages as hosts (Sequeira and Mackauer, 1994). However, few studies have examined the reproductive biology of this species. Mackauer (1969) compared *A. ervi*'s mating behaviour to two other parasitoid species that attack the pea aphid, while Battaglia et al., (2002) studied males' antennation behaviour during courtship. In this paper we present (i) a description of *A. ervi* mating behaviour that is mediated by female sex pheromones, and (ii) the results from laboratory and field experiments, run to test the hypotheses that like *A. nigripes*, age and mating status affect both female production of, and male receptivity to, sex pheromones.

2.2 Materials and Methods

Insects. The pea aphid, *A. pisum* (Harris) and *A. ervi* colonies were established using material provided by Agriculture and AgrifoodCanada, London. Parasitoids used in these experiments came from mummies collected from aphid-infested broad bean plants (*Vicia faba* L) reared at room temperature (~24°C), under natural light conditions (>15 h light per day).

Newly-formed mummies were collected, isolated in individual gelatine capsules and observed daily for emergence. Each day the adults were sexed, males and females placed in separate plastic holding cages with a 20% sucrose solution, and held until needed. In all cases, unless specifically stated, naïve virgin individuals of known age were used in all assays.

McNeil and Brodeur, (1995) found little evidence of either male or female age affecting the *A. nigripes* male response to extracts of the short distance pheromone. The absence of age effects was somewhat surprising given that there are significant age-related changes in both the production of, and response to, sex pheromones in Lepidoptera (see McNeil, 1991 and references therein). However, in their study the experimental design of the field trials did not allow for a direct assessment of female age effects on the long distance pheromone. Therefore, in the current study we included age as a factor in several of the experiments to test the hypothesis that age would affect both female attractiveness and male responsiveness to pheromones in parasitoids, as reported for moths. We also varied the time of day at which certain assays were conducted to test the hypothesis that mating success, at least in part, was

associated with a diel periodicity of both female production of, and male response to, the short-range pheromone.

Mating behaviour. A newly emerged virgin (<24 h) male and female were paired to observe the behaviours displayed during courtship and copulation. The duration of the pursuit phase, antennation during courtship and the time spent in copula were measured. To develop an ethogram, 217 naive couples were observed and terminated after 6 min, if mating did not occur, as the probability of copulation was low after 3 min (Mackauer, 1969). Observations were made at room temperature (22-25°C) between 7 and 10 am, as *A. ervi* generally mates in the morning (Mackauer, 1969) and, unless specifically stated, all experiments were carried out during this same time period.

In addition the following mating experiments were conducted, to determine:

(i) if age affected mating success, by comparing the incidence of mating and time to mate in pairs consisting of a newly-emerged virgin male or female with either a newly-emerged or 8-day-old member of the opposite sex.

(ii) the effect of mating status on subsequent mating, by pairing a 1-day-old virgin male or female with a 1-day-old individual of the opposite sex that was either a virgin or that had previously mated on the day of emergence. In the case of females, an additional experiment was carried out, where the incidence of mating of 1-day old virgins was compared with that of similar aged females recently mated <2 h prior to being paired with 1-day-old virgin males.

(iii) if there is a diel periodicity in mating activity, by comparing mating in pairs of virgin males and females set up at 08:00 and 18:00 on the day of emergence.

Twenty-five couples were observed in each experiment and the assays carried out over several days to account for any daily variability due to atmospheric conditions (Marchand and McNeil, 2000).

Bioassays with cuticular extracts. To establish the optimal extraction time, a preliminary experiment was carried out, by soaking newly emerged females in 300 μ l of methylene chloride for periods between 180 and 720 sec. The 540 sec extract gave the highest level of male response (wing fanning behaviour) and shortest reaction time following the introduction of the source, so this extraction time was used for all subsequent experiments. Unless specifically stated, all extracts were prepared soon after the lights-on period. Each extract was prepared using 3 virgin females, as McNeil and Brodeur (1995) showed that there could be considerable variability in male response to extracts of individual *A. nigripes* females.

Each assay was carried out using 3 μ l [0.01 female equivalents (FE)] placed on a 0.38-cm² filter paper disk. All experiments, testing 10 individual 24- to 48-h old naïve virgins, were run between 7:00 and 10:00 AM, and replicated three times. The extracts were tested on consecutive days, thereby minimizing any possible daily or atmospheric pressure effects which have been reported in other studies (Lanier and Burns, 1978; Steinberg et al., 1992; Roitberg et al., 1993; Marchand and McNeil, 2000). The solvent was allowed to evaporate and the disk was then introduced into an 8-dram glass shell vial containing a 24- to 48-h old virgin male parasitoid. Two variables were measured, the proportion of males that responded and the time to respond to the stimulus. Males were considered responsive if they exhibited rapid wing-fanning, the first step in the courtship behaviour (Decker et al., 1993; McNeil and Brodeur, 1995), within 60 sec of exposure to the stimulus.

The following experiments were carried out to determine:

(i) the origin of the cuticular pheromone, by comparing the response of 1-day-old virgin males to extracts from the abdomen and the remainder of the body of 1-day-old virgin females.

(ii) the degree of inter-female variability, by comparing responses to extracts of 10 different <1-day- old virgin individuals.

(iii) if there is a diel periodicity in both female production of, and male responsiveness to, the pheromone. The responses of 1-day-old virgin males to female extracts from 1-day-old virgin females, prepared at 07:00 and 18:00, were tested at both 07:00 and 18:00.

(iv) if female age affected the concentration of pheromone present, by comparing the response of 1-day-old males to extracts from 0-, 2-, 4-, 6-, 8-, 10- and 12- day-old virgin females.

(v) if the responsiveness of males was age dependent, by comparing the response of 0-, 2-, 4-, 6-, 8-, and 10- day-old virgin individuals to a standard pheromone extract from 1-day-old virgin females .

(vi) to what extent mating affected both attractiveness of, and receptivity to, the cuticular pheromone. To determine attractiveness, the response of 1-day-old virgin males to extracts from 24- to 48-h old virgin and mated (24 h earlier) was compared. For receptivity, the responsiveness of 24- to 48-hr old virgin and mated (24 h before) males to an extract from 1-day-old virgin females was compared.

Field Trapping. In all experiments Delta sticky traps were installed at 10 m intervals, 30 cm above the crop canopy, in an alfalfa plot at Agriculture and Agrifood Canada in London, Ontario. A plastic cylindrical cage (8 cm long and 4 cm of diam),

with both ends covered with an open mesh cloth to allow air circulation, was suspended from the roof of each trap. The cages contained a female, with a 20% sugar water solution as a food source or, in the case of controls, just sugar water. All trials were carried out over a 24 h period, starting at 13 h, and the number of males captured in each trap was recorded. All parasitoids caught in the sticky traps were collected, sexed and identified using the key of the hymenopterous parasites of the pea aphid found in eastern North America (Mackauer and Finlayson, 1967). A subsample of those identified as *A. ervi* were sent to Agriculture and Agrifood Canada for confirmation.

The following experiments were carried out, to determine;

(i) the level of inter-female variability. Twelve newly emerged females and three controls were randomly distributed along a trap line and the number of males caught by each female was determined over a three day period. Each day the number of males captured were counted and removed, and the position of each trap re-randomized, to account for any possible effects of trap location.

(ii) the effect of female age on pheromone production by comparing the attractiveness of newly-emerged, 5- and 10-day old virgin females. Three females in each category and three controls were tested each day. The experiment was repeated four times.

(iii) if mating affected pheromone production. The attractiveness of 2-day-old females mated on day 1 was compared with that of 2-day-old virgins. Six individuals of each mating category and three controls were tested each day. The experiment was replicated four times.

Statistical Analyses. In the laboratory assays the proportion of males responding was analyzed using chi-square ($\alpha = 0.05$). For male reaction time as a function of either male or female age a 1-way ANOVA was used, while in all other assays a t-test was used. For the field trials, the number of males captured in traps containing live females was analysed by one-way ANOVA, after normality was verified using a Shapiro-Wilk test.

2.3 Results

Mating behaviour. The sequence of events leading to copulation is shown in Figure 1. In the absence of females, males move actively around the arena, waving their antennae in various directions with their wings held over their abdomen. However, when a female is detected, the male increases walking speed and starts wing fanning. Initially there are distinct wing fanning bouts, but this behaviour becomes almost continuous by the time he contacts the female. The male mounts the female from the back or the side and aligns along her body axis. He advances and places his antennae between those of the female, rapidly moving his own from side to side and making repeated contact with the female's antennae. This antennal stroking is a pre-requisite to successful copulation and probably involves a male contact pheromone present on his antennae that influences female mate choice (see Battaglia et al. 2002). If the female is receptive, she stops moving, raises her abdomen and positions her antennae laterally, where they remain immobile for the duration of mating. Once the female takes the acceptance position, the male moves backwards and curves his abdomen downwards. Once the genitalia are locked, the female

