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**ÉTUDE COMPORTEMENTALE ET ÉCOLOGIE
CHIMIQUE DE LA RECHERCHE D'UN
PARTENAIRE SEXUEL CHEZ LE PUCERON DE LA
POMME DE TERRE, *MACROSIPHUM EUPHORBIAE*
(THOMAS) (HOMOPTERA: APHIDIDAE)**

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Résumé

Afin d'utiliser les phéromones sexuelles dans les programmes de lutte intégrée des espèces nuisibles d'insectes, il est essentiel d'identifier les composés chimiques phéromonaux et de comprendre adéquatement la biologie reproductive des espèces considérées. Cette thèse porte sur l'étude de ces deux thèmes chez le puceron de la pomme de terre, *Macrosiphum euphorbiae* (Homoptera: Aphididae), un insecte nuisible pour l'agriculture à l'échelle mondiale.

Les femelles sexuées vierges du puceron de la pomme de terre émettent leur phéromone sexuelle afin d'attirer les mâles conspécifiques. Lorsque soumises à des températures froides, elles expriment ce comportement à un plus jeune âge que dans des conditions plus chaudes. En laboratoire, pour tous les régimes thermiques, une grande différence était observée au niveau du comportement d'appel en fonction de l'âge (i.e., les vieilles femelles appelaient plus tôt après l'ouverture des lumières et passaient plus de temps à appeler). Toutefois, sur le terrain, compte tenu des effets des faibles températures, des vents élevés et de la pluie sur l'activité des femelles en appel, les changements liés à l'âge étaient beaucoup moins prononcés.

La phéromone sexuelle libérée par les femelles de *M. euphorbiae* a été identifiée comme étant un mélange de deux monoterpénoïdes, le nepetalactol (I) et la nepetalactone (II). Ces deux composés étaient présents dans un rapport de 4:1 à l'émergence des femelles. Ce ratio a changé avec l'âge, les femelles les plus âgées libérant un mélange au rapport de 2:1. Des proportions semblables de mâles de *M. euphorbiae* ont répondu aux mélanges synthétiques des rapports de 3:1, 4:1 et 5:1 et aux femelles vierges en appel. Cependant, les mâles mettaient plus de temps pour atteindre une source de phéromone synthétique que des femelles en appel.

Le comportement des mâles face aux femelles sexuées en appel a été étudié dans des bioessais en laboratoire et sur le terrain. En laboratoire, les mâles détectaient la phéromone et s'orientaient vers la source mais ne volaient pas contre le vent lorsqu'ils étaient exposés à une source de phéromone. Par contre, ils rejoignaient la source si un pont était présent entre la source de phéromone et les cages de relâche pour leur permettre de

marcher contre le vent. Sur le terrain, les comportements d'appel des femelles et de marche des mâles ont été étudiés pour différentes vitesses de vent inférieures à 5 m/s. Les femelles appellent jusqu'à une vitesse de 4 m/s mais le comportement de vol des mâles était inhibé à des vitesses supérieures à 2 m/s. Tel qu'observé en laboratoire, les mâles marchaient vers la source à des vitesses de vent élevées. Toutefois, contrairement aux résultats de laboratoire où les mâles atteignaient tous la source en marchant, 30% des mâles volaient contre le vent et se posaient sur la source. L'étude détaillée des patrons de vent a démontré qu'en présence de vent, les mâles marchaient vers la source, mais s'envolaient et progressaient vers l'avant lorsqu'une accalmie temporaire du vent se présentait.

Abstract

In order to use insect sex pheromones in integrated management programme of pest species, it is essential to have the correct identification of the chemical composition of pheromones and a solid understanding of the reproductive biology for the species in question. In this thesis, I examined the two aspects in the potato aphid, *Macrosiphum euphorbiae* (Homoptera: Aphididae), an agricultural pest of importance worldwide.

Virgin female oviparae of the potato aphid release sex pheromone to attract conspecific males. At cooler temperatures females express this behavior at a younger age than at warmer ones. Under all constant temperature regimes in the laboratory there was a significant change in calling behavior as a function of age, with older females calling sooner after the “lights on” signal and spending more time calling. However, under field conditions the age related changes were much less evident due to the effects of low temperatures, high winds and rain on female calling activity.

The sex pheromone emitted by calling females of *M. euphorbiae* was identified as a mixture of two monoterpenoids nepetalactol (I) and nepetalactone (II). The two components were present in a 4:1 ratio in the young females, but this changed with age and older females released a 2:1 ratio. A similar proportion of *M. euphorbiae* males responded to 3:1, 4:1, and 5:1 synthetic blends and to calling virgin, although the time taken to reach the source was less when conspecific females were used.

Males' behaviour to the calling females was examined in the laboratory and field bioassays. In the laboratory, males detected and oriented themselves to the source but did not fly upwind when exposed to calling females or appropriate lures, and only reached the source if there was a bridge available between the release cage and pheromone source allowing them to walk upwind. Under field conditions female calling, and male walking behavior were observed under variable wind speeds <5m/s, but male flight behavior was inhibited at variable winds >2m/s. In contrast to laboratory results where males only reached the source by walking, nearly 40% of males reaching the source did so by flying. A detailed examination of the wind patterns showed that in the presence of wind males

walked towards the source but would take flight and make forward progress when there was a temporary lull in the wind.

Avant-Propos

Les trois articles de cette thèse ont été ou seront publiés avec, à titre de co-auteur, le professeur Jeremy N. McNeil. Le chapitre II a été accepté pour publication dans la revue *Ecological Entomology*. Le chapitre III a été soumis à la revue *Journal of Chemical Ecology*; les autres co-auteurs sont Sarah Dewhirst, John A. Pickett, et Lester Wadhams du Centre de recherche de Rothamsted, Angleterre. Le chapitre IV sera soumis à la revue *Proceedings of the Royal Society of London, Series B*.

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À ma famille

Table des matières

Résumé.....	ii
Abstract.....	iv
Avant-Propos.....	vi
Remerciements.....	vii
Table des matières.....	x
Liste des tableaux.....	xii
Liste des figures.....	xiii
Chapitre I Introduction.....	1
Références.....	12
Chapitre II Calling behaviour of the potato aphid <i>Macrosiphum euphorbiae</i> (Thomas) (Homoptera: Aphididae) oviparae under laboratory and field conditions.....	21
Résumé.....	22
Abstract.....	23
Introduction.....	24
Materials and methods.....	25
Results.....	27
Discussion.....	29
Acknowledgements.....	32
References.....	33
Chapitre III Identification of the sex pheromone in the potato aphid, <i>Macrosiphum euphorbiae</i> (Thomas) (Homoptera: Aphididae).....	47
Résumé.....	48
Abstract.....	49
Introduction.....	50
Materials and Methods.....	52
Results.....	55
Discussion.....	57
Acknowledgements.....	60
References.....	61
Chapitre IV Effect of wind speed on the behaviour of sexual morphs of the potato aphid, <i>Macrosiphum euphorbiae</i> (Thomas) (Homoptera: Aphididae) under laboratory and field conditions.....	71
Résumé.....	72
Abstract.....	74
Introduction.....	76
Materials and Methods.....	78
Results.....	81
Discussion.....	83
Acknowledgements.....	86
References.....	87

Chapitre V Conclusion 99
Références 105

Liste des tableaux

Chapitre II

Table 1: Analysis of variance of the mean onset time of calling and mean time spent calling by virgin <i>Macrosiphum euphorbiae</i> females as a function of calling age under different constant temperature conditions in the laboratory.	36
Table 2: Analysis of variance of the mean onset time of calling and mean time spent calling by virgin <i>Macrosiphum euphorbiae</i> females as a function of calling age under field conditions at two different periods in the autumn of 2001.	37
Table 3: Regression analysis of the proportion of virgin <i>Macrosiphum euphorbiae</i> oviparae calling throughout the photophase under field conditions from 11 to 18 September 2001 as a function of time of day, temperature, and wind speed.	38
Table 4: Regression analysis of the proportion of virgin <i>Macrosiphum euphorbiae</i> oviparae calling throughout the photophase under field conditions from 21 to 28 September 2001 as a function of time of day, temperature, and wind speed.	39

Chapitre III

Table 1: The ratio of nepetalactol: nepetalactone reported in the sex pheromone of different aphid species.	66
Table 2: Behavioral responses ($\bar{x} \pm \text{SEM}$) of <i>Macrosiphum euphorbiae</i> males when exposed to calling females and synthetic pheromone lures, differing in the ratio of nepetalactol: nepetalactone, under controlled conditions in a wind tunnel.	67

Chapitre IV

Table 1: Proportion of <i>Macrosiphum euphorbiae</i> males responding to sexual females as a function of distance to the source at different wind speeds in a wind tunnel at $18 \pm 0.5^\circ \text{C}$, 40- 50 % RH.	91
Table 2: Two-way ANOVA results summarizing the effect of distances from source within a given wind speed on <i>Macrosiphum euphorbiae</i> males' behaviour in a wind tunnel at $18 \pm 0.5^\circ \text{C}$, 40- 50 % RH.	92

Liste des figures

Chapitre II

- Figure 1: The age (days after final moult) at which virgin *Macrosiphum euphorbiae* oviparae called for the first time at three constant temperature regimes: (a) 10 °C, (b) 15 °C, and (c) 20 °C. Arrows indicate the mean age of calling..... 40
- Figure 2: (a) Mean onset time of calling (hours after the lights-on signal) and (b) mean time spent calling (hours) of virgin *Macrosiphum euphorbiae* oviparae as a function of calling age at 10, 15, and 20 °C. 41
- Figure 3: The age (days after final moult) at which virgin *Macrosiphum euphorbiae* oviparae called for the first time when placed under field conditions in early and late September 2001. Arrows indicate the mean age of calling..... 42
- Figure 4: (a) Mean onset time of calling (hours after the lights-on signal) and (b) mean time spent calling (hours) of virgin *Macrosiphum euphorbiae* oviparae as a function of calling age under field conditions in early and late September 2001.. 43
- Figure 5: Variability in the proportion of virgin *Macrosiphum euphorbiae* oviparae calling throughout the day, as a function of temperature and wind speed on (a) 26 , (b) 27, and (c) 28 September 2001. 44
- Figure 6: Variability in the proportion of virgin *Macrosiphum euphorbiae* oviparae calling throughout the day on 18 September 2001. 45
- Figure 7: Variability in the proportion of virgin *Macrosiphum euphorbiae* oviparae calling throughout the day, as a function of rainfall on (a) 22 and (b) 25 September 2001. ... 46

Chapitre III

- Figure 1: The experimental bridge which allows *M. euphorbiae* males to walk to different pheromone sources in a wind tunnel..... 68
- Figure 2: Proportion of *M. euphorbiae* males exhibiting orientation and flight response to different odour sources in a wind tunnel (40 cm/sec) when there was (a) no physical connection, and (b) a physical connection between the release site and the source... 69
- Figure 3: Proportion of *M. euphorbiae* males orienting, walking to the source or flying downwind when exposed to five calling oviparae in a wind tunnel (40 cm/sec) when there was a physical connection between the release site and the source..... 70

Chapitre IV

- Figure 1: Effect of wind speed on the proportion of *Macrosiphum euphorbiae* oviparae calling and males taking flight ($\bar{x} \pm \text{SEM}$; 3 replicates of 15 individuals at each wind speed) in a wind tunnel at $18 \pm 0.5^\circ \text{C}$, 40- 50 % RH..... 93
- Figure 2: Effect of wind speed on the proportion of *Macrosiphum euphorbiae* males ($\bar{x} \pm \text{SEM}$; 3 replicates of 15 individuals) taking flight in the presence or absence of calling females sexuals in a wind tunnel at $18 \pm 0.5^\circ \text{C}$, 40- 50 % RH.94

Figure 3: Effect of wind speed on the proportion of *Macrosiphum euphorbiae* males ($\bar{x} \pm$ SEM) (a) orientating and (b) waking the source, as well as (c) the time to reach the source in a wind tunnel at $18 \pm 0.5^\circ$ C, 40- 50 % RH. The source was 5 calling oviparae 40 cm upwind of the release site. Number of males tested are indicated within each column.95

Figure 4: Effect of wind speed on the proportion of *Macrosiphum euphorbiae* oviparae calling and males taking flight ($\bar{x} \pm$ SEM) under variable wind conditions in the field on (a) 15/9/01 and (b) 27/9/01. 30 females and 20 males were observed at each observation period.....96

Figure 5: Response of *Macrosiphum euphorbiae* males (N = 46; $\bar{x} \pm$ SEM) to calling females located 40 cm upwind under variable wind conditions in the field assays carried out on 11 and 24 October, 2001. Males could either fly or walk to the source.97

Figure 6: Temporal pattern of wind velocity during the assay carried out on 11 October, 2001 to test the response of *Macrosiphum euphorbiae* males to calling females located 40 cm upwind under variable wind conditions in the field. The periods identified as W and F represent the conditions males either walked or flew to the source..... 98

Chapitre I

Introduction

En lutte intégrée, de manière à optimiser le contrôle des insectes ravageurs tout en ayant le moins d'effets négatifs sur l'environnement, diverses méthodes peuvent être utilisées conjointement (Flint et Van den Bosch, 1981). En matière de contrôle des insectes nuisibles, une des conditions de base à remplir est d'avoir une approche aussi spécifique que possible vis-à-vis de l'organisme d'intérêt. Jusqu'à maintenant, l'un des meilleurs exemples de spécificité et de sélectivité en aménagement des insectes l'a été par le biais des infochimiques (i.e., substances chimiques impliquée dans le transfert d'informations lors d'une interaction entre deux individus). Il a été démontré que l'utilisation des infochimiques pour manipuler le comportement des insectes est l'une des composantes les plus utiles des programmes de lutte intégrée contre les insectes nuisibles (Bjostad et al., 1993).