MATING BEHAVIOUR

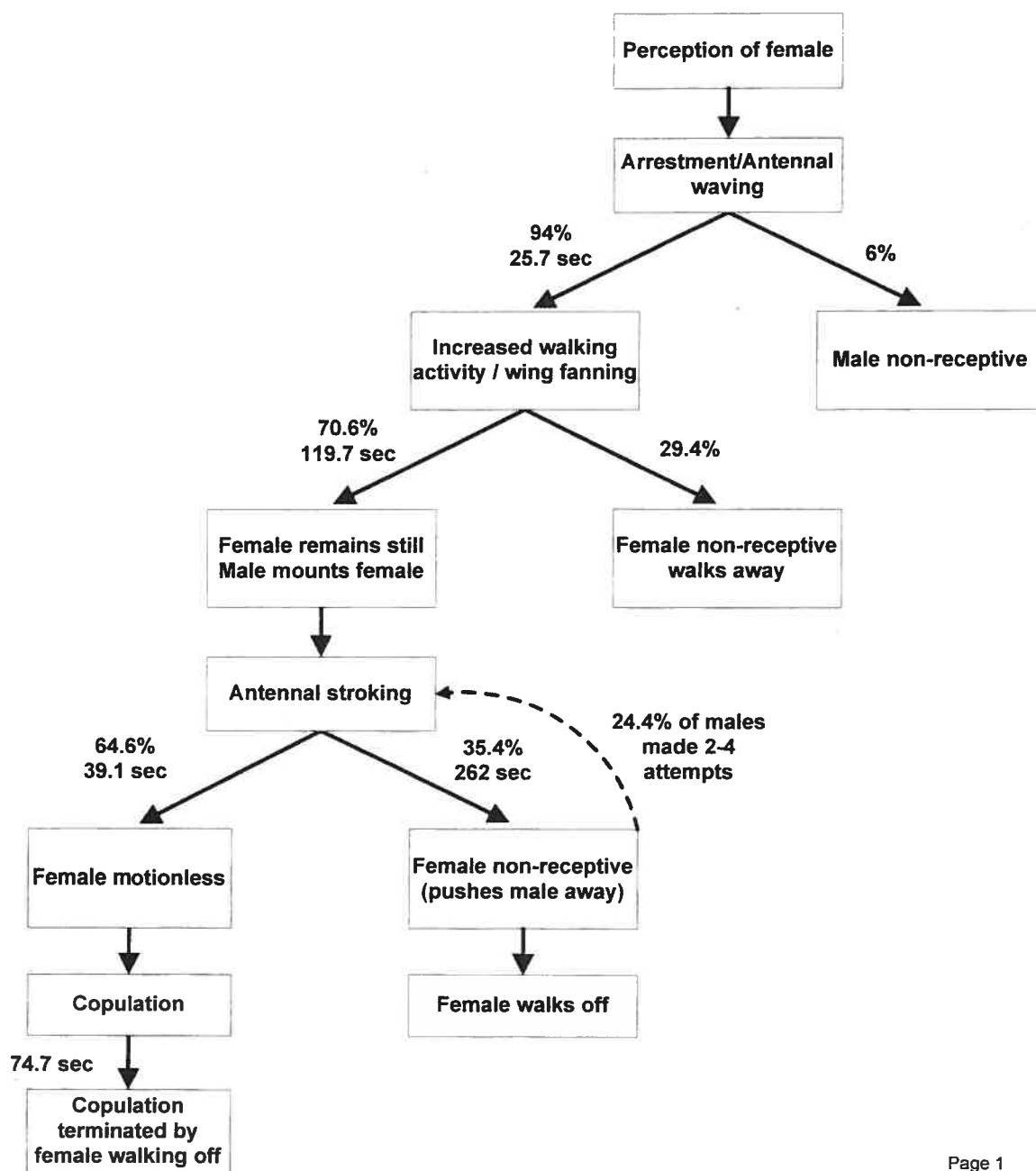


Figure 2. 1: A flow chart of mating behaviour of male *Aphidius ervi* (N=217). Percentages indicate the proportion of virgin individuals, < 24 hours old, displaying each behaviour and the mean duration (in seconds)

remains completely immobile. Initially, the male continues to slowly antennate the female's antennae/head, but in the final seconds of mating he stops and places his antennae, immobile, above his head. Mating terminates when the female starts walking forward, at which time the male dismounts. Unreceptive females often fly or walk away upon first contact. However, if the male succeeds in mounting an unreceptive female, she continues to move her antennae, flit her wings, lower her head, curve her abdomen forward between her legs and push the male with her hind legs. About 70% of males tested were successful in mounting the female (Figure 2.1) and about 80% of those subsequently succeeded in mating (Figure 2.2). If a male was unsuccessful on the first attempt after he mounted the female he often sustained antennal stroking, which may occur prior to or following the first attempt to engage her genitalia. However, the probability of success on subsequent attempts decreases considerably (Figure 2.2). Unsuccessful males dismount and move away.

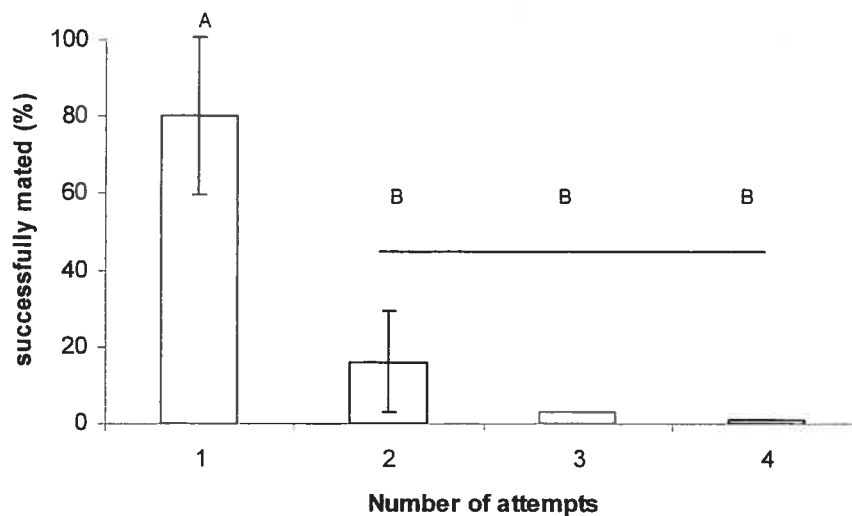


Figure 2. 2: The incidence of mating success of *Aphidius ervi* males (Mean \pm SEM), as a function of the number of attempts to copulate with the same virgin female during a courtship bout (N=217)

There is a significant effect of both male and female age, as well as the time of day, on male responsiveness (wing fanning, time to respond and time to attempt mating) and mating success of *A. ervi* (see Table 2.1). The highest response level and shortest time to respond, as well as incidence of successful mating, were observed with pairs made up of newly-emerged adults. Responsiveness and mating success declined, and the time to respond increased, when older males or females were tested, as well as when the bioassays were carried out at the end of the afternoon rather than soon after lights-on (Table 2.1). The duration of mating varied in the different assays but no consistent pattern was evident.

Mating, even 2 h before, significantly reduces female attractiveness, as seen by a lower incidence of male response (Table 2.1). When males did attempt to mate, females generally moved away, exhibited wing flitting and curving of her abdomen forward between her legs. Thus, there was a very low incidence of remating (Table 2.1). In no choice situations, the mating success of previously mated males did not differ significantly from that of similar aged virgins. It did, however, take significantly longer for them to react to the stimulus, but the mating duration was not significantly different (Table 2.1).

Table 2. 1: The effect of female and male age and mating status, as well as time of day assays were conducted, on the number of *A. erivi* males (N=25) that responded to the presence of a female, attempted and succeeded to mate, as well as the time (in seconds) it took them to do so. Values represent averages \pm standard deviation

	Number	Time to wing fanning	Number attempting to mate	Time to mating attempt	Number succeeding	Duration of copulation
1-day old virgin female	24	19.7 \pm 5.5	17	132.1 \pm 36.4	12	73.2 \pm 8.3
8-day old virgin female	18*	47.7 \pm 14.3*	5*	90.4 \pm 39.7	0*	.
1-day old virgin male	25	20.0 \pm 6.7	19	104.4 \pm 23.6	11	66.3 \pm 5.8
8-day old virgin male	11*	106.6 \pm 45.1*	4*	18.7 \pm 5.9*	1*	41.4
1-day old virgin male	23	23.6 \pm 5.6	19	107.5 \pm 21.1	14	69.7 \pm 6.3
1-day old mated male	20	62.4 \pm 25.1*	13	161.6 \pm 51.2*	10	75.7 \pm 10.1
1-day old virgin female	25	22.7 \pm 8.4	19	133.1 \pm 24.6	13	71.2 \pm 5.9
1-day old female mated 24h prior	16*	45.6 \pm 23.0*	7*	117.2 \pm 63.4	4*	99.8 \pm 20.2
1-day old virgin female	24	26.7 \pm 10.4	21	113.2 \pm 25.0	13	74.4 \pm 6.5
1-day old female mated 2h prior	17*	19.6 \pm 4.2	8*	211.0 \pm 83.1*	0*	.
1-day old virgins, tested in a.m.	21	26.8 \pm 7.8	17	126.8 \pm 23.4	13	73.2 \pm 9.5
1-day old virgins, tested in p.m.	16	16.7 \pm 3.2*	9*	160.4 \pm 45.5	4*	58.4 \pm 13.4

* : significant at $p < 0.005$

Bioassays with cuticular extracts. The extracts of virgin females elicited a strong wing fanning response from males, while the whole body extract of a male did not (90% VS 0%). The source of pheromone appears to be in the abdomen, as abdominal extracts elicited significantly higher responses than those of the head/thorax (66.67 ± 5.77 VS 6.67 ± 11.55 SEM; $X^2=23.254$; $df = 1$; $P < 0.001$). However, when males did respond, the mean reaction time was similar regardless of the origin of the extract ($t=2.307$; $df = 20$; $P=0.144$; data not shown). The proportion of males responding to individual female extracts varied, but these differences were not significant ($X^2=12.329$; $df= 9$; $P=0.195$; data not shown) and mean reaction time was similar for all females ($F=1.379$; $df1=9$, $df2=101$; $P=0.207$; data not shown).

The number of males that responded changed significantly depending on the time of day the female extract was prepared and the time at which the assays were carried out ($X^2=16.175$; $df = 3$; $P=0.001$; Figure 2.3). These results suggest a diel periodicity in both female production of the short range sex pheromone and the male's receptivity. However, for those males that did respond their response time did not differ significantly, regardless of the time the extracts were taken or the time the assays were carried out ($F=0.568$; $df1=3$, $df2=34$; $P=0.640$).

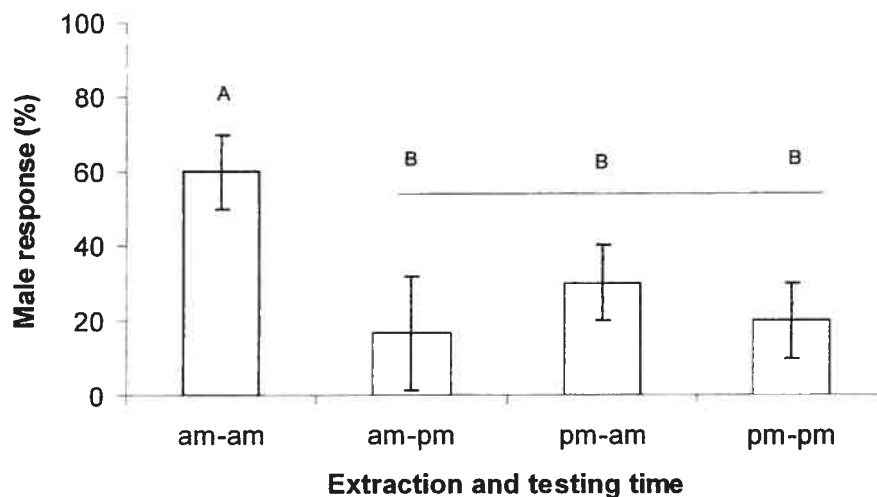
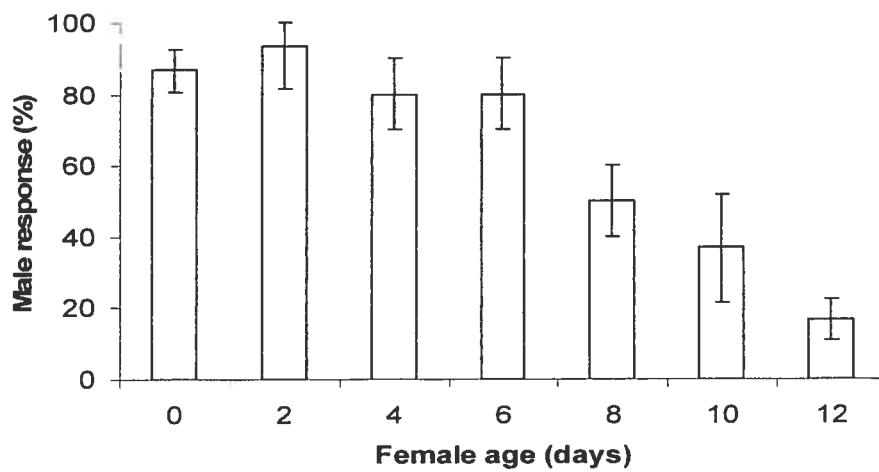


Figure 2. 3: The proportion of *Aphidius ervi* males (N=30) responding at 7 am and 6 pm to 3- μ l (0.01 FE) of methylene chloride extracts from 0 to 24-hr old virgin females at the same hours (Mean \pm SEM). On the X axis, the first value for each trial indicates the time at which the female extract was made and the second is the time at which the males were tested.

The responsiveness of 24h-old males to extracts of virgin females changed with female age ($X^2= 65.455$; $df =6$; $P<0.001$; Figure 2.4A), with a noticeable decline occurring with females > 6-days-old. This is associated with an increase in time to wing fan, with females >8-days-old ($F=2.533$; $df1=6$, $df2=114$; $P=0.024$; Figure 2.4B). Similarly, male age affected responsiveness to a standard virgin extract, both with respect to the proportion that responded ($X^2= 19.945$; $df =5$; $P=0.001$; Figure 2.5A) as well as the time to respond ($F=3.487$; $df1=5$, $df2=123$; $P=0.006$; Figure 2.5B), and this was due to males >6-days-old.

A)



B)

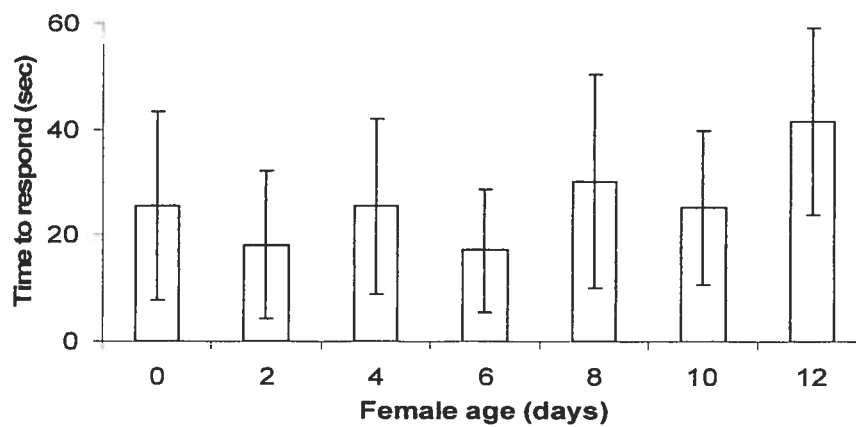
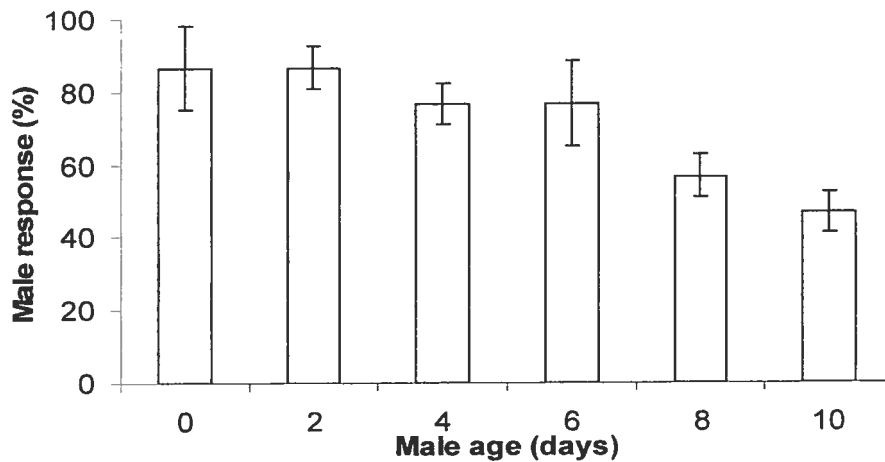


Figure 2. 4: The effect of female age on a) the proportion (N=30) of *Aphidius ervi* males responding and b) the reaction time of responding males to 3- μ l (0.01 FE) methylene chloride extracts from virgin females (Mean \pm SEM)

A)



B)

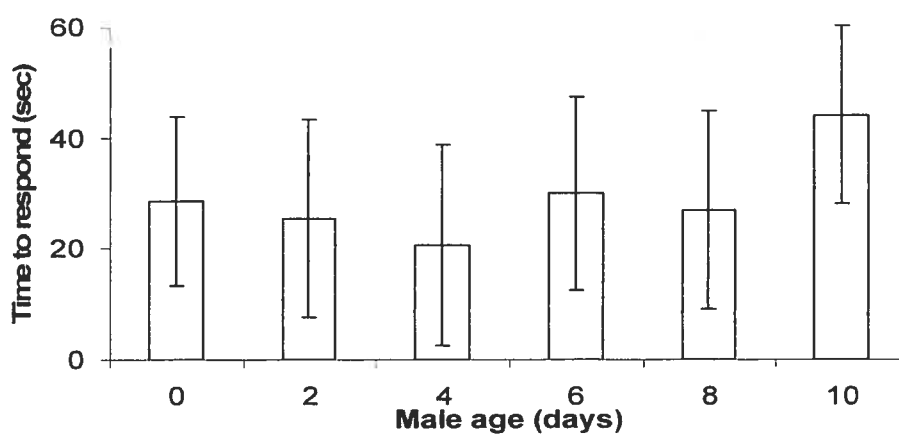


Figure 2. 5: The effect of male age on a) the proportion (N=30) of *Aphidius ervi* males responding and b) the reaction time of responding males to 3-μl (0.01 FE) methylene chloride extracts from 0 to 24-hr old virgin females (Mean±SEM)

Extracts from mated females were considerably less attractive than those of similar aged virgin females (6.67 ± 5.77 VS 86.67 ± 5.77 ; $X^2=38.571$; $df = 1$; $P < 0.001$) but, as seen in the mating trials, the mean reaction time of those males that did

respond was unaffected (26.71 ± 22.06 VS 25.55 ± 17.84 ; $t=0.008$; $df=26$; $P=0.929$). In contrast, previous male mating history did not affect the level of response (73.33 ± 5.77 VS 86.67 ± 11.55 ; $X^2=1.667$; $df=1$; $P=0.197$) or the time to respond (23.76 ± 16.97 VS 28.6 ± 15.34 ; $t=0.123$; $df=46$; $P=0.727$) to a standard female extract.

Field Trapping. The number of males captured per day (ranging from 0 to 8 per trap) suggests that there is considerable inter-female variability in the long distance pheromone produced by newly-emerged virgins ($F=3.500$; $df_1=14$, $df_2=30$; $P=0.002$; Figure 2.6). Furthermore, there was a significant decline in the attractiveness of virgin females with age ($F=10.014$; $df_1=3$, $df_2=56$; $P=0.000$; Figure 2.7). Mating also reduced female attractiveness, with mated females capturing significantly fewer males than virgins of the same age ($F=6.092$; $df_1=2$, $df_2=54$; $P=0.004$; Figure 2.8).