Un des groupes d'infochimiques les plus connus et utilisés avec succès en lutte intégrée est celui des phéromones sexuelles, des infochimiques qui modulent les interactions entre deux individus de la même espèce. Celles-ci se sont révélées efficaces tant dans les programmes de dépistage, que dans la capture de masse, ou encore, pour créer une confusion sexuelle chez des insectes ravageurs (Ridgway et al., 1990; Minks et Cardé, 1997; Vickers, 2002). Toutefois, malgré les nombreuses tentatives d'utilisation des phéromones sexuelles en lutte intégrée, ces dernières n'ont pas été utilisées à leur plein potentiel. De plus, dans certains cas, une utilisation couronnée de succès une première fois a parfois échoué lorsque répétée (Sanders, 1997). Parmi les causes pouvant expliquer ces insuccès, des lacunes ont été identifiées au niveau de la reconnaissance des principes biologiques fondamentaux et de leur incorporation dans les programmes appliqués (Lewis, 1981), l'identification chimique incomplète des infochimiques (Tumlinson, 1988) et le manque d'information concernant les facteurs biotiques et abiotiques pouvant influencer la communication via les phéromones sexuelles (McNeil, 1991). Par conséquent, de meilleures connaissances sur la chimie, l'écologie, et le comportement de l'insecte sont nécessaires à l'élaboration de méthodes de contrôle efficaces des insectes nuisibles.

Les mélanges de phéromones et leur dosage sont des facteurs déterminants de la spécificité des phéromones et sont donc deux des aspects les plus importants de la biosynthèse phéromonale (Prestwich et Blomquist, 1987). L'identification des phéromones nécessite plus qu'une simple caractérisation des composés chimiques biosynthétisés par des insectes. La plupart des phéromones sont produites en quantité allant de l'ordre du nanogramme au picogramme. Plusieurs systèmes phéromonaux sont formés de mélanges à composés multiples, majeurs et mineurs, dans lesquels la proportion des différentes substances est déterminée avec précision. Quoiqu'ils puissent être des éléments clés de la compréhension des interactions comportementales chez les insectes, habituellement, les composés mineurs, par définition, ne forment qu'un faible pourcentage des mélanges. Par exemple, E10,Z12-16:Ald et E10,Z12-16:Ac sont les composés majeurs du mélange de phéromones sexuelles chez plusieurs espèces du genre *Hemileuca* (Lepidoptera: Saturniidae). Toutefois, E10,Z12-16:OH, un composé mineur, est essentiel à l'attraction des mâles de *H. maia* (McElfresh et al., 2001).

Chez les taxons apparentés, ce n'est pas seulement la présence de composés vraiment différents qui peut accorder une grande spécificité. Une différence importante peut être associée avec la stéréochimie d'une composante. Par exemple, deux races phéromonales de la pyrale du maïs, *Ostrinia nubilalis* (Lepidoptera: Pyralidae), sont retrouvées en Amérique du Nord. La phéromone utilisée par cette espèce est un mélange d'acétates de (E)- et de (Z)-11-tetradecenyl. Une lignée se sert d'un mélange au rapport E:Z de 96:4, alors que l'autre répond à un rapport E:Z de 3:97 (Kochansky et al., 1975). Dans les régions où les deux races existent, les mâles répondent préférentiellement à la phéromone de leur propre race et ainsi, les populations ne s'hybrident pas librement (Cardé et al., 1978). Un autre exemple peut être trouvé chez les races orientale et occidentale de *Ips pini* (Coleoptera: Scolytidae). Dans ce cas, la population de l'ouest produit et répond préférentiellement au (-)-ipsdienol, alors que celle de l'est utilise un mélange à 65:35 des deux énantiomères. Ainsi, les deux races répondent surtout à leur propre phéromone et les hybrides répondent davantage à leur propre mélange phéromonal hybride (Lanier et al., 1980).

Les récepteurs antennaires des insectes sont très spécifiques et de légers changements dans la géométrie d'une molécule peuvent rendre le composé inactif ou, voire

même, inhibiteur (Subchev et al., 2002). En conséquence, la chiralité peut jouer un rôle de premier plan dans la spécificité des phéromones (Silverstein, 1988). En effet, lorsqu'un énantiomère est utilisé pour la communication entre individus, l'autre peut engendrer une réponse positive, inhiber, ou encore n'entraîner aucune réponse (Prestwich et Blomquist, 1987). Le plus grand nombre de phéromones chirales a été découvert chez les coléoptères (Leal, 1998). Par exemple, aussi peu que 1% de l'énantiomère S-(+) de la lactone (Z)-5-(1-décenyl) dihydro-2(3H)-furanone réduit grandement la réponse des mâles du scarabée japonais, *Popillia japonica* (Coleoptera: Scarabaeidae) à sa phéromone. La phéromone produite naturellement par la chrysomèle des racines du maïs occidentale, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae), est la (R,R)-8-méthyle-2-2-décyle propanoate et les mâles ne sont ni inhibés, ni attirés par les isomères 2R,8S ou 2S,8S. Cependant, l'isomère 2S,8R crée une attirance, quoiqu'à un degré beaucoup moindre que le 2R,8R trouvé naturellement (Guss et al., 1984). La chrysomèle des racines du maïs septentrionale, *D. barberi*, une espèce très apparentée, est fortement inhibée par le stéréoisomère 2S,8R, mais se sert du 2R,8R pour communiquer (Dobson et Teal, 1987).

Dans l'identification d'une phéromone, une des choses les plus importantes est de déterminer avec exactitude la composition de la phéromone libérée dans l'atmosphère par l'insecte émetteur. Il est possible que la glande produisant la phéromone contienne plusieurs autres substances, comme des précurseurs, pouvant être inhibiteurs (Teal et al., 1984). Par exemple, les aldéhydes jouent un rôle de premier plan dans les systèmes phéromonaux sexuels chez toutes les espèces du genre *Heliothis* (Lepidoptera: Noctuidae). Toutefois, les extraits des glandes phéromonales femelles de *H. virescens* ont révélé la présence d'alcool correspondant, en termes de structure, aux molécules phéromonales aldéhydiques. Teal et Tumlinson (1986) ont démontré que les composés aldéhydiques de la phéromone de *H. virescens* étaient produits par l'action d'une oxydase alcoolique sur le précurseur d'alcools sécrétés par la glande phéromonale. Ainsi, les alcools primaires sont synthétisés dans la glande phéromonale sexuelle, pour ensuite être oxydés en aldéhydes lorsqu'ils traversent la cuticule. Ceci explique pourquoi seuls les aldéhydes ont été retrouvés parmi les substances volatiles récoltées chez *H. virescens* (Teal et al., 1986), même si les alcools sont trouvés en assez grande quantité dans les extraits de glandes. De plus, le rapport entre les composés du

mélange libéré dans l'atmosphère peut être différent de celui trouvé dans la glande (Teal et al., 1986).

La recherche portant sur la biosynthèse phéromonale permet de rehausser le niveau de nos connaissances, ainsi que la compréhension que nous avons des systèmes phéromonaux des insectes. Par ailleurs, compte tenu de l'accroissement de nos connaissances sur les mécanismes régissant ces systèmes, il semble raisonnable d'imaginer que nous devrions être capables de trouver des moyens permettant d'inhiber la biosynthèse phéromonale ou, du moins, de l'altérer pour ainsi développer de nouvelles techniques de lutte intégrée (Villorbina, 2003). La biosynthèse des phéromones reçoit une attention grandissante de la part de plusieurs groupes de recherche, et ce, sur une multitude d'insectes appartenant surtout aux ordres des Lépidoptères, des Diptères et des Coléoptères (Hoskovec et al., 2002). Chez les différents ordres, les voies et les sites de biosynthèse varient grandement. Alors que plusieurs mouches synthétisent leurs phéromones au niveau de la cuticule abdominale (Zurek et al., 2002), les phéromones de plusieurs espèces de coléoptères sont synthétisées au niveau de l'intestin (Vanderwel et Oehlschlager, 1987; Seybold et Tittiger, 2003). Toutefois, les Lépidoptères étudiés jusqu'à maintenant synthétisent leur phéromone sexuelle au niveau de glandes habituellement situées près de l'extrémité de l'abdomen (Bjostad et al., 1987; Choi et al., 2002).

L'ensemble des facteurs biotiques et abiotiques doivent être considérés (McNeil, 1991 et références citées) dans tout programme de lutte intégrée utilisant les phéromones sexuelles.

D'un point de vue biotique, il y a plusieurs aspects importants, incluant l'âge des individus et les plantes hôtes. L'âge au premier appel, le moment de la journée où débutent les appels, le temps passé à appeler et le titrage de la phéromone sont quelques-uns des aspects de la biologie phéromonale des femelles qui peuvent changer significativement avec l'âge (voir McNeil, 1991; Spurgeon, 2003). La réceptivité des mâles envers une source phéromonale peut aussi varier avec l'âge. Habituellement, les individus démontrent une augmentation de leur degré de réponse au cours des premiers jours suivant leur émergence (Turgeon et al., 1983). Les évidences disponibles suggèrent que les femelles Lépidoptères n'utiliseraient pas les composés végétaux secondaires spécifiques acquis pendant

l'alimentation au stade larvaire comme précurseurs phéromonaux, mais qu'elles les synthétiseraient *de novo* (Roelofs et Wolf, 1988). Toutefois, la qualité de la plante hôte peut avoir un effet significatif sur les différents paramètres physiologiques et comportementaux liés à la production phéromonale ainsi que la réponse des mâles (Usui et al., 1988; McNeil et Delisle, 1989; Landolt et Phillips, 1997).

Par ailleurs, les facteurs abiotiques peuvent aussi influencer l'émission et la détection des phéromones. Les conditions de température peuvent déterminer l'âge auquel les femelles débutent leurs appels pour la première fois. Chez les Lépidoptères, de manière générale, la période comprise entre l'émergence et le premier appel s'allonge avec une baisse de la température (Turgeon et McNeil, 1983; Delisle et McNeil, 1987a; Gerber et Howlader, 1987). Lorsque les femelles sont conservées sous différentes températures constantes depuis leur émergence (Delisle et McNeil, 1987a; Gerber et Howlader, 1987) ou qu'elles sont soumises à des variations de température à un certain moment suivant leur émergence (Cardé et Roelofs, 1973; Webster et Cardé, 1982; Haynes et Birch, 1984; Delisle et McNeil, 1987b), d'importants changements dans la périodicité des appels sont notés. Les conditions de température peuvent aussi modifier divers aspects de la réceptivité des mâles, tels que le moment où elle débute, l'activité quotidienne et le retard du frémissement des ailes chez les mâles en réponse aux phéromones (Turgeon et al., 1983; Cardé et Hagaman, 1983; Song et Ridel, 1985).

La durée du jour peut influencer l'âge auquel les femelles initient leurs appels, la périodicité de ces derniers, de même que leur durée. La période précédente les appels était nettement allongée lorsque les femelles de la légionnaire uniponctué, *Pseudaletia unipuncta* (Lepidoptera: Noctuidae) (Delisle et McNeil, 1986) et de la légionnaire orientale, *Mythimna separata* (Han, 1988 d'après McNeil, 1991) étaient soumises à des conditions de jours courts. La durée du jour serait encore plus importante pour la périodicité des espèces multivoltines, pour lesquelles la photopériode est nettement différente entre les périodes successives de vol (Haynes et Birch, 1984). Delisle et McNeil (1986) suggèrent que la durée du jour pourrait aussi être très importante pour les espèces qui subissent des changements rapides de photopériode suite à une migration de longue distance.

Très peu de recherches ont porté sur l'influence de l'humidité relative et de la vitesse des vents sur la communication phéromonale (voir McNeil, 1991). Webster et Cardé (1982) ont noté une diminution de la proportion de femelles en appel chez la pyrale du maïs, *Ostrinia nubilalis* (Lepidoptera: Pyralidae), lorsque celles-ci étaient transférées dans des conditions de faible humidité au deuxième ou au troisième jour suivant leur émergence, et que le comportement d'appel était amorcé plus tardivement au cours de la scotophase sous des conditions plus sèches. De plus, au cours de nuits d'appel successives, elles avaient moins de séquences d'appels et passaient moins de temps à appeler à des conditions de faible humidité comparativement à des conditions d'humidité élevée (Royer et McNeil, 1991). Royer et McNeil (1993) ont trouvé que la réponse des mâles de la pyrale du maïs à une phéromone synthétique dans un tunnel de vol était significativement affectée par l'humidité relative. Sur le terrain, une relation inverse a été observée entre le succès de capture par piège et le taux d'humidité relative dans un champ de coton chez *Earias vittella* (Lepidoptera: Noctuidae), capturée à l'aide de pièges appâtés avec une phéromone sexuelle (Naik et al., 1997). Cependant, il est bien certain que les effets de l'humidité relative peuvent varier selon les espèces. Par exemple, ni la période d'activité du mâle chez la tordeuse à bandes rouges, *Argyrotaenia velutinana* (Lepidoptera: Tortricidae) dans les vergers (Comeau et al., 1976), ni le taux de recapture de *Spodoptera litura* (Lepidoptera: Noctuidae) dans les pièges à phéromones sexuelles (Shih et Chu, 1995) étaient significativement affectés par l'humidité relative.

Le vent est également un facteur important pouvant influencer la communication chimique entre deux partenaires sexuels (McNeil, 1991). Kaae et Shorey (1972) ont trouvé que la proportion de femelles en appel de *Trichoplusia ni* (Lepidoptera: Noctuidae) était plus faible à des vents de 0 et 3 m/s qu'à 0.3 et 1 m/s et que les appels cessaient à des vitesses supérieures à 4 m/s. Une relation négative significative a été trouvée entre le succès de capture et la vitesse du vent chez les mâles de *Helicoverpa zea* et de *Heliothis virescens* (Lepidoptera: Noctuidae) attrapés à l'aide de pièges à phéromones sexuelles (Parajulee et al., 1998).