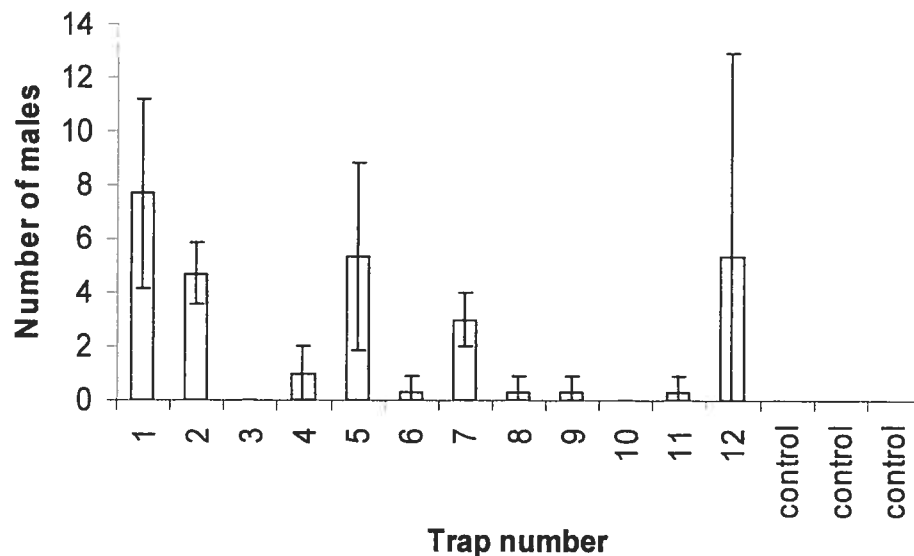


Figure 2. 6: Evidence of individual variability in the attractiveness of newly-emerged virgin *Aphidius ervi* females under field conditions in London, Ontario (Mean±SEM)

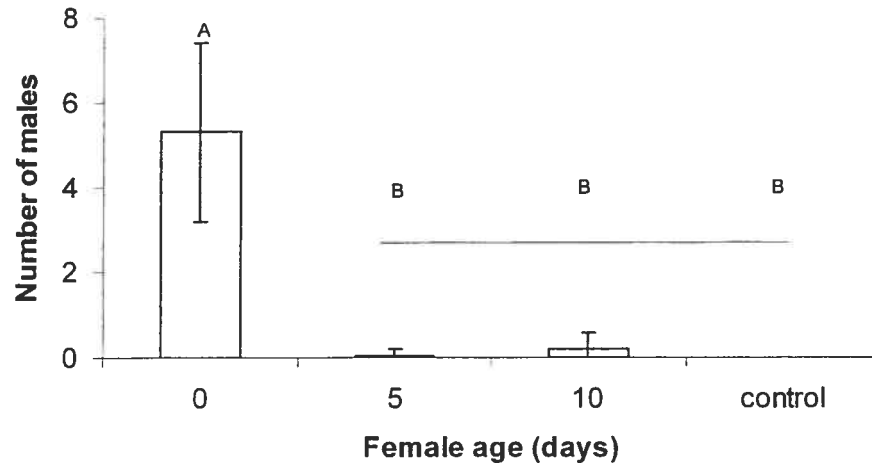


Figure 2. 7: The effect of age on the attractiveness of virgin *Aphidius ervi* females under field conditions in London, Ontario (Mean±SEM)

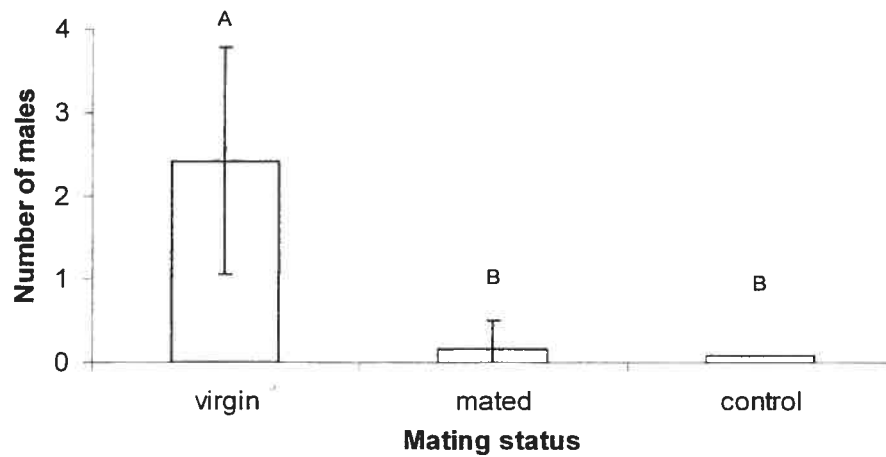


Figure 2. 8: The effect of mating on the attractiveness of *Aphidius ervi* females under field conditions in London, Ontario (Mean±SEM)

2.4 Discussion

The mating behavior of *A. ervi* is very similar to that of *A. nigripes*, with female pheromones clearly modulating attraction and courtship behavior (McNeil and Brodeur, 1995; Marchand and McNeil, 2000), and that both short and long distance pheromones are involved. The female short-distance pheromone affecting courtship (male wing fanning and attempted copulation) is located in the female abdomen and, as suggested for *A. nigripes*, the low levels of response observed to head/thorax extracts may result from female grooming behavior (McNeil and Brodeur, 1995). In both species the production of, and response to, the short-distance pheromone shows diel periodicity, being maximal in the morning. In *A. ervi* this periodicity was also evident in the mating assays, with the number of attempted and successful matings being significantly higher in the morning than in the afternoon. Similarly, there was evidence of diel periodicity associated with the long distance pheromone in *A. nigripes*, as the majority of males captured in traps baited with virgin females occurred between 06:00 and 10:00 (McNeil and Brodeur, 1995). Mating early in the morning would be quite advantageous for small insects like *A. ervi*, as the cool temperatures and high relative humidity to reduce the risk of desiccation. Furthermore, the generally calmer wind conditions at this time of day would be good for foraging males, as flight is inhibited by winds $>1\text{m/s}$ (Marchand and McNeil, 2000; McClure and McNeil, unpublished data).

A number of parameters affect female production/emission of sex pheromones in *A. ervi*. For example, field trials provide evidence of individual variability in the production of the long distance pheromone although, while variation was observed,

there were no significant differences between females in the laboratory bioassays testing the short range pheromone. A similar pattern of individual variation between females was noted for *A. nigripes*, in field (McNeil and Brodeur, 1995), as well as in wind tunnel experiments where female body size (related to the size of the host) played a significant role (Cloutier et al, 2000). There was a significant effect of age on long distance attraction by *A. ervi* females, presumably due to a decline in pheromone production as reported for the age-related decline in pheromone production in virgin *Helicoverpa zea* females (Teal et al, 1990). There was also a significant decline in male responsiveness to short range pheromone as a function of female age, but only to extracts from individuals >6 day old, similar to the non-significant trend observed for *A. nigripes* (McNeil and Brodeur, 1995). The results of this study support the hypothesis that female age affects pheromone production in parasitic Hymenoptera, especially the long distance one that stimulates male upwind flight.

It would also appear that females of *Aphidius* species are generally monandrous and repeated matings are rare (results of this study, McNeil and Brodeur, 1995). Thus, a post-mating decline in pheromone production (pheromonstatis) is not surprising and has previously been suggested for other parasitoids (Van den Assam, 1986). This phenomenon has generally been studied in Lepidoptera (e.g. Raina *et al.*, 1994; Ramaswamy *et al.*, 1996, Marcotte *et al.*, 2003, 2005), and Diptera (e.g. Ottiger *et al.* 2000) and the length of the female refractory period may be related to a number of factors, including the quality of the male ejaculate (see Kingan *et al.*, 1993, 1995; Fan *et al.*, 1999a, b; Delisle & Bouchard, 1995; Delisle and Hardy, 1997; Marcotte *et al.*, 2005). Very few *A. ervi* females remated, and as it was only observed

in those that had mated 24h earlier, possibly the result of an unsuccessful mating or due to a low quality mate.

There appear to be fewer, or less pronounced, changes in the responsiveness of *A. ervi* males to the female short-distance pheromone. There is a decline in responsiveness by males > 6d and could be the result of reduced sensitivity of peripheral pheromone receptors and/or the integration at the level of the central nervous system (see Hansson 1999). Furthermore, the mating success of 8-day-old males that do respond to the sex pheromone is significantly lower than for 1-day-old individuals, possibly associated with the production of male infochemicals involved in female mate choice (Battaglia et al., 2002). However, while the responsiveness of young, previously mated *A. ervi* males is significantly slower than similar aged virgins, previous mating history does not affect acceptance by females in a no choice situation. Whether this is true in a competitive situation, or when older virgin males are involved, remains to be determined. Furthermore, even if females did not discriminate the longer response time of previously mated males could result in them being out-competed by virgin conspecifics.

Clearly, additional research is required to better understand the causes of the observed changes in the emission of, and response to, sex pheromones in *A. ervi*, as well as determining to what extent they impact on reproductive success. Furthermore, we need to know how factors such as temperature, wind speed and humidity also affect the pheromone-mediated communication system. The information generated would not only add to our basic understanding of insect reproductive biology, but could help determine the optimal conditions for parasitoid release and their effective deployment in pest management programs. Furthermore, the chemical composition of

these pheromones must be elucidated if one wishes to test their potential in “push-pull” management strategies (Pyke et al., 1987; Miller and Cowles. 1990; Khan et al., 2000; Nalyanya et al., 2000).

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3. Article 2: The effect of abiotic factors on the male mate searching behaviour and the mating success of *Aphidius ervi* (Hymenoptera: Aphidiidae)

Article soumis dans Journal of Animal Behaviour

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Abstract- Males of *Aphidius ervi* respond to two types of female pheromones; a long distance pheromone that stimulates upwind flight in males and a short distance one that elicits courtship behaviour. The effect of wind speed and distance from the source on the male response of the aphid parasitoid, *A. ervi* (Hymenoptera: Aphidiidae), to a pheromone source was studied in a wind tunnel. The number of males taking flight, entering the plume and successfully reaching the source, decreased at wind speeds >50 cm/s. Furthermore, the proportion of those attempting upwind flight that fell to the ground increased with increasing wind speed. In contrast, tested distance from the source had no significant effect on any of the parameters examined. While male flight behavior was significantly reduced at

70cm/s, some males walked to the source when there was a bridge connecting the pheromone source and the release platform. This suggests that ambulatory behavior could be a significant component of male mate searching in *A. ervi* when wind conditions are too strong for upwind flight.

The possible effects of variation in atmospheric pressure on male flight behaviour to the long distance pheromone, as well as to the short distance one, were also investigated. The only significant effect observed was that the higher the atmospheric pressure at the time the assay was conducted, the greater the number of males reaching the source. These findings differ significantly from those previously reported for another aphid parasitoid, *A. nigripes*, and the reasons for such differences are discussed.

Key Words- *Aphidius ervi*, parasitoid, wind, atmospheric pressure, mate searching behaviour

3.1 Introduction

There is evidence that insects modify their behaviours in response to changes in atmospheric pressure (Lanier and Burns 1978; Steinberg et al. 1992; Roitberg et al. 1993) and it has been hypothesised that such changes are used as cues of impending changes in weather conditions. Insects generally stop flying at quite low wind velocities (e.g. Nealis 1986; Kennedy 1990; Fink and Volk 1995; Messing et al. 1997; Weisser et al. 1997; Storer et al. 1999). Weak flyers would have little or no control over their flight path, and be subject to a higher mortality, in fast and/or turbulent air conditions.

However, wind is one of the important abiotic factors influencing pheromone-mediated reproduction in insects (McNeil 1991), not only affecting parameters associated with the actual chemical message but also the behavior of the animals. Wind velocity and turbulence will determine the structure of the plume, influencing the concentration of pheromone at different distances from the source (e.g. Elkinton and Cardé 1984; Murlis et al. 1992). In addition, wind velocity may impact on the emission of sex pheromone by females (Kaae and Shorey 1972; Conner et al. 1985) and the mate searching behaviour of males (Johnson 1969; Shorey 1976; Elkinton and Cardé 1984; Elkinton et al. 1987; Willis and Baker 1994). Thus, periods of reduced flight could negatively impact overall reproductive success, as the time available to locate mates would be significantly reduced. However, to date, the majority of work looking at wind velocity on mate location has been carried out with large, strong flying species, with little attention being given to smaller species, such as many parasitoids.

Aphidius ervi has been widely used as a biological control agent of the pea aphid (*Acyrthosiphon pisum*) (Gonzalez et al. 1978; Schwörer and Völkl 2001). There have been several studies on the foraging behaviour of *A. ervi* females (Du et al. 1998; Schwörer et al. 1999; Guerrieri et al. 2002), but few studies have examined the reproductive biology of this species (Mackauer 1969; Battaglia et al. 2002), although McClure et al. (2006) have shown that mating is mediated by female sex pheromones and that a long range pheromone stimulates upwind flight and a short distance pheromone elicits courtship behaviour. As there is evidence that variations in wind and barometric pressure may alter female parasitoid foraging behaviour (Steinberg et al. 1992; Roitberg et al. 1993; Fink and Völkl 1995; Weisser et al. 1997) and pheromone-mediated mating (Marchand and McNeil 2000) we undertook studies to test the hypotheses that (i) the frequency of upwind flight of male *A. ervi* to a pheromone source would decline with increasing wind velocity and with distance from the source, (ii) walking behaviour would be important for mate location when wind velocities inhibited flight behaviour, and (iii) daily variations in atmospheric pressure would influence the responsiveness of males to females in a wind tunnel, incidence of mating and the responsiveness of *A. ervi* males to an extract of female pheromone in laboratory assays.

3.1 Materials and Methods

Insects. Agriculture Canada provided the parent material for the aphid and parasitoid colonies, which were subsequently maintained on potted broad bean plants (*Vicia faba* L.) at 20°C, 60% relative humidity (RH) and under a 16L: 8D

photoperiod. Aphid mummies were collected regularly from the rearing cage and isolated in individual gelatine capsules to ensure that all insects used were of known age and mating status. Each day the newly emerged adults were sexed, and the sexes placed in separate plastic holding cages containing a 20% sugar water solution.

The experiments to test the hypotheses that male responsiveness to the long distance female sex pheromone was influenced by both wind velocity and distance from the source were carried out at 20°C and 60% R.H. in a laminar airflow flight tunnel (140 cm long, 66 cm wide, and 66 cm high). Lighting was provided by 40 W fluorescent lights above the ceiling of the wind tunnel, resulting in a light intensity around 130 lux at the height the assays were conducted. All experiments were carried out during the first three hours of the photophase, as this was the period of maximum mating activity for *A. ervi* under controlled laboratory conditions (Mackauer 1969; McClure et al. 2006). Each naïve, virgin 24-48 hr old male was placed in an individual release cage (10 cm long and 4.5 cm in diameter, covered with gauze at each end), and when tested the cage was placed on a wooden platform, 35 cm off the floor of the tunnel downwind from the pheromone source. For each male we measured the time to take-off and the presence or absence of oriented upwind flight (for at least 20 cm), and whether or not they reached the cage containing the virgin females. We also noted the proportion of those leaving the release cage that fell to the ground, unable to sustain flight activity. If males did not take off within 3 min, they were considered unresponsive and were removed from the wind tunnel. In all experiments individual males were only tested once.

The *A. ervi* long distance sex pheromone has not been isolated so the pheromone source consisted of 10 virgin, 24-to 48-hr old females in a plastic cylinder

(10 cm long and 4.5 cm in diameter, covered with gauze at each end). Ten females were used to ensure that there would be a source of pheromone throughout the entire assay. The cage with females was placed on a support 35 cm above the floor at the upwind end of the tunnel 15 min prior to the start of each assay, allowing them to acclimate to the prevailing conditions.

Four wind speeds, 30, 50, 70 and 100 cm/sec (measured with a portable air velocity meter at the male release cage) were tested, and this was repeated for males placed 50 and 90 cm from the source (Marchand and McNeil, 2000). Ten males were tested at both distances during each assay, with different wind speeds tested on different days. There were three replicates tested on different days for the two distances and four wind speeds.

The experiment at 50 cm was repeated at wind velocities 30, 70 and 100 cm/s, in which males had the option of either flying or walking upwind to the source. In this situation there was a string “bridge” connecting the stands on which the male and female cages were placed (Goldansaz and McNeil, 2006). Ten males were used for each test (e.g. with and without the bridge), at each of the three wind speeds on the same day. This experiment was replicated on three different days.

We conducted one set of experiments to test the hypothesis that there would be a relationship between atmospheric pressure and male responsiveness to the long distance pheromone in the wind tunnel. The responsiveness of 10 different virgin, 24- to 48-hr old males, placed at 50 cm from the pheromone source with a wind speed of 30 cm/sec was determined every day over a 14-day period, recording the same parameters mentioned previously.

We also tested the hypothesis with respect to the response to short range pheromone using two different pheromone sources. In the first we recorded the mating success of 10 pairs, consisting of newly-emerged (<24h) virgin individuals, set up in individual gelatine capsules each day, for 20 consecutive days. Assays were conducted between 08:00 and 10:00, and were terminated after 6 min if mating did not occur, as the probability of copulation was low after 3 min (Mackauer 1969). In the second we tested the response of 24 hr-old naïve virgin males to an extract of the close range sex pheromone (a cuticle extract that is only attractive at a few centimetres). The extracts were obtained by soaking 3 newly-emerged (<24h) virgin females in 300 µl of methylene chloride for 540-sec, as this was the optimal extraction time (McClure et al. 2006). Three µl [0.01 female equivalents (FE)] of extract was placed on a 0.38- cm² filter paper disk, and the solvent allowed to evaporate before introducing the pheromone source into an 8-dram glass shell vial with the test male. As with mating pairs, 10 assays with different individuals were conducted each day for 20 days. Two variables were measured; the proportion of males that responded and the time to respond to the stimulus. Males were considered responsive if they exhibited rapid wing-fanning, the first step in the courtship behaviour (McClure et al. 2006), within 60 sec of exposure to the stimulus.

In all assays the various male behaviours measured were correlated to the atmospheric pressure at the time the tests were run, as well as to the changes in pressure over the 12 and 24h prior to the trials as both the direction and the magnitude of barometric changes may influence parasitoid behaviour (Steinberg et al. 1992; Marchand and McNeil 2000). The atmospheric pressure data were obtained from Environment Canada.

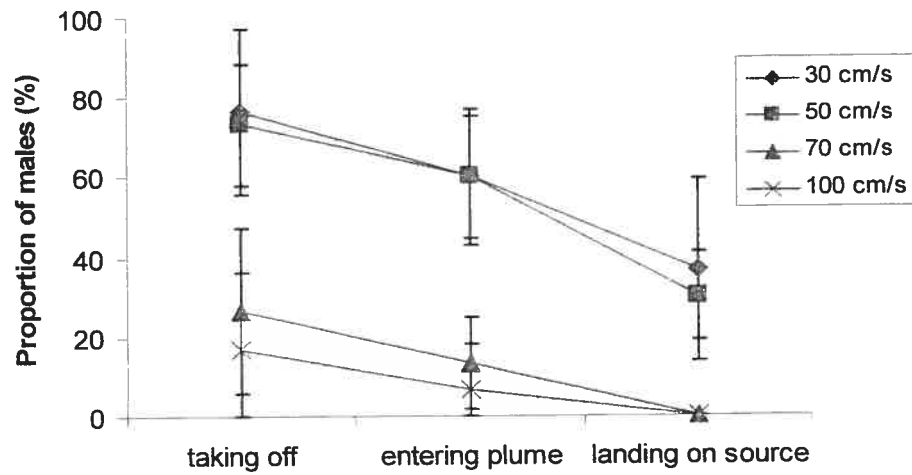
Statistical analyses. The proportion of males taking off and falling to the ground after take-off at different wind speed and distance combinations were compared using chi-square analyses. Categorical (in the plume/not in the plume; landing on the source/not landing on the source; sustaining flight/falling to the ground) responses among distances and wind speed were compared using logistic regression. The proportion of males responding by wing fanning to a female pheromone extract, successfully copulating, entering the plume and landing on the source as a function of atmospheric pressure variations were analysed by linear regressions. Time to take-off as a function of wind speed was analysed using a one-way ANOVA. An error rate of 0.05 was used in all tests.

3.2 Results

Wind speed significantly and negatively affected the proportion of males leaving the release cage ($Z=-6.464$; $df=239$; $P<0.001$), entering the plume ($Z=-2.580$; $df=109$; $P=0.0098$) and reaching the source ($Z=-2.147$; $df=109$; $P=0.032$) with a marked decline in the number of males taking off and consequently reaching the source at wind velocities of 70 and 100 cm/s, regardless of the distance to be covered (Figs. 3.1 A,B). Furthermore, the proportion of males that took flight and then fell to the ground immediately upon take-off increased with increasing wind speed ($Z=3.774$; $df=109$; $P<0.001$; Fig. 3.2). Although few individuals were active at the higher wind speeds, the proportion of those actually leaving the release cage and exhibiting upwind flight did not differ significantly as a function of wind speed regardless of whether males were 50 ($\chi^2=6.009$; $df=3$; $P=0.111$) or 90 cm ($\chi^2=3.183$;

df= 3; P=0.364) from the source (data not shown). Furthermore, there was no interaction between wind speed and distance ($Z=1.052$; $df=$; $P=0.292$). The response time of males (defined as the time to take-off) was not affected by either wind speed ($F=0.614$; $df1=3$; $df2=54$; $P=0.609$) or distance to the source ($F=0.112$; $df=1$; $df=45$; $P=0.739$), with an overall average and standard deviation of 66.3 ± 10.8 sec.