Les infochimiques, tout particulièrement les phéromones sexuelles, ont été étudiés intensivement et, offrent un très grand potentiel en ce qui concerne les stratégies de contrôle des insectes nuisibles. Ils peuvent être employés seuls ou en combinaison avec

d'autres approches pour les stratégies de lutte intégrée. Lorsqu'employés seuls, ils peuvent s'avérer être inefficaces ou insuffisamment robustes pour procurer un contrôle satisfaisant (Lewis, 1981). En cas de combinaison avec d'autres techniques de lutte intégrée, l'objectif est de réunir ces approches dans une stratégie de "push-pull", aussi connue sous le nom de "stimulo-deterrent diversionary strategy" (SDDS) (Miller and Cowles, 1990). Dans une SDDS, la récolte est protégée par des substances (i) qui masquent l'odeur de l'hôte et ainsi évite l'invasion par l'herbivore, (ii) qui sont repoussantes et qui entravent l'alimentation par des insectes nuisibles, et/ou (iii) qui attirent les prédateurs et les parasitoïdes dans l'aire en question. Par la même occasion, des infochimiques agrégatives peuvent être utilisées, dans cette stratégie, pour stimuler la colonisation des insectes nuisibles dans des « cultures pièges » ou favoriser leur attraction dans des pièges où des agents pathogènes peuvent être déployés (Pickett et al., 1994; Hartfield et al., 2001). Par exemple, dans le cas des scolytes *Ips paraconfusus* (Coleoptera: Scolytidae), l'utilisation combinée du verbenone (phéromone répulsive) au niveau des zones à protéger et du (-)-ipsdienol (phéromone d'agrégation) dans des zones pièges permet de grandement diminuer l'impact négatif de ce ravageur (voir Borden, 1997 et références citées).

Les Pucerons

Les pucerons (Homoptera: Aphididae), particulièrement ceux de la sous-famille des Aphidinae, sont parmi les insectes nuisibles les plus dommageables pour les cultures et les forêts (Dixon, 1998; Blackman et Eastop, 2000). Non seulement en raison des effets directs des populations à densité élevée, mais aussi indirectement, en tant que vecteurs de nombreux virus affectant les végétaux (Kennedy et al., 1962; Alyokhin et al., 2002). Les caractéristiques propres au cycle de vie des pucerons sont parmi les plus remarquables du monde animal. Entre autres, se trouvent des générations issues de la parthénogénèse et de la reproduction sexuée, des polymorphismes complexes et l'obligation d'alterner entre des plantes-hôtes de taxons non-apparentés (Moran, 1992; Margaritopoulos et al., 2002). Différents patrons de cycle de vie ont été identifiés chez les pucerons. Ces cycles sont soit holocycliques ou anholocycliques selon le fait qu'une génération à reproduction sexuée soit

présente ou non, ou encore, qualifiés d'hétéroïque ou de monoïque s'il y a ou non alternance d'hôtes (Miyazaki, 1987).

Plusieurs espèces de pucerons produisent des morphes sexuels l'automne afin de permettre la ponte d'oeufs en diapause sur la plante-hôte primaire pour l'hiver (Moran, 1992). Les femelles sexuées aptères qui pondent ainsi leurs oeufs, libèrent des phéromones sexuelles qui attirent les mâles de la même espèce en vue de l'accouplement. Ces dernières années, les phéromones sexuelles de plusieurs espèces de pucerons ont été identifiées comme étant un mélange variable de deux monoterpénoïdes: le nepetalactol et le nepetalactone (Dawson et al., 1990). La proportion des deux composés diffère d'une espèce de puceron à une autre et malgré le fait que les mâles soient attirés par un éventail plus ou moins large de rapports (Hardie et al., 1990) ou que les phéromones sexuelles de différentes espèces puissent attirer à l'intérieur d'olfactomètres des mâles de nombreuses espèces (Guldmond et Dixon, 1994), jusqu'à un certain point, une spécificité a pu être démontrée. L'utilisation des phéromones sexuelles des pucerons est envisagée pour contrôler les populations de pucerons à leurs sites d'hivernage par piégeage (Campbell et al., 1990; Hardie et al., 1992), pour manipuler les parasitoïdes des pucerons (Lilley et al., 1994; Gabryś et al., 1997) et pour augmenter l'efficacité de certains agents pathogènes en favorisant le contact pathogène/ravageur (voir Pickett et al., 1994; Hartfield et al., 2001). Ainsi, les phéromones sexuelles peuvent jouer un rôle de grande importance dans les programmes visant à contrôler les pucerons. Par ailleurs, les composés chimiques secondaires végétaux, en tant qu'infochimiques de premier niveau trophique, jouent un rôle important dans les programmes de lutte intégrée. Par exemple, quelques-unes des substances anti-appétantes dérivées de végétaux les plus actives sont les drimanes sesquiterpènes extraits de l'arbre africain *Warburgia sp.*. Ceux-ci sont très efficaces contre la colonisation par les pucerons et la transmission des virus (Asakawa et al., 1988). Le salicylate de méthyle est un composé volatil produit par le cerisier à grappes, l'hôte hivernal de *Rhopalosiphum padi*. Certains ont suggéré que même si ce composé semble attirer les individus migrants à l'automne, il repousse ceux qui migrent au printemps ; le salicylate de méthyle pouvant significativement réduire la colonisation (Pettersson et al., 1994) ou retarder l'immigration et l'établissement (Ninkovic et al., 2003) de *R. padi* dans les cultures céréalières

Plusieurs des différents types d'infochimiques impliqués dans les relations "pucerons-plante(s) hôte(s)", "pucerons-pucerons" et "pucerons-ennemi(s) naturel(s)" ont été identifiés et synthétisés. Dans cette continuité, des tentatives visant à combiner les effets d'attraction et de répulsion par les infochimiques pour obtenir des systèmes efficaces de lutte intégrée par les méthodes de "push-pull" sont maintenant en cours (Pickett et al., 1997). Toutefois, des lacunes sont présentes tant au niveau de l'identification des phéromones, qu'en ce qui concerne les aspects biologiques fondamentaux et comportementaux, en particulier sur le terrain, de la reproduction sexuée des pucerons. Afin de maximiser les chances de succès dans la gestion des populations de pucerons par l'entremise des infochimiques, ces connaissances sont essentielles.

Le puceron de la pomme de terre

Le puceron de la pomme de terre, *Macrosiphum euphorbiae* (Thomas), est un puceron holocyclique et hétéroïque chez qui les formes sexuées s'observent à l'automne sur certaines Rosacées (*Rosa nitida*, *R. palustris* et *R. rugosa*) servant d'hôtes primaires, en vue de la reproduction sexuée et de la ponte d'œufs hibernants (Shands et al., 1972; Alford, 1991). Plusieurs plantes herbacées, souvent des espèces cultivées, sont utilisées comme hôtes secondaires au cours de l'été. Ce puceron est donc nuisible tant pour les cultures de pomme de terre que celles de tomates, en plus d'être un important vecteur de nombreux virus affectant les végétaux (Alyokhin et al., 2002).

Description des chapitres

Dans cette thèse, en prenant le puceron de la pomme de terre, *M. euphorbiae*, comme modèle, j'ai entrepris des études sur l'écologie phéromonale de cette espèce, au laboratoire (i.e., conditions contrôlées) ainsi que sur le terrain. Les objectifs étaient d'obtenir une connaissance fondamentale de la biologie reproductive encore peu connue de cette espèce, et d'interpréter les résultats dans le contexte de l'importance de la spécificité de la phéromone sexuelle pour éviter l'hybridation entre des espèces sympatriques. De plus, je voulais voir si une meilleure connaissance du comportement reproducteur de cette espèce permettra l'utilisation efficace des phéromones dans un programme de lutte contre ce ravageur.

Dans le premier chapitre j'ai examiné le comportement d'appel des femelles ovipares sous différents régimes de températures constantes afin de tester plusieurs hypothèses: (i) l'âge de premier appel est affecté par la température, et, contrairement aux espèces estivales les femelles atteindront la maturité sexuelle à un plus jeune âge lorsqu'elles sont soumises à des températures plus froides car les formes sexuées des pucerons sont présentes en automne, (ii) il y a une fenêtre temporelle spécifique pour l'expression du comportement d'appel, et (iii) le temps passé en appel augmente avec l'âge d'appel de la femelle, ceci étant, comme chez des Lépidoptères, une adaptation pour augmenter la probabilité d'accouplement. Par la suite j'ai examiné le comportement d'appel sur le terrain afin de voir si les patrons observés sous des conditions contrôlées en laboratoire étaient maintenus sous des conditions abiotiques variables. Les résultats seront discutés en fonction de l'hypothèse proposée par Guldmond and Dixon (1994), selon laquelle la séparation temporelle des périodes d'appels peut être un mécanisme efficace d'isolement reproductif pour diverses espèces de pucerons sympatriques.

Dans le deuxième chapitre j'ai entrepris d'identifier la phéromone sexuelle du puceron de la pomme de terre et de voir s'il y avait des changements dans sa composition en fonction de l'âge des femelles. Trois hypothèses ont été testées: (i) la phéromone sexuelle de puceron de la pomme de terre est composée de deux monoterpénoïdes, le nepetalactol et le nepetalactone avec un ratio différent de celui des autres espèces, (ii) comme la phéromone est un moyen de communication spécifique pour maintenir l'isolement reproducteur, le ratio des composantes de la phéromone ne change pas avec l'âge des femelles, et (iii) les mâles détectent la phéromone sexuelle et répondent en volant vers une source de phéromone. Nos résultats suggèrent comme certaines des études précédentes, que l'isolement reproductif chez les pucerons est plus complexe que prévue initialement.

Le troisième chapitre porte sur l'effet du vent sur la capacité de vol des mâles vers une source de phéromone sexuelle dans des conditions contrôlées en laboratoire et sous des conditions variables sur le terrain. Étant donné les résultats disponibles dans la littérature sur la réponse des mâles aux phéromones sexuelles chez d'autres insectes (e.g., les Lépidoptères), mes hypothèses de départ ont été que les mâles, de par leur petite taille et leur faible capacité de vol, vont (i) s'activer seulement à des faibles vitesses de vent, (ii)

entreprendre un vol orienté vers une source en présence des faibles vents, et (iii) entreprendre des vols à des plus hautes vitesses du vent quand ils sont en présence de la phéromone que lorsqu'il n'y a pas de source de phéromone.

L'ensemble des résultats de ma thèse indique clairement la nécessité d'étudier différents aspects de la biologie reproductive des pucerons (études chimiques et comportementales) en plus grand détail afin de clarifier si les phéromones permettent des mécanismes efficaces d'isolement reproducteur pour diverses espèces sympatriques. De plus, certaines approches proposées pour l'utilisation des phéromones sexuelles dans la lutte contre les espèces nuisibles (Pickett et al., 1994) devront être revisitées s'il est confirmé que la marche plutôt que le vol est le comportement principalement utilisé par les mâles pour trouver des femelles.

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Chapitre II

Calling behaviour of the potato aphid *Macrosiphum euphorbiae* (Thomas) (Homoptera: Aphididae) oviparae under laboratory and field conditions

Résumé

1. Le comportement d'appel des femelles sexuées vierges du puceron de la pomme de terre, *Macrosiphum euphorbiae* (Thomas) (Homoptera: Aphididae), a été étudié en laboratoire sous trois températures constantes différentes. L'âge moyen au premier appel décroît avec la température et va de 2,9 jours pour une température de 20°C à 2,1 jours pour une température de 10°C. Pour tous les régimes thermiques, le temps moyen du début des appels était devancé de 5 à 3 h suite à l'ouverture des lumières, et le temps moyen passé à appeler augmentait de plus de 4 h au cours des 8 jours.

2. Les cohortes de femelles sexuées ont aussi été observées à deux différents moments sur le terrain vers la fin de l'été-début de l'automne pour évaluer l'effet des changements dans les conditions abiotiques (température, vitesse du vent et pluie) et de l'âge sur le comportement d'appel. Tout comme en laboratoire, l'âge moyen au premier appel diminuait avec la température pour être de 3,7 jours au début septembre et de 1,6 jours à la fin du même mois. Les changements liés à l'âge pour l'heure de début d'appel et le temps moyen passé à appeler étaient beaucoup moins constants sur le terrain, étant donnés les effets inhibiteurs des basses températures, des forts vents et de la pluie sur l'activité d'appel des femelles.

3. Les résultats sont discutés dans un contexte de succès reproductif et en fonction d'une hypothèse proposée antérieurement qui suggère qu'une fenêtre temporelle d'appels spécifique à chaque espèce peut servir de mécanisme d'isolement reproductif pour des espèces sympatriques de pucerons.

Abstract.

1. The calling behaviour of virgin oviparae of the potato aphid *Macrosiphum euphorbiae* (Thomas) (Homoptera: Aphididae) was studied at three different constant temperatures under laboratory conditions. The mean age of calling for the first time decreased with a decrease in temperature from 2.9 days at 20 °C to 2.1 at 10 °C. At all temperature regimes, the mean onset time of calling advanced from about 5 to 3 h after the onset of the photophase, and the mean time spent calling increased by > 4 h over the first 8 days of calling.

2. Cohorts of oviparae were also observed at two different periods in late summer-early autumn in the field, to examine the effects of fluctuating abiotic conditions (temperatures, wind velocity, rain) and age on calling behaviour. As under constant laboratory conditions, the mean age of calling for the first time declined with declining temperature, from 3.7 days in early September to 1.6 days at the end of September. Age related changes in the mean onset time of calling and the mean time spent calling were much less evident under field conditions, due to the inhibitory effects of low temperatures, high winds, and rain on female calling activity.

3. The results are discussed within the context of reproductive success and address a previously proposed hypothesis suggesting that species-specific calling windows may serve as a reproductive isolating mechanism for sympatric aphid species.

Introduction

Species-specific sex pheromones may serve as one of the reproductive isolation mechanisms for closely related, sympatric insect species (e.g. Lofsted & Van Der Pers, 1985; McLellan *et al.*, 1991), however, the sex pheromone of many of the aphid species studied to date is composed of the same two monoterpenoids, nepetalactol and nepetalactone, differing only in blend ratio (Dawson *et al.*, 1990; Pickett *et al.*, 1997). In addition, bioassays have shown that males may respond to a range of ratios (Hardie *et al.*, 1990; S. H. Goldansaz & J. N. McNeil, unpublished), as well as to interspecific pheromones in an olfactometer (Guldemon & Dixon, 1994). Therefore, unless there are specific differences in the stereochemistry of the two monoterpenoids (Hardie *et al.*, 1997) or additional pheromone components that have not been identified, other factors must play a role to avoid interbreeding. Guldemon and Dixon (1994) studied the calling behaviour associated with the release of the sex pheromone in three species of *Cryptomyzus* and hypothesised that interspecific differences in the temporal calling patterns may reduce hybridisation, an idea that has also been proposed in Lepidoptera (Teal & Byers, 1980; Lofstedt & Van Der Pers, 1985). Clearly the reliability of a temporal calling window as a component of reproductive isolation requires that the calling period remains fairly constant under varying environmental conditions.

Calling behaviour has been well documented in Lepidoptera (e.g. Swier *et al.*, 1977; Turgeon & McNeil, 1982; West *et al.*, 1984; Delisle & McNeil, 1987a; Delisle, 1992; Hirashima *et al.*, 2001) and there is clear evidence that the periodicity of calling may vary with respect to age, the presence or absence of suitable host plants, and an array of abiotic factors, such as temperature, photoperiod, and relative humidity (see McNeil, 1991, and references therein). In contrast, there is a paucity of detailed information about the calling behaviour of aphids (although see Eisenbach & Mittler, 1980; Guldemon & Dixon, 1994), and the studies have generally been carried out under controlled laboratory conditions.

In the work reported here, the calling behaviour of virgin oviparae (sexual females) of the potato aphid *Macrosiphum euphorbiae* is examined under three constant temperature conditions in the laboratory and under variable field conditions.

Materials and methods

Insects

All aphids used in the experiments came from a multiclonal laboratory culture, established and restocked annually using parthenogenic individuals collected from potato fields in the vicinity of Quebec City, Canada. Colonies were maintained on potato seedlings *Solanum tuberosum* c.v. Norland, at 20 ± 1 °C, $60 \pm 10\%$ RH, under a LD 16:8 h photoperiod, conditions that ensure continuous asexual production. Potato seedlings at the four to six leaf stage were used as host plants in all experiments, and new plants were provided every 3 days.

It requires two generations reared at 20 ± 1 °C, $60 \pm 10\%$ RH, under a LD 10:14 h photoperiod to obtain sexuals. The gynoparous females (which produce the sexual females) are produced in the first generation; these were held in groups on host plants and their offspring were collected daily to ensure production of known-age oviparae. All experiments were carried out under these short day conditions.

Laboratory experiments

Newly moulted virgin oviparae were placed on individual potato plants and were reared, simultaneously, at 10, 15 or 20 (± 0.5) °C. There were 30 females in each temperature treatment and the experiment was replicated three times, rotating the temperature treatments among the three incubators. Preliminary experiments confirmed that *M. euphorbiae* female calling behaviour is only expressed during the photophase, as reported for *Schizaphis graminum* (Eisenbach & Mittler, 1980) and *Cryptomyzus spp.* (Guldmond & Dixon, 1994). Therefore, the oviparae were observed every 30 min throughout the photophase at the three temperature regimes to determine the mean age of first calling, the mean onset time of calling, and the mean time spent calling on the first 8 days of calling.

Females were considered as calling when the abdomen and hind legs were raised and not in contact with the host plant surface. Once initiated, calling is generally

continuous, with females rarely exhibiting interrupted calling bouts, contrary to the patterns reported in many moths (e.g. Turgeon & McNeil, 1982; McNeil & Delisle, 1989). If a female was in the calling position during two consecutive observation periods, it was considered to have called for 1 h but only for 30 min if it called in only one of two consecutive observations.

Field experiments

Two series of field experiments were conducted on the campus of Laval University, Ste-Foy, Quebec (Latitude: 46°, 48' N; Longitude: 71°, 23' W) in early and late September 2001, using sexuals produced from the laboratory culture. Newly moulted adult oviparae were placed on individual potato plants and transferred to a large table in the field, 72 on 8 September and 74 on 21 September. Observations were made every 30 min 2 h after sunrise to 1 h before sunset (preliminary observations had indicated that calling did not occur outside this window) on each day for 5 days to determine the mean age of first calling. In order to determine the mean onset time of calling and the mean time spent calling over the first 8 days of calling, a randomly selected subsample of 30 individuals that all initiated calling on the same day (11 and 21 September) was used, to ensure that females were all of the same calling age throughout the observation period. Temperature and wind speed measurements beside the plants were taken coincident with each observation period, using a portable thermometer/wind meter (TA 3000T, Airflow Developments, Ltd., Toronto, Canada).

Statistical analysis

An analysis of variance, followed by a LSD test, was used for the mean age of calling for the first time data in the laboratory experiment while a t-test was used to compare the mean age of calling for the first time of females placed in the field in early and late September. The data of the mean onset time of calling and the mean time spent calling from all experiments were subjected to ANOVA with repeated measures. In field experiments, the effects of wind speed and ambient temperature on the proportion of calling females throughout the day were examined using a multiple regression analysis on transformed (logit) data (SAS, 1999).

Results

The mean age of calling for the first time was affected significantly by temperature under laboratory conditions ($F = 7.08$, d.f. = 2,4, $p < 0.05$), with females at 20 °C calling for the first time at a significantly older age than those at the lower temperatures (Fig. 1).

Once initiated, the calling behaviour followed a similar temporal patterns at all constant temperature conditions in the laboratory. The mean onset time of calling (Fig. 2a) occurred significantly earlier on successive days of calling and the significant temperature \times age interaction resulted from the earlier onset of calling of females during the first few days at 10 °C (Table 1, Fig. 2a). The mean time spent calling (Fig. 2b) increased significantly on successive days of calling, resulting more from an increase in the duration of individual calling bouts rather than an increase in the number of bouts, as these ranged from 1.3 to 1.8 bouts per day at all temperatures (data not shown). There was also a significant effect of temperature on the mean time spent calling (Table 1), with females at 20 °C calling more than those at lower temperatures (Fig. 2b).

Females placed in the field in early September (mean day time temperature: 24.2 ± 1.8 °C) called for the first time at a significantly older age (3.7 vs 1.6 days; $t = 3.18$, d.f. = 5, $p < 0.05$) than the cohort started 12 days later, when the mean daily temperature was 15.8 ± 0.1 °C. No individuals initiated calling on the day following emergence in early September, compared with 42% of females placed in the field in late September (Fig. 3). The age-related changes in the mean onset time of calling and the mean time spent calling seen in the laboratory (Fig. 2) were less evident under field conditions (Fig. 4). This is due to marked day to day variations associated with changing climatic conditions, and is reflected by significant cohort \times age interactions (Table 2).

In both field experiments, the best models (explaining 45 and 58% of the overall variability in the proportion of females calling) included temperature, wind speed, and time of day (Tables 3 and 4). The relationship between temperature and time of day differed between the two experimental periods, with a significant quadratic relationship and temperature \times time interaction present in early (Table 3) but not in late (Table 4) September.

This difference may be due to the higher daytime temperatures in mid-September, which result in longer calling windows than at the lower temperatures in late-September. There was a significant inverse relationship between calling and wind speed in both experiments (Tables 3 and 4), with calling behaviour ceasing at wind speeds $> 4 \text{ m s}^{-1}$ (S. H. Goldansaz & J. N. McNeil, unpublished), and in the trial carried out in late September there was a marginally significant temperature \times wind interaction (Table 4).

An examination of the temporal patterns of the proportion of females calling on specific days provided certain insights into the manner in which different abiotic factors may influence calling behaviour. For example, the majority of the cohort called for about 8 h on 26 September (Fig. 5a) when the air temperature was very stable and wind speeds never exceeded 2 m s^{-1} . In contrast, on 27 and 28 September, females only called for about 4 h. On the 27 September, females called in the morning but a slight drop in temperature and a significant increase in wind speed inhibited calling in the afternoon (Fig. 5b). On the 28 September, however, the majority of calling activity occurred in the afternoon when air temperatures had risen and wind speeds declined (Fig. 5c).

In the models (Tables 3 and 4), time of day was a significant parameter. Clearly there is a significant time of day \times temperature interaction in early September (Table 3) but it is possible that this may also be the result of changes in abiotic factors, such as light intensity, not measured in this study. An example of this is seen from the data for 18 September. While individuals in the population called throughout the day, there was a marked temporary decline in the early afternoon (Fig. 6), during a period when both temperature and wind conditions were relatively constant and there were no evident climatic changes, such as a sudden rain shower.

Rainfall also influenced female calling behaviour. On 22 September, all calling behaviour stopped in early afternoon (Fig. 7a), coinciding with a 1 h shower when 10 mm of rain fell; however calling resumed immediately once it stopped raining. On 25 September it rained continuously all day (average 4.5 mm h^{-1}), and $< 10\%$ of the females called, and only then for a short period around 12:00 hours (Fig. 7b). However, as the rain was also accompanied by high winds it is not possible to attribute the absence of calling just to the continuous precipitation.

Discussion

The age at which females initiate calling for the first time is temperature dependent in *M. euphorbiae*, although contrary to the patterns generally reported for Lepidoptera (McNeil, 1991, and references therein) as oviparae call at a younger rather than older age as ambient temperatures decline. This is not particularly surprising however when considered within the context of the aphid's biology, as mating to produce the overwintering egg stage occurs late in the season when temperatures are low. A similar case of greater pheromone-related activities at lower than higher temperatures has been reported in the winter moth, *Operophtera brumata* (L.), which also mates late in the autumn (Roelofs *et al.*, 1982).

Possible interspecific differences in the mean age of calling for the first time may exist, for at 15 °C females of three *Cryptomyzus* species (Guldemon & Dixon, 1994) and two subspecies of the bean aphid *Aphis fabae* (Thieme & Dixon, 1996) initiated calling, on average, at a older age than observed for the potato aphid; however in this study observations were carried out at LD 10:14 h while those of Guldemon and Dixon (1994) and Thieme and Dixon (1996) were at LD 12:12 h. Therefore, it is possible that temperature effects on the mean age of calling for the first time were modulated by prevailing photoperiodic conditions, similar to the temperature x photoperiodic interactions seen in the true armyworm *Pseudaletia unipuncta* (Delisle & McNeil, 1987b). Decreasing day length would be a more reliable environmental cue of future habitat deterioration than temperature under autumn conditions and may serve to accelerate the pheromone production and the onset of calling, possibly through physiological changes, as demonstrated in the true armyworm (Cusson *et al.*, 1990).

Once pheromone emission has been initiated both mean onset time of calling and mean time spent calling of *M. euphorbiae* oviparae change as a function of age at all temperatures, although the differences are more pronounced under the constant laboratory conditions rather than variable field conditions. These changes, which result in an increasingly greater calling window over successive days of pheromone emission, have been reported in a few aphid species (Guldemon & Dixon, 1994) and a large number of Lepidoptera (see McNeil, 1991, and references therein). Swier *et al.* (1977) suggested that

these changes may increase the probability of an ageing virgin female attracting a mate, when competing with younger conspecifics. This idea is supported by work on the oblique banded leafroller *Chroistoneura rosaceana*, in which younger females had higher pheromone titre (Delisle & Royer, 1994) and under field conditions attracted many more males than did older conspecifics (Delisle, 1992). A similar scenario could hold for aphids, as there is evidence that the pheromone titre/ratio (the vetch aphid *Megoura viciae*: Hardie *et al.*, 1990) and attractiveness to males (the greenbug *Schizaphis graminum*: Eisenbach & Mittler, 1980) may change as females age. To address this question, however, it is necessary to know how long, under variable field conditions, receptive females take to attract a suitable mate. In the laboratory, females mate readily on the first day of calling and if this holds true in the field, competition between different-aged females for available mates would be relatively low. At the lower temperatures generally encountered during the autumn reproductive season, females call for less time than at higher temperatures but the actual calling behaviour is more pronounced, with the abdomen and back legs being much further off the leaf surface. If this is associated with a higher release of pheromone, it could increase the probability of rapidly attracting a mate.

Reduced calling windows at the lower temperatures encountered in the autumn and mating as soon as possible after sexual maturation would not only reduce intraspecific competition for males and ensure the rapid onset of oviposition, it could also reduce predation and parasitism. Aphid sex pheromones may act as kairomones in attracting predators and parasitoids (Dawson *et al.*, 1987; Boo *et al.*, 1998, and references therein) so any reduction in the time of emission by virgins, and the onset of pheromonostasis (inhibition of pheromone production) in mated females (Eisenbach & Mittler, 1980) should decrease the probability of being detected by natural enemies.

As seen in this study, the rather consistent calling patterns observed under controlled laboratory conditions are much less evident in the field, with both the time of calling and the time spent calling varying markedly from one day to the next in response to variations in factors such as temperature, wind speed, and rainfall. The high degree of inter-day variability seen for *M. euphorbiae* suggests species specific calling windows may play a minor role as a reliable reproductive isolating mechanism if there were other closely related sympatric species present. In fact, this may also be the case for species that are not

that closely related as (i) the sex pheromone of a number of quite different aphids are composed of differing ratios of the same two monoterpenoids (Dawson *et al.*, 1990; Pickett *et al.*, 1997), (ii) intraspecific ratios may change with female age (Hardie *et al.*, 1990; S. H. Goldansaz *et al.*, unpublished), and (iii) males may respond to a range of ratios (Hardie *et al.*, 1990; S. H. Goldansaz *et al.*, unpublished) and interspecific pheromones (Guldemon & Dixon, 1994) under laboratory conditions. Therefore, detailed field studies are essential to actually determine if, and to what extent, temporally specific calling windows reduce the probability of interspecific mating under natural conditions, as initially proposed by Guldemon and Dixon (1994).

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Table 1: Analysis of variance of the mean onset time of calling and mean time spent calling by virgin *Macrosiphum euphorbiae* females as a function of calling age under different constant temperature conditions in the laboratory.

	Effect	d.f.	F value	P
Mean onset time of calling	Block	2,4	12.46	0.0191
	Temperature	2,4	10.54	0.0254
	Age	7,42	98.66	< 0.0001
	Temperature × Age	14,42	3.22	0.0017
Mean time spent calling	Block	2,4	10.6	0.0252
	Temperature	2,4	25.03	0.0055
	Age	7,42	98.97	< 0.0001
	Temperature × Age	14,42	1.31	0.2419

Table 2: Analysis of variance of the mean onset time of calling and mean time spent calling by virgin *Macrosiphum euphorbiae* females as a function of calling age under field conditions at two different periods in the autumn of 2001.

	Effect	d.f.	F value	P
Mean onset time of calling	Cohort	1,58	177.69	< 0.0001
	Age	7,341	138.23	< 0.0001
	Cohort x Age	7,341	112.37	< 0.0001
Mean time spent calling	Cohort	1,58	18.99	< 0.0001
	Age	7,406	65.58	< 0.0001
	Cohort x Age	7,406	38.54	< 0.0001

Table 3: Regression analysis of the proportion of virgin *Macrosiphum euphorbiae* oviparae calling throughout the photophase under field conditions from 11 to 18 September 2001 as a function of time of day, temperature, and wind speed.