A)



B)

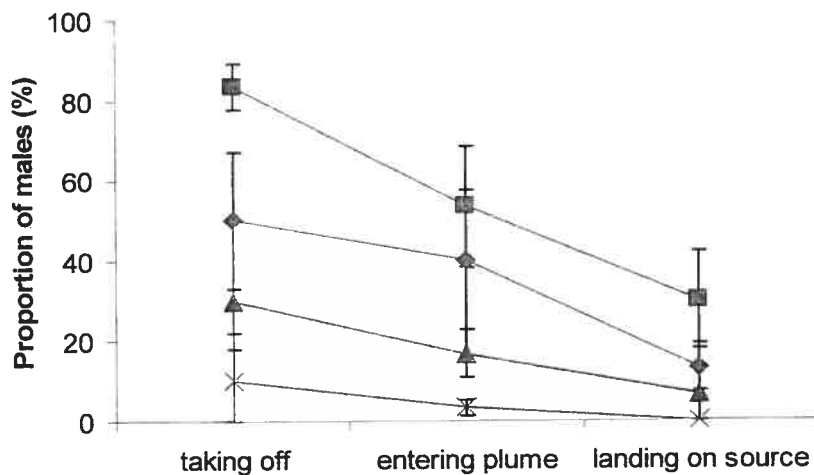


Figure 3. 1 : Percentage of *Aphidius ervi* males taking off, entering the pheromone plume and landing on the pheromone source as a function of wind speeds at A) 50 cm (N=120) and B) 90 cm (N=120) from a source of female sex pheromone (Mean \pm SEM)

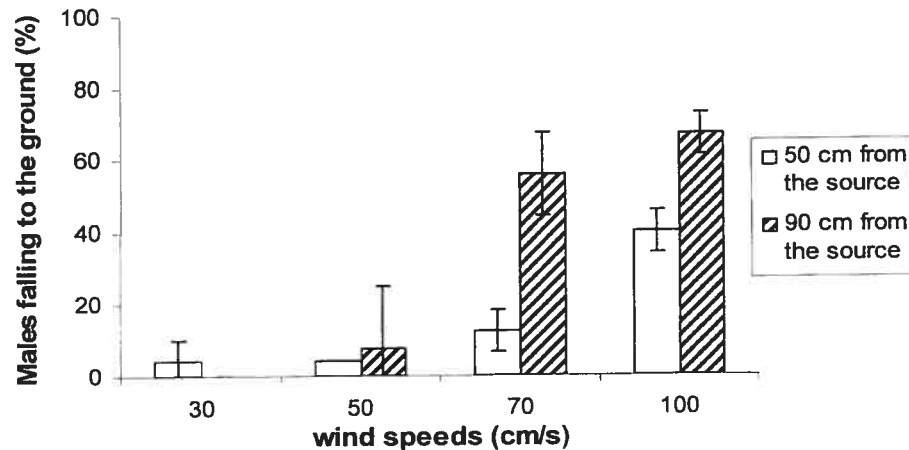
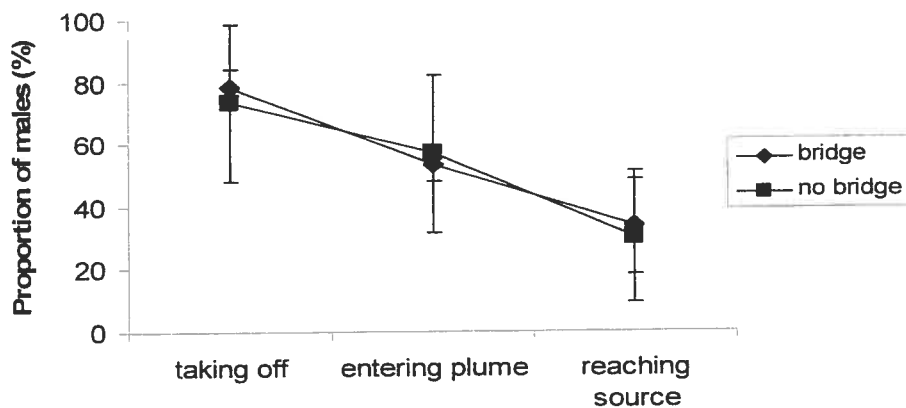


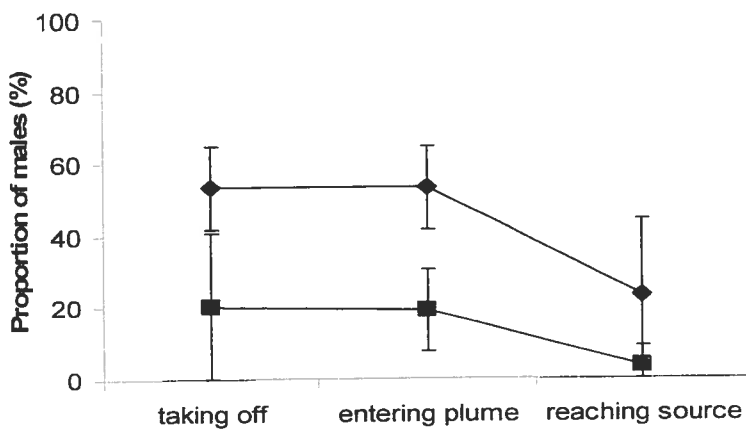
Figure 3. 2 : Percentage of *Aphidius ervi* males falling to the ground as a function of wind speeds at 50 cm (N=120) and 90 cm (N=120) from a source of female sex pheromone (Mean±SEM)

At wind speeds of 30 (Fig. 3.3A) and 100 cm/s (Fig. 3.3C) the presence or absence of a bridge did not influence the proportion of males leaving the release cage ($\chi^2=0.608$; df= 1; P=0.436), entering the plume ($\chi^2=0.046$; df= 1; P=0.830) or reaching the pheromone source ($\chi^2=0.087$; df= 1; P=0.768). However, at 70 cm/s, significantly more males left the release cage ($\chi^2=5.284$; df= 1; P=0.022), entered the plume ($\chi^2=7.886$; df= 1; P=0.005) and reached the source ($\chi^2=4.000$; df=1; P=0.046) when a bridge was available (Fig. 3.3B). Furthermore, at this wind speed a significantly higher proportion of the males leaving the release cage reached the source by walking than by flying (7 ± 1.5 VS 2 ± 1.15 ; $\chi^2=5.760$; df= 1; P=0.016; Fig. 3.4).

A)



B)



C)

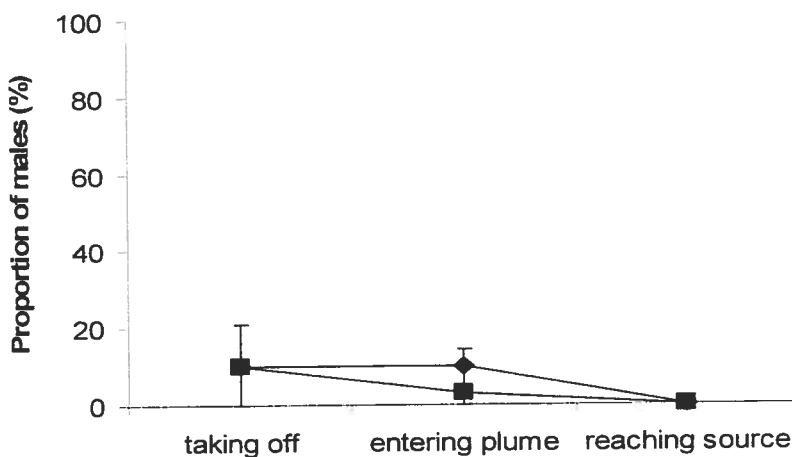


Figure 3. 3 : Percentage of *Aphidius ervi* males (N=180) leaving the release cage, entering the pheromone plume and landing on the pheromone source at a wind speed of A) 30 cm/s B) 70 cm/s and C) 100 cm/s when positioned at 50 cm from a source of female sex pheromone with and without a bridge (Mean±SEM).

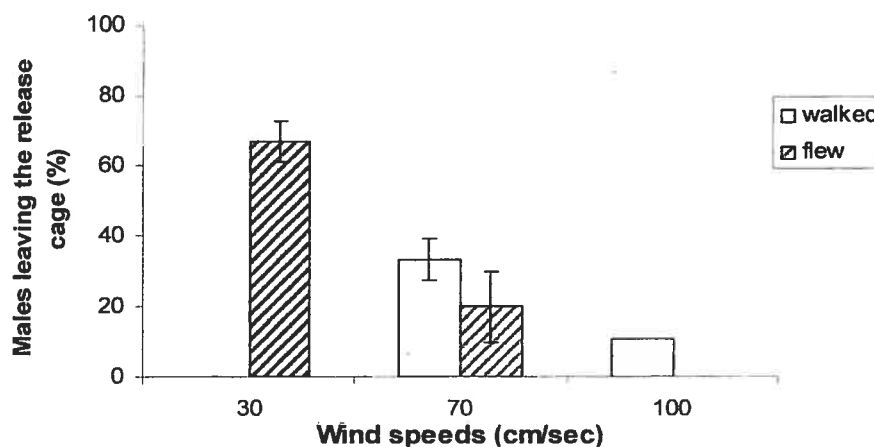
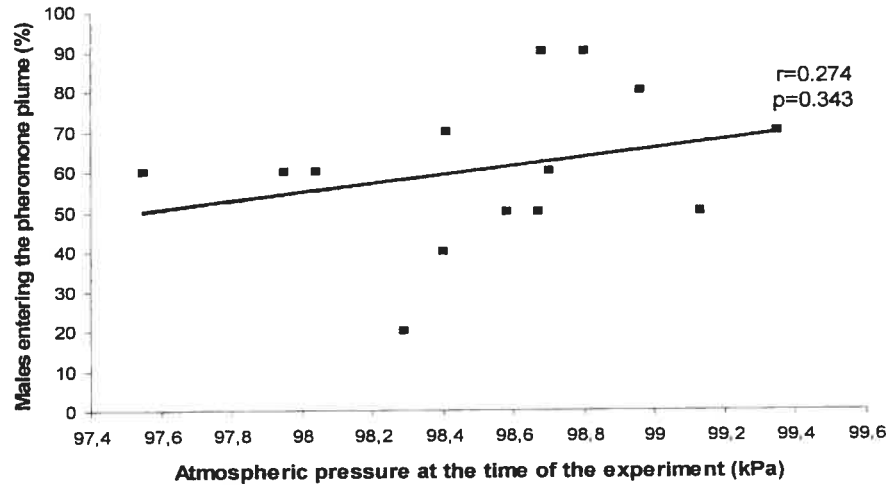