Factor	d.f.	PE [†]	SE	t	p
Intercept	1	-75.27968	5.68389	-13.24	< 0.0001
Temperature	1	-1.46042	0.36383	-4.01	< 0.0001
Temperature ²	1	0.35281	0.04424	7.97	0.0079
Wind	1	-0.22374	0.08324	-2.69	< 0.0001
Time of day	1	13.68732	1.14238	11.98	< 0.0001
Time of day ²	1	-0.50922	0.04045	-12.59	< 0.0001
Temperature ² × Time	1	-0.04706	0.00494	-9.53	< 0.0001
Temperature ² × Tim ²	1	0.00171	0.00017	10.05	< 0.0001

† Parameter estimate.

Table 4: Regression analysis of the proportion of virgin *Macrosiphum euphorbiae* oviparae calling throughout the photophase under field conditions from 21 to 28 September 2001 as a function of time of day, temperature, and wind speed.

Factor	d.f.	PE [†]	SE	t	p
Intercept	1	-20.41268	2.67675	-7.63	< 0.0001
Temperature	1	-0.4343	0.08391	-5.18	< 0.0001
Time of day	1	4.17263	0.42297	9.87	< 0.0001
Time of day ²	1	-0.1536	0.01548	-9.92	< 0.0001
Wind	1	-1.24656	0.3101	-4.02	< 0.0001
Temperature x Wind	1	0.04364	0.02207	1.98	0.0495

† Parameter estimate.

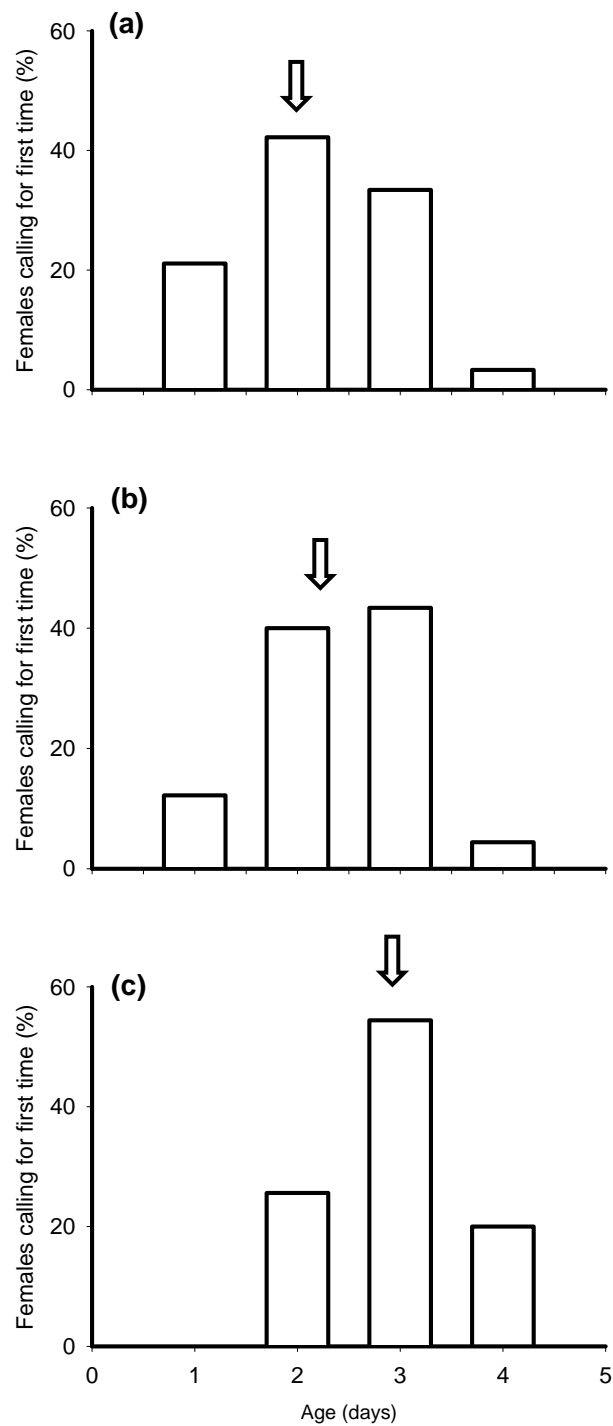


Figure 1: The age (days after final moult) at which virgin *Macrosiphum euphorbiae* oviparae called for the first time at three constant temperature regimes: (a) 10 °C, (b) 15 °C, and (c) 20 °C. Arrows indicate the mean age of calling

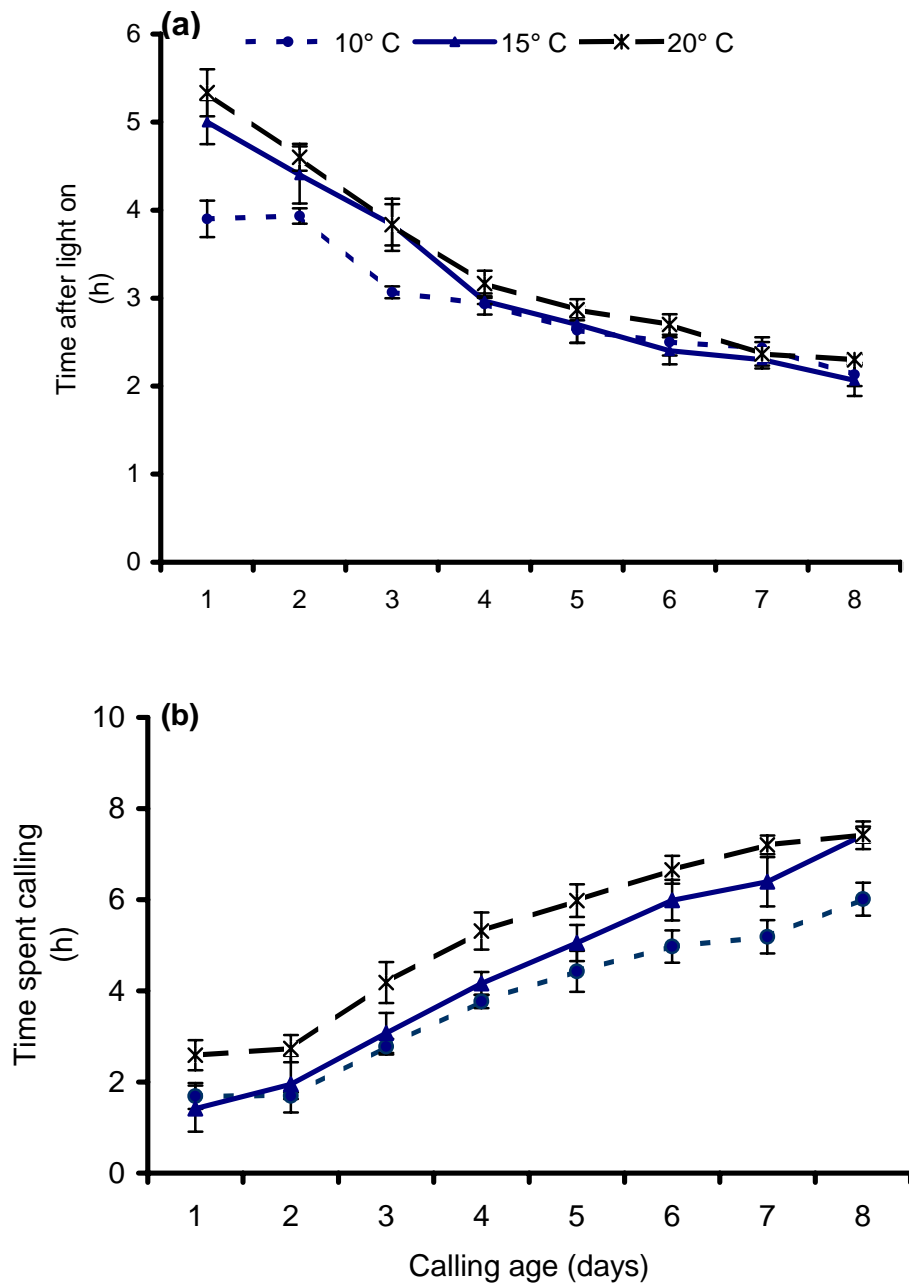


Figure 2: (a) Mean onset time of calling (hours after the lights-on signal) and (b) mean time spent calling (hours) of virgin *Macrosiphum euphorbiae* oviparae as a function of calling age at 10, 15, and 20 °C.

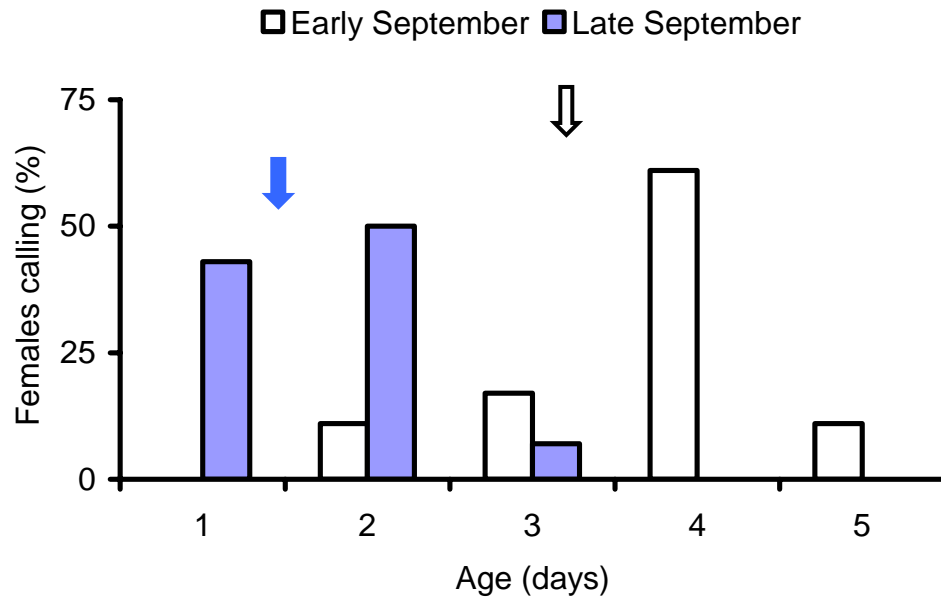


Figure 3: The age (days after final moult) at which virgin *Macrosiphum euphorbiae* oviparae called for the first time when placed under field conditions in early and late September 2001. Arrows indicate the mean age of calling.

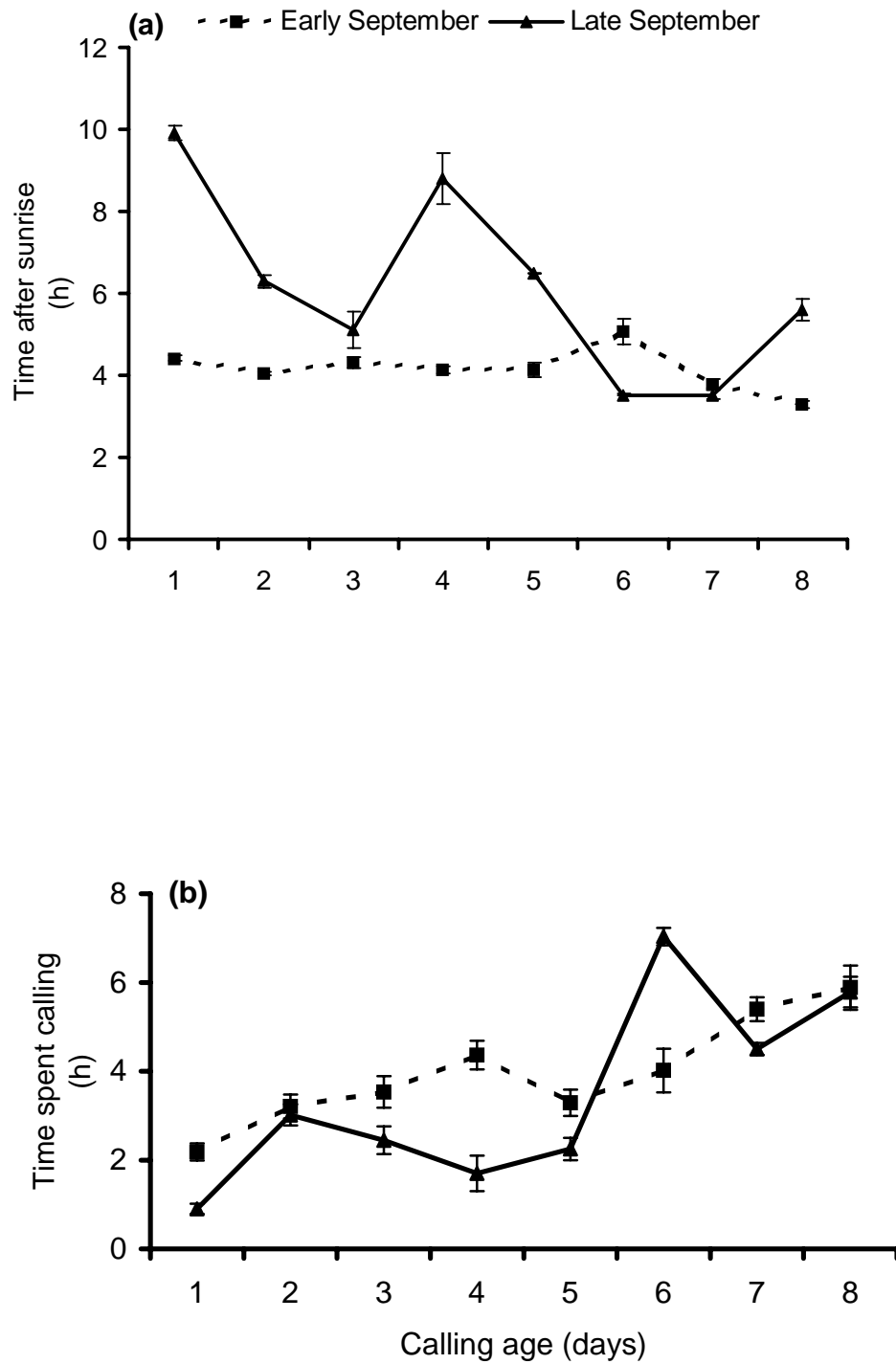


Figure 4: (a) Mean onset time of calling (hours after the lights-on signal) and (b) mean time spent calling (hours) of virgin *Macrosiphum euphorbiae* oviparae as a function of calling age under field conditions in early and late September 2001.