Figure 3. 4 : Percentage of *Aphidius ervi* males (N=90) leaving the release cage at 50 cm from a source of female sex pheromone by walking or flying when a bridge is provided at wind speeds of 30 cm/s, 70 cm/s and 100 cm/s (Mean±SEM)

While the proportion of males entering the plume was not significantly affected by the atmospheric pressure conditions at the time the bioassays were carried out (Fig. 3.5A), there was a significant positive correlation between atmospheric pressure and the number of males reaching the source (Fig. 3.5B). However, there were no significant changes in either of the male behaviours measured as a function of atmospheric pressure changes in the 12h ($r=0.142$; $df= 1$; $P=0.62$) or 24h ($r=0.207$; $df= 1$; $P=0.478$) prior to the assay being conducted (data not shown).

A)



B)

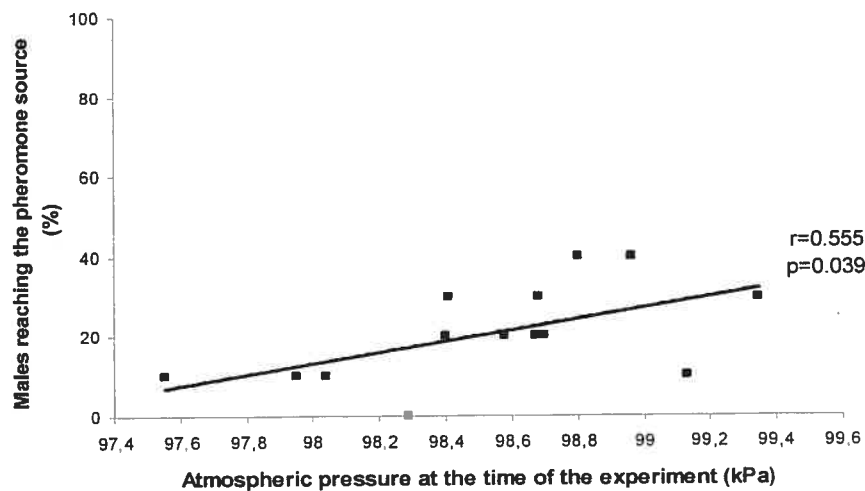


Figure 3.5 : Percentage of *Aphidius ervi* males (N=140) A) taking flight and B) landing on the pheromone source as a function of the atmospheric pressure (kPa) at the time of the experiment

There were no effects of atmospheric conditions on the incidence of mating, either with respect to the prevailing pressure at the time the assays were carried out ($r=0.130$; $df= 1$; $P=0.596$; data not shown) or with changes in the preceding 12 hr ($r=-0.422$; $df= 1$; $P=0.064$; data not shown) or 24 h ($r=-0.117$; $df= 1$; $P=0.635$; data not

shown). Similarly, the proportion of males responding to the female extract did not change significantly with atmospheric pressure at the time of the experiments ($r=0.394$; $df=1$; $P=0.95$; data not shown) or with variations occurring in the 12h ($r=-0.103$; $df=1$; $P=0.664$; data not shown) or 24 hr ($r=0.127$; $df=1$; $P=0.605$; data not shown) prior to the assays.

3.3 Discussion

Oriented flight in response to pheromone cues is a major component of mate location by *A. ervi* males but would only be an effective means of foraging at wind speeds <70 cm/s. This upper limit falls well within the range reported for other species of parasitoids, be it for female foraging or male mate location (Juillet 1964; Kaae and Shorey 1972; Fink and Völkl 1995; Messing et al. 1997; Schwörer et al. 1999, Marchand and McNeil 2000). At higher wind speeds there is a marked change in the behavior of *A. ervi* males, with individuals showing a significantly lower propensity to initiate flight towards a pheromone source at wind velocities >50 cm/s. This behavioural modification is not surprising, given that the small size (2.4-3.0 mm) of an *A. ervi* male would certainly limit its ability to sustain upwind flight at high wind speeds. Such an attempt would not only be energetically costly (see Keller 1990), there is also a high probability of being carried down wind and, thus, further from the potential mate. Furthermore, as suggested by the positive relationship between the proportion of males falling to the ground and increasing wind velocity, attempting flight at high wind speed could lead to injury or death. Thus, relying uniquely on flight to locate mates when the flight is inhibited by strong winds could

have a markedly negative impact on a small insect's overall reproductive success (Weisser et al. 1997).

However, Bell (1991) postulated that environmental conditions should impact on the evolution of foraging strategies, and the results from the wind tunnel experiments when a bridge is present would support this idea. Walking towards a pheromone source increased at 70 cm/s even though upwind flight was markedly inhibited, thus allowing males to forage over a wider range of wind velocities than by flight alone. These findings are similar to those reported for the response of potato aphid, *Macrosiphum euphorbia* (Thomas) males to calling oviparous females at varying wind speeds (Goldansaz and McNeil 2006). The significantly higher proportion of males leaving the release cages when a bridge is present at 70 cm/s suggests that there may be a close range visual component, allowing males to assess the substrate actually available for foraging. If this is the case then one could predict that a broader surface, a leaf rather than a thin string, may result in a higher incidence of walking behavior, even at velocities such as 100 cm/s where, with only a string bridge, no such activity was observed. However, periods of strong wind may still result in a considerable time loss for *A. ervi* adults, if prevailing wind conditions force them to remain on particular plants without potential mates (Schwörer et al. 1999). Under such conditions females could still exploit available hosts but this could have an important impact on subsequent sex ratios if the situation lasted for an extended period.

In the current experiments there was no significant effect of distance from the pheromone source on any stage of the *A. ervi* male foraging behavior. This is rather different from the situation reported for *A. nigripes* (Marchand and McNeil 2000),

where the proportion of males entering the plume generally declined with distance from the source, and that within the wind velocity range permitting flight, the response of males at further distances from the source increased with wind velocity. However, the assays testing *A. nigripes* were carried out in a bigger wind tunnel, where it was possible to test a wider range of distances. Thus, similar differences might have been observed with *A. ervi* if we had included a distance of 130cm. While there was no overall significant effect of distance, in the 90 cm assays the proportion of males entering the plume was higher at wind speeds of 50 than at 30 cm/s (Figure 1B), a pattern similar to the one reported for *A. nigripes* (Marchand and McNeil 2000)

The higher the atmospheric pressure at the time the assays were conducted, the higher the proportion of *A. ervi* males that reached the pheromone source, a pattern previously reported for the response of *C. glomerata* females to host plant volatiles (Steinberg et al. 1992). The fact that there was no significant difference in the proportion actually entering the pheromone plume would suggest that the higher proportion reaching the source under such weather conditions are the result of increased female emission of, and/or, male sensitivity, to the pheromone. These non-exclusive possibilities will only be resolved once the long distance *A. ervi* sex pheromone has been identified, but whatever the cause there will be a higher incidence of mating under such favourable climatic conditions.

Nevertheless, there were no significant effects of atmospheric changes in either the 12 or 24hr period prior to the assays on *A. ervi* male foraging. Again, this is different from the situation reported for *A. nigripes*, where changes in atmospheric pressure during the 24 hr preceding the assay affected both the proportion of males

entering the plume and reaching the source (Marchand and McNeil 2000). In the case of the proportion entering the plume the significant differences were observed when the pressure changes were an increase or decrease of > 5 mb. Yet, over the 14 day period of the *A. ervi* assays there were only 3 days with such large fluctuations, the remainder having very minor or no fluctuations in pressure. Similarly, the changes in proportion of *A. nigripes* males reaching the source was related to moderate fluctuations (>2 and <5 mb) and during our study these conditions only occurred in about 30% of all assays conducted.

The apparent absence of atmospheric pressure changes on the response to the short distance pheromone, either assessed by mating, or by male responses to the female cuticular extracts must also be viewed with caution, for 13 of the 20 days had atmospheric pressure fluctuations <2 mb, and only two >5 mb. However, it is possible that changes in atmospheric pressure may actually have little impact on male responses to the short distance pheromone, for these are only detected from about 5 cm. Under such conditions the male would most probably be within walking distance of the source and not courting a potential mate would be counterproductive.

Further research is needed to address how variable wind speeds, a much more normal situation in the field, affects *A. ervi* male foraging. Goldansaz and McNeil (2006) found that while males of the potato aphid would walk, but never fly, to a calling female under constant wind conditions in a wind tunnel, under field conditions individuals walking to a source would fly upwind when there was a lull in the wind. This may well be a common mate location strategy used by weak flying insects. Additional experiments will also be required to further investigate whether the relatively low impact of changes in atmospheric pressure observed in this study

reflect real interspecific differences or if this resulted from the rather stable conditions during the summer months when the present study was conducted. Real interspecific differences cannot be ruled out, as *A. ervi* males took nearly a minute to respond to a pheromone source and this was independent of either wind speed or distance. In contrast, for *A. nigripes* mean response times never exceeded 30 s, and were affected by both wind velocity and distance from the source (Marchand and McNeil 2000). Nonetheless, given the fact that males of both species show similar behavioural modifications in mate searching flight behavior in response to different wind velocities, it seems rather unlikely that *A. ervi* would have such a markedly different response to fluctuating atmospheric conditions than *A. nigripes*. Furthermore, being able to respond to cues signalling the advent of adverse conditions, such as rainfall that has been shown to negatively impact foraging *A. rosea* females (Fink and Völkl 1995), would certainly increase survival and, thus, reproductive success.