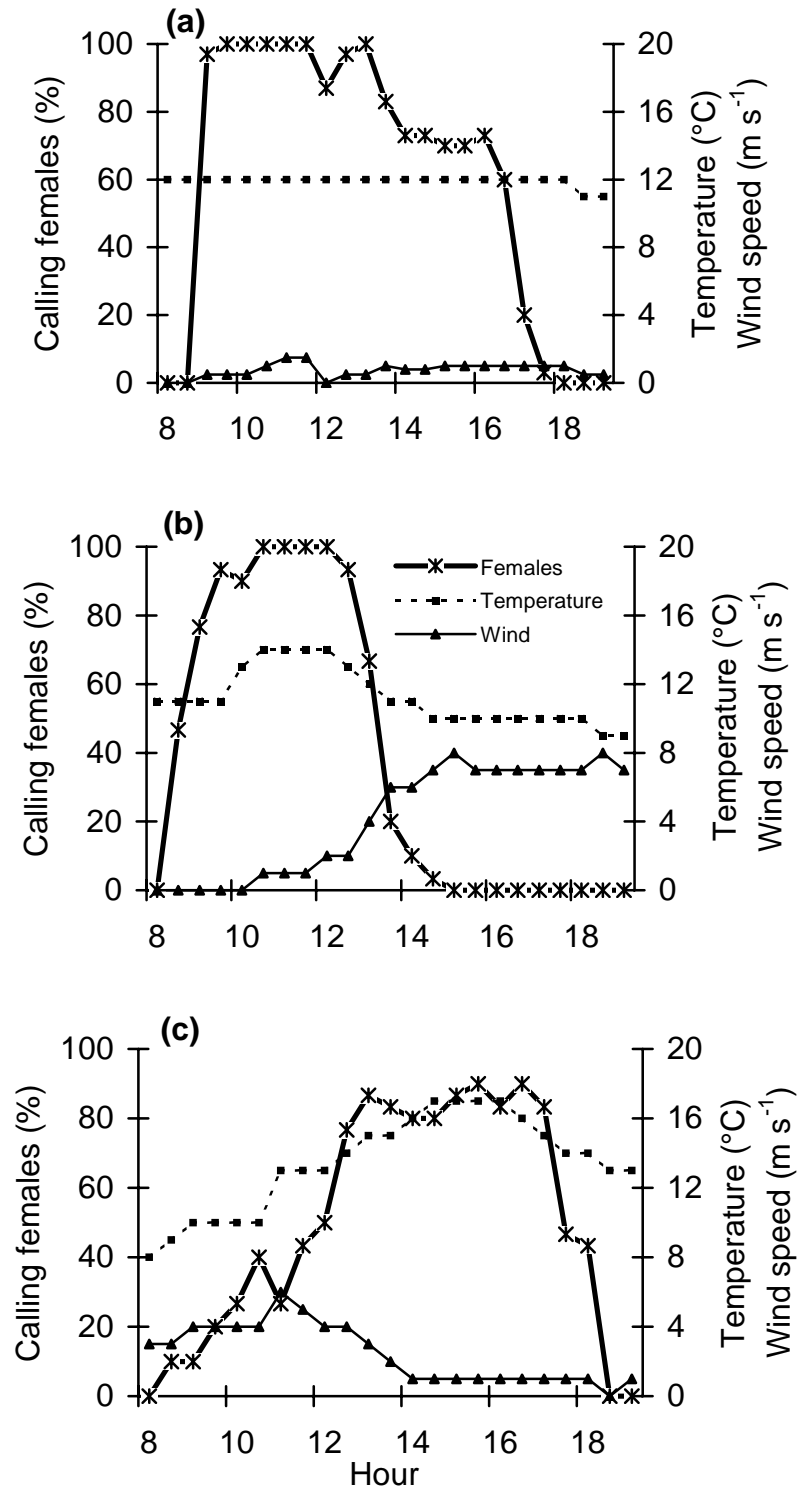


Figure 5: Variability in the proportion of virgin *Macrosiphum euphorbiae* oviparae calling throughout the day, as a function of temperature and wind speed on (a) 26 , (b) 27, and (c) 28 September 2001.

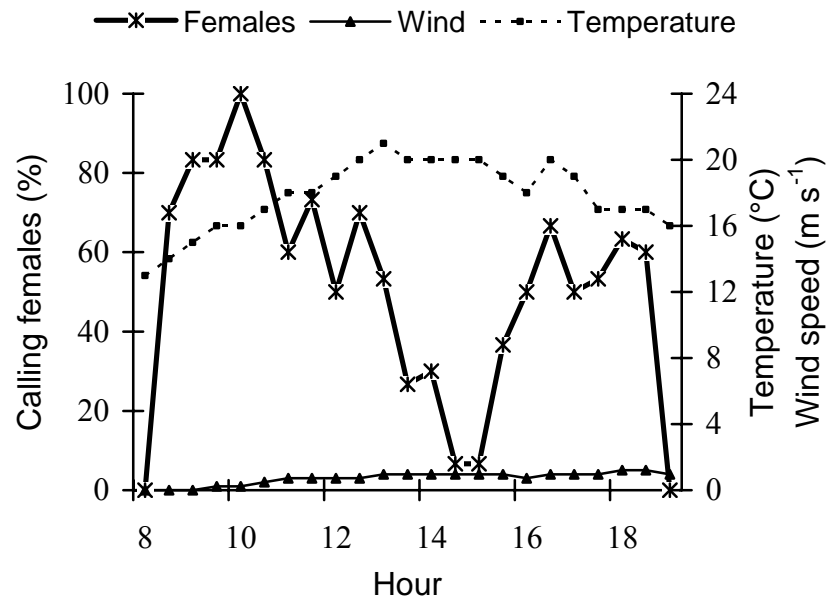


Figure 6: Variability in the proportion of virgin *Macrosiphum euphorbiae* oviparae calling throughout the day on 18 September 2001.

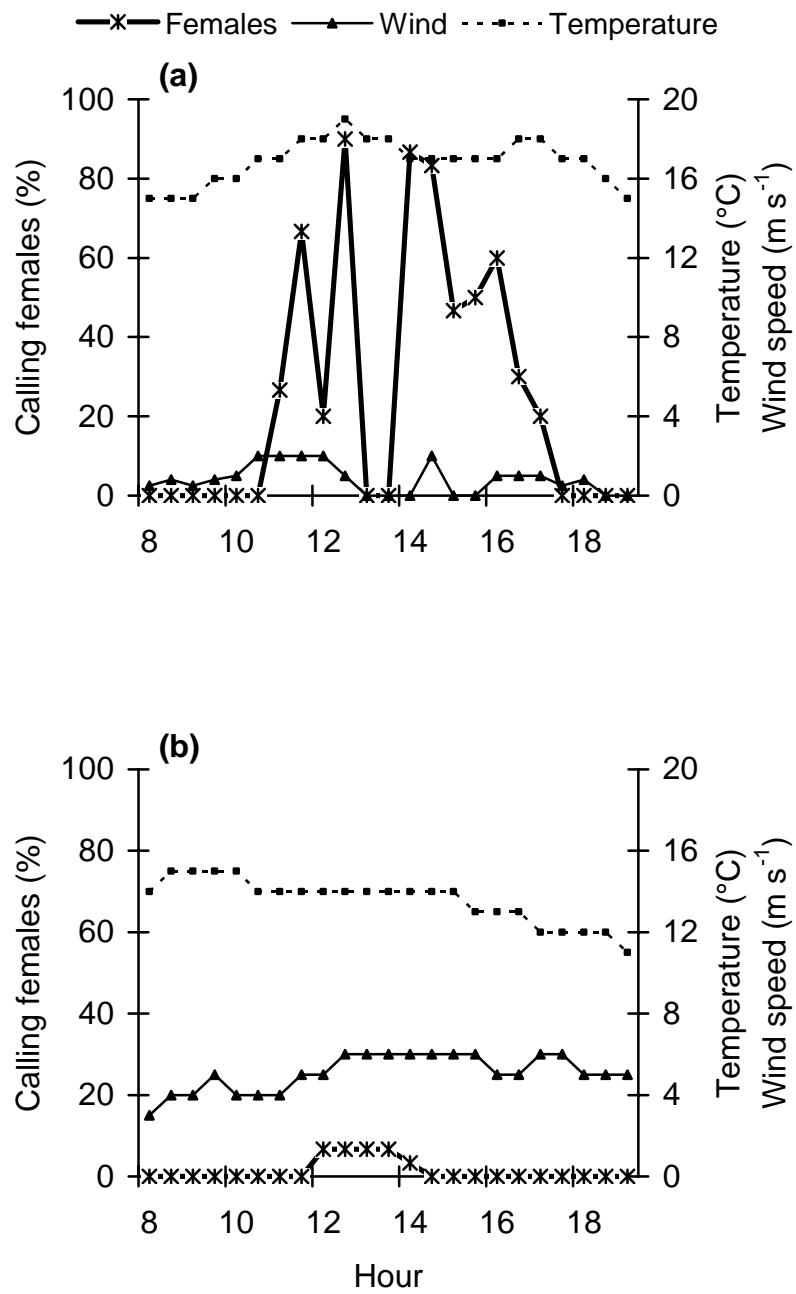


Figure 7: Variability in the proportion of virgin *Macrosiphum euphorbiae* oviparae calling throughout the day, as a function of rainfall on (a) 22 and (b) 25 September 2001.

Chapitre III

Identification of the sex pheromone in the potato aphid, *Macrosiphum euphorbiae* (Thomas) (Homoptera: Aphididae)

Résumé

Les femelles du puceron de la pomme de terre, *Macrosiphum euphorbiae*, démontrent un comportement d'appel typique. Chez celui-ci, les femelles sexuées vierges soulèvent leurs pattes postérieures du substrat pour libérer la phéromone sexuelle contenue dans les glandes trouvées sur les tibias. Les échantillons aériens 'airborn' de femelles sexuées en appel ont été analysés par le 'GC' et le 'GC-MS'. La phéromone sexuelle a été identifiée comme étant un mélange de (4aS, 7S, 7aR)-nepetalactol (I) et de (4aS, 7S, 7aR)-nepetalactone (II). Toutefois, le rapport des deux composés variait de 4:1 à 2:1 (I:II) avec l'augmentation de l'âge des femelles. Lors d'essais biologiques en tunnel de vol, les mâles de *M. euphorbiae* répondaient aux plants de patate portant des femelles sexuées, mais non à ceux portant des aptères parthénogénétiques ou aux plants seuls. Le même degré de réponse a été observé vis-à-vis de mélanges synthétiques allant de 3:1 à 5:1 (nepetalactol:nepetalactone). Toutefois, le temps nécessaire à l'atteinte de la source en question était significativement plus long qu'avec des femelles vierges. Dans tous les cas, les mâles marchaient vers la source au lieu d'y voler. Les mâles démontraient une réponse réduite face à un mélange synthétique de 1:1 et aucune réponse envers l'un des deux composés pris isolément. En conditions de terrain, très peu de mâles de *M. euphorbiae* ont pu être capturés dans les pièges appâtés avec divers rapports de la phéromone sexuelle.

Abstract

Females of the potato aphid *Macrosiphum euphorbiae* exhibit typical calling behaviour, with virgin female oviparae raising their back legs of the substrate to release sex pheromone from the glands on the tibia. Airborn collections from calling oviparae were analysed by GC and GC-MS and the sex pheromone was identified as a mixture of (4*aS*, 7*S*, 7*aR*)-nepetalactol (I) and (4*aS*, 7*S*, 7*aR*)-nepetalactone (II). However, the ratio of the two components changes 4:1 to 2:1 (I:II) as females aged. In bioassays in the wind tunnel *M. euphorbiae* males responded to potato plant with oviparae but not those with parthenogenetic apterae or to plant alone. The same levels of response was observed to 3:1 to 5:1 synthetic blends of nepetalactol: nepetalactone, but the time taken to reach the source was significantly longer than to virgin females. In all cases males walked rather than flew to the source. Males showed lower responses to a 1:1 synthetic mixture and none either of the components alone. Under field conditions, very few *M. euphorbiae* males were captured in traps baited with different ratios of the synthetic pheromone.

Introduction

Many aphid species (Homoptera: Aphididae) have complex seasonal life cycles, with a number of parthenogenetically reproducing asexual generations during the summer and one fall generation of sexually reproducing morphs that produces the overwintering eggs (Moran, 1992). They may be monoecious, exploiting the same host throughout the year or heteroecious, alternating between primary overwintering and secondary summer hosts. The asexual summer generations of aphids are amongst the most serious pests of agricultural and horticultural crops in the world, not only from the direct effects of feeding but also as vectors of plant diseases (Kennedy et al., 1962; Blackman and Eastop, 2000; Radcliffe and Ragsdale, 2002). Current aphid control is strongly dependent on the use of insecticides, but as this obviously carries the undesired side effects of insecticide resistance, reduction of beneficial insect populations, and environmental contamination (Metcalf, 1980; Clark et al., 1995; Foster et al., 2002; Hemingway et al., 2002; Kranthi et al., 2002; Foster et al., 2003), alternative approaches must be sought.

Sex pheromones have been successfully deployed for monitoring, mass trapping or mating disruption within management programmes of many pest species, especially Lepidoptera (Ridgway et al., 1990; Minks and Cardé, 1997; Walker and Welter, 2001; Svensson et al., 2002; Vickers, 2002). There have been several reports of sexual female aphids attracting males with a sex pheromone released from the hind tibiae (Pettersson, 1970, 1971; Marsh, 1972; Eisenbach and Mittler, 1980; Dawson et al., 1990; Lilley and Hardie, 1996; Boo et al., 2000) and the pheromones of several aphid species have since been identified (Dawson et al., 1987; Dawson et al., 1988; Dawson et al., 1989; Campbell et al., 1990; Lilley et al., 1994/1995; Gabryś et al., 1997; Boo et al., 2000). However, they have not been widely exploited on a commercial level. This is due, in part, to the fact that the sexual morphs occur late in the season rather than in the summer when crops are being invaded by the asexual alate females. Yet, aphid sex pheromones have several potential uses, such as monitoring aphid populations, as well as manipulating aphid parasitoids and disseminating pathogens (Campbell et al., 1990; Hardie et al., 1992; Lilley et al., 1994; Pickett et al., 1994; Gabryś et al., 1997; Hartfield et al., 2001). However, if sex pheromones

are to be used effectively, then a solid understanding of the pheromone biology for the species in question is essential (see McNeil, 1991 and references therein).

The potato aphid, *Macrosiphum euphorbiae* (Thomas), may be an important pest on both potato and tomato crops (Shands et al. 1965; Lange and Bronson, 1981), damaging plants by sucking phloem sap and by transmitting viral diseases (Kennedy et al., 1962; Alyokhin et al., 2002). The present paper identifies the sex pheromone of *M. euphorbiae*, examines the rates of emission as a function of female age, as well as reports on male responses to putative female sex pheromone blends under controlled conditions in a wind tunnel and field.

Materials and Methods

Insects.

All aphids used in these experiments came from an annually restocked, multiclonal laboratory culture which was established using parthenogenetic individuals collected from potato fields near Quebec City. Colonies were maintained on potato seedlings, *Solanum tuberosum* c.v. Norland, at $20 \pm 1^\circ \text{C}$, $60 \pm 10\% \text{RH}$, under a 16 L: 8 D photoperiod, conditions which ensure continuous asexual production. Potato seedlings at the 4-6 leaves stage were used as host plants in the all experiments, and new plants were provided every three days.

Sexuals were obtained by rearing two consecutive generations at $20 \pm 1^\circ \text{C}$, $60 \pm 10\% \text{RH}$ under a 10L: 14D photoperiodic regime. Gynoparous (which produce the sexual females) and androparous (which produce the winged males) females were produced in the first generation and were held in groups on host plants. Their offspring were collected daily to ensure a production of known age oviparae and males. The sexes were maintained separately to ensure that females were virgin and that males had never been exposed to pheromone prior to being used in bioassays.

Isolation of volatiles.

Twenty five virgin oviparae of *M. euphorbiae* (1 day old) were placed on a potato seedling in a glass culture vessel (Quickfit FV range, 1 litre). Air that had been dried and purified by passage through an activated 5 Å molecular sieve and charcoal, was drawn at 1 l/min through the container. Volatiles were entrained onto Porapak Q which had been purified by washing with ether (5 ml) and heating at 150°C for 12 h under a stream of nitrogen. After 4 days the volatiles were desorbed from the Porapak Q by elution with freshly distilled ether (4ml). The resulting extract was concentrated under a stream of nitrogen and stored in sealed glass ampoules at -20°C . The procedure was repeated for two additional 4 day periods, replacing the host plant each time, to examine for possible age related changes in pheromone emission. Females may live up to four weeks but we limited

sampling to the first 12 days, as it seemed very unlikely that a female would remain unmated for any greater length of time under field conditions. The entire experiment was replicated three times.

Analysis of volatiles.

Air entrainment samples were sampled by GC on both polar (HP-wax, 30m x 0.23mm ID x 0.5 μ m film thickness) and non-polar (HP-1, 50m x 0.32mm ID x 0.52 μ m film thickness) capillary columns using a HP6890 GC (Agilent Technologies, UK) fitted with a cool-on-column injector and a flame ionisation detector (FID). Further analysis was by GC-MS using a VG Autospec (VG Analytical, UK) coupled to a HP5890 GC.

Wind tunnel bioassays.

Experiments were conducted in a laminar airflow wind tunnel (290 cm long, 120 cm wide, and 100 cm high) located in an environmental chamber maintained at $18 \pm 0.5^\circ$ C with RH 40- 50 %. Tunnel lighting was provided by 8 (40 W) fluorescent light bulbs uniformly placed above the tunnel ceiling, providing a light intensity of 150 lux where bioassays were carried out. All assays, using 5-6 day old naïve virgin males, were carried out in the 3rd to 6th hours of the photophase as this was the period of maximum calling activity under controlled laboratory conditions (Goldansaz and McNeil, 2003). Males were considered unresponsive if they did not respond within 3 min. Individuals were only used once in all the experiments.

In a first series of experiments individual naïve males were placed in a release cage 40 cm down wind of the source under a constant wind speed of 40 cm/sec. The proportion of males orienting to the source and taking flight, as well as the time to exhibit these responses, were recorded when males were exposed to (i) clean wind, (ii) an uninfested potato plant, (iii) 5 5-day-old adult parthenogenetic apterae, or (iv) 5 calling 5-day-old oviparae on a potato plant. The experiment was a randomised block design with 3 replicates and 15 males per replicate.

However, under these conditions males flew downwind rather than upwind whenever they took flight. We, therefore, connected the platforms bearing the source and

the release cages with a thin string bridge (Fig. 1), a technique previously used by Eisenbach and Mittler (1980), and repeated the experiment mentioned above.

This “bridge” approach was also used to test the response of *M. euphorbiae* males to synthetic sex pheromone, applied on filter paper discs that were replaced every 15 min. The two pheromone components, identified by GC-MS, nepetalactol: nepetalactone, were prepared following the procedures described by Dawson et al. (1989). Six ratios of the two compounds were tested: 1:0, 0:1, 1:1, 3:1, 4:1, 5:1 together with the ether solvent as a control, using a randomised block design with 3 replicates of 15 males.

Testing synthetic pheromone lure under field conditions.

A series of water traps were baited with 1:0, 0:1, 1:1, and 5:1 nepetalactol: nepetalactone lures, with release rates for both components at 80-85 µg/h. These were placed at the edges of commercial potato fields near Pont Rouge, QC and near a rose garden in the Jardin Van Den Hende on the Laval University Campus. A minimum of 5 traps per site/ per year were used and emptied every three days from July through October in 1997, 1998 and 2000. Lures were changed every two weeks.

Statistical analyses.

Categorical responses (orient/not orient; take flight/not taking flight; reach the source/not reach the source) of males exposed to different odour sources were compared with a logit model using the GENMOD procedure of SAS, while the time to reach the source was compared with ANOVA using GLM procedure (SAS, 1999). The proportion of male orienting in the presence or absence of a bridge was compared with an analysis of contrast, using the GENMOD procedure (SAS, 1999).

Results

Pheromone identification.

GC analysis of the air entrainment samples from virgin sexual females of *M. euphorbiae* showed the presence of two peaks that were identified by coupled GC-MS and GC coinjection with authentic standards as (-)-(1R, 4aS, 7S, 7aR)-nepetalactol and (+)-(4aS, 7S, 7aR)-nepetalactone. The ratio changed as a function of age being *ca* 4:1 on days 1-4 but declined to *ca* 3:1 and 2:1 on days 5-8 and 9-12, respectively. If one assumes that all females were calling, then the hourly release rates ranged from 50-20 and 15-10 ng/aphid/h of the nepetalactol and nepetalactone over the 12 day collection period.

Bioassays.

In the absence of any odour source, when there no bridge for individuals to walk upwind, males moved to the top of the release cage and immediately flew downwind. In contrast, when an odour source was present males that oriented moved to the upwind edge of the release cage to face the source, walked down the side and moved back and forth on the upwind edge of the platform before taking flight downwind. *M. euphorbiae* males oriented upwind significantly more when exposed to calling virgin females than to asexual females or a potato plant (d.f. = 3, $X^2 = 83.20$, $p < 0.0001$; Fig. 2). Furthermore, males exposed to calling oviparae spent longer orienting, resulting in fewer individuals actually taking off during the assay (d.f. = 3, $X^2 = 40.12$, $p < 0.0001$; Fig. 2a) When a bridge was available between the source and release platforms the proportion of males orienting to the different sources were similar to those seen in the previous experiment (d.f. = 1, $X^2 = 0.23$, $p = 0.635$). In this case significantly more males walked to calling oviparae on plants than parthenogenetic females or plants only (d.f. = 3, $X^2 = 70.75$, $p < 0.0001$, Fig. 2b). However, there is some individual variability in response to calling females, as some males moved to the source while others still flew downwind (Fig. 3).

The proportions of males orienting to and reaching the source when exposed to 3:1, 4:1 and 5:1 nepetalactol: nepetalactone lures were generally similar to those observed when

virgin females were used (Table 2). The responses were significantly lower when a 1:1 blend was used and very little response was observed when either of the components was presented alone. In all cases the time taken to reach the synthetic lures was greater than to virgin females (Table 2).

Field trapping.

We captured in excess of 2000 male aphids, most of which could not be identified with any certainty beyond the Family or Genus, as males for most species have not been previously described (Eastop, personal communication). Only three were *M. euphorbiae* and all were captured near potato fields in late August, in traps baited with a 5:1 nepetalactol: nepetalactone lure, which of all the combinations used was closest to the ratio emitted by calling females.

Discussion

The sex pheromone of *M. euphorbiae* is made up of a blend of nepetalactol and nepetalactone, the same two major components reported for a number of other aphid species (Table 1). It has been proposed that these different ratios of the two compounds may provide species specific pheromones (Guldemon et al., 1993; Hardie et al., 1994; Thieme and Dixon, 1996). The fact that response of *M. euphorbiae* males to the 3:1- 5:1 synthetic lures is similar to that of calling virgin females, but significantly less to the other ratios or either of the components alone, would lend some support to the hypothesis. However, there is at least one aspect of the present study that would raise some doubts whether a pheromone, varying only in the ratio of two common components, could actually provide an effective species specific mating signal.

The ratio of the potato aphid sex pheromone changes with age, as reported for the vetch aphid, *M. viciae* (Hardie et al., 1990), so there is not a constant species specific signal. Thus fluctuations in ratio might increase the probability of interspecific mating. For example, older *M. euphorbiae* emit a 2:1 nepetalactol: nepetalactone ratio, similar to that reported for the green peach aphid, *Myzus persicae* (Pickett et al., 1992). While both aphids share common secondary host plants (like potato) during the summer, they exploit different primary hosts as overwintering sites. These primary hosts could be spatially separated yet these may occur within the same habitat. In the latter scenario, unless there was marked temporal separation between the appearance of the two species the production of very similar pheromones could result in interspecific attraction, as reported for *Acyrtosiphon pisum* and *M. viciae* (Hardie et al., 1990). However, if females mated soon after the adult moult, before there are age-related changes in pheromone blend ratio then species integrity might be retained.

There are, obviously, other aspects of the chemical ecology of aphid reproduction that may reduce interspecific mating. There may be differences in the actual pheromone concentrations released by different species, aspects of the stereochemistry of the two pheromone components (see Hardie et al., 1997), or yet unidentified minor components in the pheromone, as suggested for *Cryptomyzus* species by Guldemon et al. (1993). The

possibility of trace compounds may be supported by work on the pheromones of *Brevicoryne brassicae* (Gabryś et al., 1997), *Sitobion avenae* (Lilley et al., 1994/1995) and *S. fragariae* (Hardie et al., 1992), where nepetalactone is the only compound to be identified from the oviparae of all three species. Consequently, unless there is very strong spatial separation and/or temporal differences in the reproductive periods it is hard to imagine a single compound providing a reliable species-specific mate recognition. This would especially be the case if there is high variability in the day to day calling periodicity as reported for the potato aphid under field conditions (Goldansaz and McNeil, 2003). The idea of trace compounds is also supported by the finding that synthetic sex pheromones, in the ratios reported from calling females were unattractive to males of *Aphis fabae* and *A. pisum* (Hardie et al., 1990), as well as to *Cryptomyzus galeopsidis* males (Thieme and Dixon, 1996). Similarly, the fact that *M. euphorbiae* males took significantly less time to reach calling females than any of the synthetic lures suggests some aspect of the pheromone blend may remain to be elucidated.

It is also possible that host plant volatiles, alone or in concert with the female sex pheromone, provide more species specific cues for aphid mate location, as reported for the hop aphid, *Phorodon humuli*, and bird-cherry aphid, *R. padi* (Campbell et al., 1990; Hardie et al., 1994). Logically, one would expect volatiles from a large suitable host tree to be more effective at long distance than the sex pheromone emitted by a small calling female. However, this is not always the case as host plant volatiles appear to low ranking cues for males of *Cryptomyzus* spp. and two subspecies of *Aphis fabae* (Guldmond et al. 1992; Thieme and Dixon, 1996). In the case of *M. euphorbiae* we cannot comment as we were obliged to use the secondary host plant given the high number of plants required in the bioassays. We did attempt two series of assays with rose plants but they were inadvertently sprayed with insecticide just before use. If host plant volatiles are important for the potato aphid, we would expect to see a higher proportion of males reaching the source in bioassays (Fig. 3) had calling females been on the primary host plant.

The very poor trap catch could be used in support of missing minor components, or that the higher release rates from the lures deterred males. However, these possibilities must be interpreted with some caution. The number of male *M. euphorbiae* captured in suction traps over several weeks each fall during a study of several years was very low

(<10/year) in Quebec (Cloutier, personal communication). This was markedly different from the high densities of males from other aphid species, suggesting that densities of *M. euphorbiae* sexuals are not high. However, this is not the only possible explanation. Experiments carried out under both laboratory and field conditions clearly demonstrated that *M. euphorbiae* males often walk rather than fly to a pheromone source at quite moderate wind speeds (Goldansaz and McNeil, in prep). If walking behaviour is a major component of mate location then the traps used may have a very low probability of capturing potato aphid males. Campbell et al. (1990) captured hop aphid, *P. humuli* males 20 m from the hop garden, indicating males are able to move of greater distances than few cm initially proposed (see Dawson et al., 1990). However, there is little evidence to date showing that such movement is entirely the result of oriented flight to the pheromone source.

Clearly, we need to gain considerably more information on the reproductive biology of *M. euphorbiae*, and of many other species, to obtain a realistic understanding about the role of the female sex pheromones in species specific mating of aphids. Such research should include further investigations of the chemical and ecological aspects of female pheromone production and release, as well as the male behaviours when locating pheromone sources. In addition the importance of potential male pheromones, auditory signals and physical factors such size, shape, colour, as well as species-specific courtship behaviours (Hardie et al., 1990; Steffan, 1990) that may influence mate choice once the two sexes are in close proximity need to be clarified. Furthermore, the information obtained from such studies would provide the basis for the most effective use of aphid pheromones in any management programme.