3.4 References

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4. Conclusion générale

Le moyen le plus couramment utilisé pour diminuer l'impact des pucerons en milieu agricole demeure l'utilisation d'insecticides chimiques. Mais depuis peu on prend conscience de l'importance de protéger l'environnement et de trouver des méthodes alternatives. Il est possible de lutter contre un ravageur de culture à l'aide de lâchers (inondatifs ou inoculatifs) d'ennemis naturels, tels que les parasitoïdes, sur le terrain. Une meilleure compréhension de l'écologie chimique des parasitoïdes serait utile pour choisir la période et l'endroit convenant le mieux au déploiement et à l'utilisation de pièges à phéromone pour quantifier leur abondance dans les milieux. Ce mémoire visait à mieux comprendre et à faire ressortir différents facteurs influençant la biologie de la reproduction d'*Aphidius ervi*, un important parasitoïde du puceron du pois (*Acrythosiphon pisum* (Harris)). Une connaissance approfondie de l'effet des facteurs biotiques et abiotiques sur l'émission de la phéromone sexuelle par les femelles, sa réceptivité par les mâles et le comportement de recherche des mâles permettrait une utilisation efficace de cet agent de lutte biologique.

Dans cette étude, nous avons démontré qu'une phéromone sexuelle provenant de l'abdomen des femelles d'*A. ervi* module le comportement d'accouplement et le comportement de recherche d'un partenaire sexuel chez le mâle, tel que démontré chez d'autres espèces de parasitoïdes (Vinson, 1972; Yoshida, 1978; Decker et al., 1993; Shu et Jones, 1993; Syvertsen et al., 1995; Nazzi et al., 1996; Tillman et al., 1999). Les extraits faits à partir de femelles initiaient la parade nuptiale chez les mâles ainsi que la copulation à très courte distance en cagette. Néanmoins, ces extraits ne semblent pas attractifs à longue distance et n'initient pas le vol dirigé du

mâle vers la source de phéromone dans le tunnel de vol. Ces résultats suggèrent que certains composés de la phéromone sexuelle, essentiels à l'expression des divers comportements associés à la reproduction ne sont pas prélevés avec les extraits, ou indique, comme cela a déjà été suggéré pour *A. nigripes* (McNeil et Brodeur, 1995; Marchand et McNeil, 2000), l'existence d'une deuxième phéromone.

Nos résultats démontrent que l'émission de la phéromone sexuelle chez *A. ervi* et la réponse à cette dernière peuvent être influencées par des facteurs biotiques (âge, accouplement, périodicité) et abiotiques (vent, pression atmosphérique), tel que déjà décrit pour d'autres insectes, et qu'il serait important d'en tenir compte lors de l'introduction dans l'habitat. Par exemple, le succès d'introduction du parasitoïde pourrait varier selon l'utilisation de momies ou d'adultes de sexe et d'âge connus. Il serait plus facile d'introduire des plantes avec des momies, car il y a moins de manipulations à faire, mais il existe de nombreux hyperparasitoïdes et prédateurs qui attaquent les momies (Brodeur et Rosenheim, 2000; Snyder et Ives, 2001; Snyder et Ives, 2003). L'occurrence de la prédation sur les parasitoïdes adultes a été moins étudiée mais semblerait être moins importante (Brodeur et Rosenheim, 2000). De plus, en relâchant des adultes il est possible de s'assurer que certaines femelles soient accouplées et de garantir un sexe ratio équilibré de la progéniture. Cloutier et al. (1981) ont démontré que la taille des femelles avait un impact sur leur longévité et leur fécondité et que les femelles produisaient moins de progéniture femelle avec l'âge. Il pourrait donc être préférable d'introduire de jeunes femelles accouplées et de taille supérieure pour maximiser leur potentiel reproducteur.

Les vitesses de vent inhibant le vol étant fréquentes sur le terrain, il serait préférable d'introduire les parasitoïdes tôt le matin avant que le vent devienne un

facteur trop important (Dupont, 2001). De plus, les indicateurs des conditions climatiques à venir telle que la pression atmosphérique sont des indices fiables et utiles pour déterminer les moments les plus propices pour l'introduction des parasitoïdes. Il serait donc profitable de prendre en considération les facteurs météorologiques sur une période définie dans le cadre de programmes de lutte biologique afin d'en augmenter l'efficacité. Étant donné que les différentes conditions météorologiques ont un effet significatif sur le vol et le déplacement des parasitoïdes, il est important de comprendre que les résultats obtenus dans les pièges sur le terrain peuvent varier. Afin d'interpréter efficacement les résultats obtenus dans les pièges, il est impératif de prendre en considération les conditions ambiantes (Juillet, 1964; Jönsson et Anderbrant, 1993).

Peu d'études se sont penchées sur l'effet de la température sur la locomotion des parasitoïdes, bien que Gilchrist (1996) ait mesuré la performance locomotrice des mâles *A. ervi* à diverses températures. Or il pourrait exister un compromis entre le vol et la marche selon la température ambiante et il est possible que la marche prédomine à des températures plus fraîches. Ceci pourrait servir à la thermogenèse musculaire pour atteindre les températures internes nécessaires pour voler (Bell, 1991). Les températures ambiantes pourraient ainsi être un facteur limitant du vol, même en présence de vitesses de vent adéquates. Le comportement ambulateur des mâles d'*A. ervi* étant soutenu à des vitesses de vent inhibant le comportement de vol, l'emplacement des pièges dans la végétation devient important si on ne veut pas sous-estimer les populations des parasitoïdes. Il est probable que le fait de marcher vers la source de phéromone soit un comportement important dans la recherche d'un partenaire sexuel et des études sont nécessaires pour mesurer l'efficacité du

comportement ambulateur sur la capacité d'atteindre une femelle et donc sur le succès d'accouplement sur le terrain. De plus, il serait intéressant d'étudier l'impact de l'architecture des végétaux sur la capacité des mâles à atteindre les femelles, et ce même lorsque les conditions sont défavorables pour le vol. Schwörer et Völkl (2001) ont démontré que lors de la recherche d'un hôte les femelles d'*A. ervi* utilisaient différentes stratégies de recherche et que les conditions atmosphériques avaient un effet différent sur leur comportement selon la structure de l'habitat. Il se pourrait que lors de la recherche d'un partenaire sexuel les femelles se placent à des endroits différents selon la structure de l'habitat et qu'il existe un compromis entre un endroit plus propice à rencontrer des mâles et un endroit assurant une transmission efficace de la phéromone sexuelle.

Lors de nos bioessais, il n'a pas toujours été facile de distinguer l'effet des facteurs biotiques et abiotiques sur l'émission de la phéromone par les femelles et la capacité des mâles de la détecter. Par exemple, on ne sait pas si la diminution du taux d'accouplement lors de certaines variations des conditions atmosphériques est la conséquence d'un changement dans l'émission de la phéromone par les femelles, de sa réceptivité par les mâles, ou les deux. En conséquence, une avenue de recherche future serait l'identification des infochimiques qui sont responsables de la communication à longue et à courte distance. Lorsqu'une phéromone synthétique sera disponible, on sera en mesure de répondre à ce type de question biologique. De plus, l'habileté de synthétiser les phéromones sexuelles de parasitoïdes, tel qu'*A. ervi*, nous permettront d'évaluer le potentiel de ces produits dans les programmes de lutte biologique. En combinant l'utilisation de substances chimiques de synthèse telles que les kairomones (miellat et phéromone sexuelle produits par *A. pisum*) et les

phéromones de synthèse, il serait envisageable d'attirer respectivement les femelles et les mâles d'*A. ervi* au niveau des colonies d'*A. pisum* (McNeil et Brodeur, 1995; Nazzi et al., 1996) et d'avoir par la suite une population d'ennemis naturels suffisamment stable pour contrôler l'impact du ravageur au-dessous d'un seuil acceptable (Debach et Rosen, 1991).

De nombreuses études sont encore nécessaires afin d'élucider le comportement d'accouplement d'*A. ervi*, non seulement quant à l'identification de la phéromone sexuelle femelle, mais aussi quant à la phéromone mâle. Battaglia et al. (2002) ont démontré que les mâles d'*A. ervi* sécrètent une phéromone au niveau de leurs antennes qui, par frottement contre les antennes de la femelle, stimulerait leur acceptation par cette dernière. Or il est possible que la production de cette phéromone mâle change avec l'âge et/ou suite à un accouplement ou en réponse à des conditions abiotiques, tels que les changements de pression atmosphérique. Il serait donc souhaitable d'identifier cette phéromone pour être en mesure de comprendre son rôle possible dans le choix de la femelle, de déterminer l'effet de l'âge et d'un accouplement préalable sur la production de la phéromone mâle sous diverses conditions abiotiques. Des analyses de profils cutanés sont présentement en cours par notre laboratoire afin de déterminer les composantes de cette phéromone.

Finalement, un aspect non considéré lors de l'introduction de parasitoïdes est la possibilité d'hybridation avec des espèces indigènes. Les résultats de cette étude ainsi que celle de McNeil et Brodeur (1995) démontrent qu'il existe de nombreuses ressemblances dans la biologie reproductive d'*A. ervi* et d'*A. nigripes*. Une comparaison des phéromones mâles et femelles combinée avec des études comportementales de ces deux parasitoïdes, respectivement introduits (*A. ervi*) et

indigènes (*A. nigripes*), permettrait de déterminer le rôle des phéromones en tant que mécanisme d'isolation reproductive. De telles études sont présentement en cours par notre laboratoire et permettront de déterminer s'il existe un risque d'hybridation, ce qui aurait des implications non seulement pour la biodiversité mais aussi pour le biocontrôle.

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