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Table 1: The ratio of nepetalactol: nepetalactone reported in the sex pheromone of different aphid species.

Species	Ratio	Reference
<i>Acyrtosiphon pisum</i>	1 : 1	(Pickett et al., 1992)
<i>Aphis fabae</i>	1 : 29	(Pickett et al., 1992)
<i>Brevicoryne brassicae</i>	0 : 1	(Gabryś et al., 1997)
<i>Cryptomyzus spp.</i>	30 : 1	(Guldemonnd et al., 1993)
<i>Macrosiphum euphorbiae</i>	4 : 1	(Goldansaz et al., this paper)
<i>Megoura viciae</i>	1 : 4	(Dawson et al., 1987)
<i>Myzus persicae</i>	2 : 1	(Pickett et al., 1992)
<i>Phorodon humuli</i>	1 : 0	(Campbell et al., 1990)
<i>Schizaphis graminum</i>	8 : 1	(Dawson et al., 1988)
<i>Sitobion avenae</i>	0 : 1	(Lilley et al., 1995)
<i>Sitobion fragariae</i>	0 : 1	(Hardie et al., 1992)
<i>Tuberocephalus momonis</i>	1 : 4	(Boo et al., 2000)

Table 2: Behavioral responses ($\bar{x} \pm \text{SEM}$) of *Macrosiphum euphorbiae* males when exposed to calling females and synthetic pheromone lures, differing in the ratio of nepetalactol: nepetalactone, under controlled conditions in a wind tunnel.

Ratio	Orienting to source (%)*		Reaching the source (%)*		Time to reach the source, (sec.) *	
0 : 0	0	a	0	a	-	
0 : 1	6 ± 2.8	ab	0	a	-	
1 : 0	14 ± 2.8	b	0	a	-	
1 : 1	47 ± 12.3	c	24.6 ± 8.5	b	181.2 ± 9.8	a
3 : 1	94 ± 2.7	e	53 ± 7.5	c	174.6 ± 6.6	a
4 : 1	92 ± 0.5	de	53.3 ± 3.4	c	187.5 ± 3.1	a
5 : 1	75 ± 0	d	44.3 ± 5.8	c	193.7 ± 0.9	a
Sexual females	80 ± 4.6	de	50 ± 4.7	c	114.9 ± 9.6	b

* Values within a column followed by different letters are significantly different ($p < 0.05$)



Figure 1: The experimental bridge which allows *M. euphorbiae* males to walk to different pheromone sources in a wind tunnel.

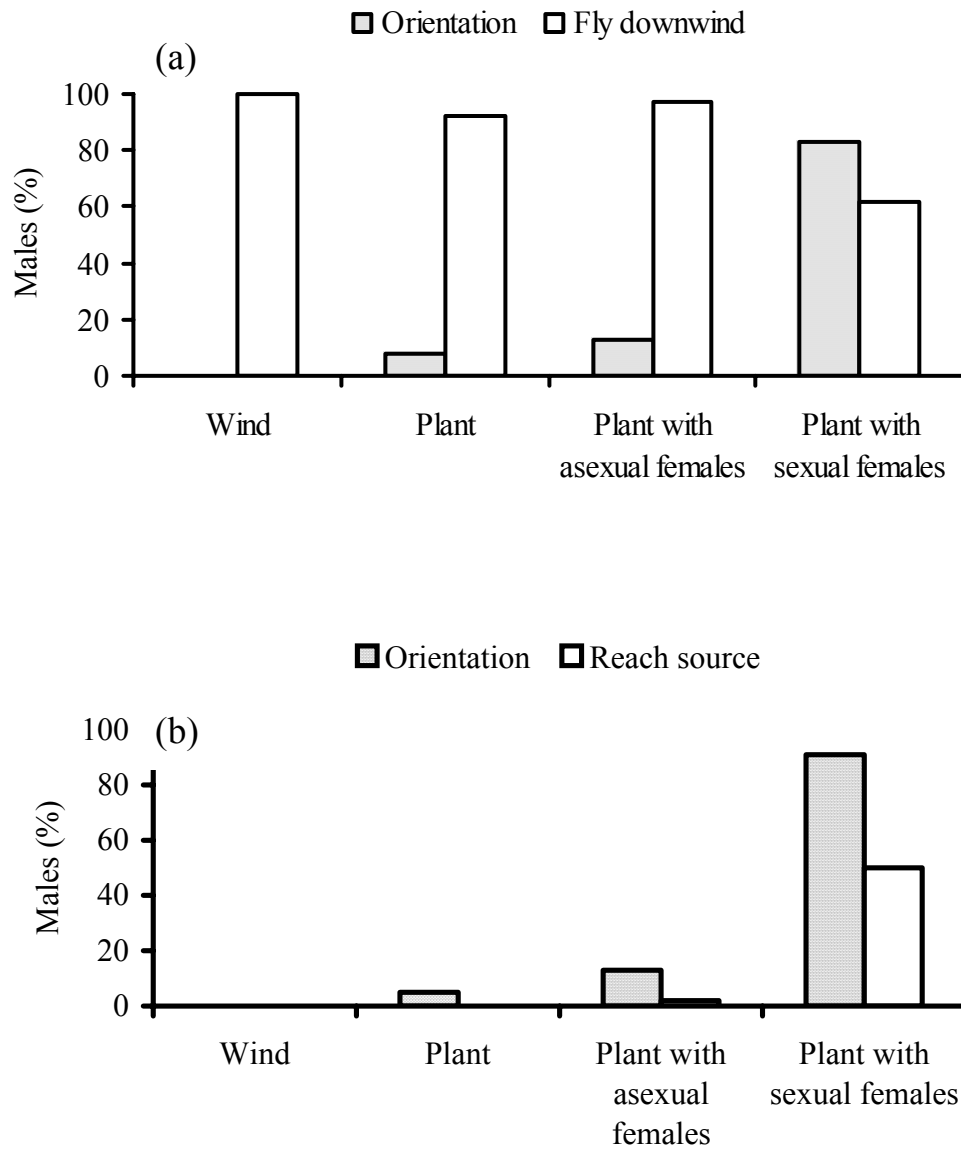


Figure 2: Proportion of *M. euphorbiae* males exhibiting orientation and flight response to different odour sources in a wind tunnel (40 cm/sec) when there was (a) no physical connection, and (b) a physical connection between the release site and the source.

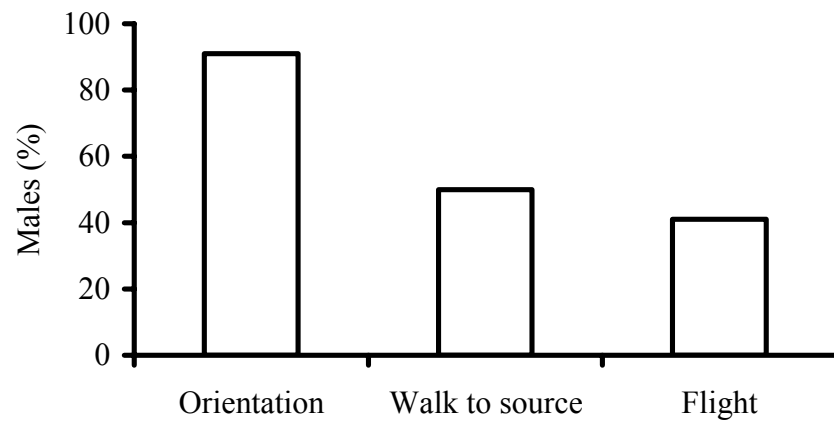


Figure 3: Proportion of *M. euphorbiae* males orienting, walking to the source or flying downwind when exposed to five calling oviparae in a wind tunnel (40 cm/sec) when there was a physical connection between the release site and the source.

Chapitre IV

Effect of wind speed on the behaviour of sexual morphs of the potato aphid, *Macrosiphum euphorbiae* (Thomas) (Homoptera: Aphididae) under laboratory and field conditions.

Résumé

Lors d'une étude précédente (Goldansaz et McNeil, sous presse), le vent a été identifié comme étant l'un des facteurs abiotiques influençant le comportement d'appel des femelles sexuées vierges du puceron de la pomme de terre, *Macrosiphum euphorbiae*, en conditions naturelles de terrain. Étant données les aptitudes au vol relativement faibles des petits insectes comme les pucerons, nous avons entrepris une étude détaillée visant à observer le comportement de vol des mâles mis en présence ou en absence d'une source de phéromones à des vitesses de vent constantes et variables, en laboratoire et sur le terrain, respectivement. Le comportement d'appel des femelles sexuées vierges a aussi été étudié et ainsi, les effets du vent sur les deux sexes ont pu être comparés.

Dans un tunnel de vent avec un air propre (absence d'une source de phéromone), les mâles ont été exposés à un éventail de vitesses de vent allant de 0 à 120 cm/s. La proportion de mâles (90%) s'envolant en aval du vent augmentait de paire avec la vitesse du vent jusqu'à une vitesse de 40 cm/s, mais montrait une importante réduction à des vitesses supérieures à 60 cm/s pour finalement ne plus voler du tout à 120 cm/s. La réponse des mâles a été comparée en air propre et avec des femelles en appel placées en amont du vent à une distance de 40 cm des cages de relâche pour des vitesses de 40, 80 et 120 cm/s. À 40 cm/s, beaucoup moins de mâles s'envolaient lorsqu'une source de phéromones était présente (<70% vs >90%), et ceux volant l'ont fait vers l'aval au lieu d'aller vers l'amont en direction des femelles en appel. À des vitesses de 80 et 120 cm/s, très peu de mâles prenaient leur envol (<20%), la source de phéromones n'altéraient pas la proportion de répondants et ceux qui s'envolaient, encore une fois, le faisaient vers l'aval. Il était évident que les mâles s'orientaient vers la source de phéromones (>80% pour les trois vitesses de vent) et lorsqu'un pont était placé entre les cages de relâche des mâles et la source de phéromones, une grande proportion d'individus marchait vers la source (42, 78 et 83% à 40, 80 et 120 cm/s, respectivement). Le temps nécessaire à l'atteinte de la source était significativement plus long à 120 cm/s (>160s) qu'à des vitesses plus faibles (<125s). Les effets de la vitesse du vent demeuraient constants lorsque l'expérience était répétée pour une même vitesse de vent

et en faisant varier la distance trouvée entre les cages de relâche et la source (40, 70 et 100 cm). Malgré les effets très marqués du vent sur le vol des mâles, aucune des vitesses de vent expérimentées avait un effet inhibiteur sur le comportement d'appel des femelles.

Sur le terrain, à des vitesses variables de vent, le vol des mâles était inhibé à des vitesses supérieures à 2 m/s. Toutefois, l'activité de marche continuait jusqu'à 4 m/s, le même éventail pour lequel les femelles continuaient d'appeler, et les mâles s'orientaient et marchaient vers la source de phéromones si un pont était présent. Cependant, contrairement aux résultats de laboratoire, 30% des mâles volaient contre le vent et atteignaient la source. Une étude plus détaillée a montré que les mâles marchaient vers la source en présence de vent, mais volaient vers l'amont lorsqu'une accalmie se présentait dans la vitesse du vent.

Les résultats obtenus en laboratoire et sur le terrain suggèrent que la marche est un comportement plus important que le vol vers la source phéromonale impliquée dans la localisation des femelles réceptives par les mâles du puceron de la pomme de terre. Toutefois, il reste à vérifier si cette stratégie de recherche de partenaire en est une qui est répandue chez les insectes à faible capacité de vol.

Abstract

In a previous study (Goldansaz and McNeil, in press) wind speed was one abiotic factor affecting the calling behaviour of virgin oviparae of the potato aphid, *Macrosiphum euphorbia*, under field conditions. Given the rather limited flight capacity of small insects such as aphids, we undertook a detailed study to examine the flight behaviour of males in the presence and absence of a pheromone source under constant wind speeds in the laboratory and under variable wind in the field. The calling behaviour of virgin oviparae was also examined so that the effects of wind velocity on the two sexual forms could be compared.

In the wind tunnel males were tested in clean air over a range of constant wind speeds from 0-120 cm/s. The proportion of males initiating downwind flight increased with increasing constant wind velocities up to 40cm/s (>90%) but showed a marked decline at speeds > 60 cm/sec and flight was totally inhibited at 120cm/s. The response of males were compared at 40, 80 and 120cm/s in clean air and when calling females were placed 40 cm upwind of the release cages. At 40cm/s significantly fewer males took flight when a pheromone source was present (<70 vs >90%), yet those flying still moved downwind rather than upwind towards the calling females. At 80 and 120 cm/s very few males took flight (<20%) thus a pheromone source did not alter the proportion responding, and again any individuals taking off flew downwind. It was evident that males oriented to the pheromone source (> 80% at all three wind speeds) and when there was a bridge between the male release cage and the pheromone source a significant proportion of individuals walked to the source (42, 78 and 83% at 40, 80 and 120 cm/s, respectively). The time to reach the source took significantly longer at 120 cm/s (>160 s) than at the two lower wind speeds (<125 s). The effects of wind speed remained the same when the assays were repeated using the same wind velocities while varying the distance between the release cage and the source (40, 70 and 100 cm). Despite the very marked effects of wind on male flight, none of the constant wind speeds tested in the wind tunnel had an inhibitory effect on female calling behaviour.

Under the variable wind conditions in the field male flight was inhibited at wind speeds > 2 m/s. However, walking activity continued up to 4m/s, the same range over which

females maintained calling, and males would orient and walk to a pheromone source if a bridge was present. However, in contrast to laboratory results 30 % of males were observed to fly to the source. A more detailed study of male behaviour showed that males would walk towards a source in the presence of wind but flew in an “upwind” direction when there was a lull.

The results from both the laboratory and the field suggest that walking is a more important behaviour in the location of receptive females by males of the potato aphid than oriented upwind flight to a pheromone source. Whether this is a common mate searching strategy for weak flying insect species remains to be verified.

Introduction

Many abiotic and biotic factors influence chemically mediated mating in insects (McNeil, 1991). Wind is one of the important abiotic factors and may affect reproductive success in two distinctly different ways, as exemplified by research generally carried out on Lepidoptera and parasitic Hymenoptera. The first relates to the actual temporal and spatial movement of the infochemicals, for the presence or absence of wind, wind velocity and the level of turbulence will markedly influence parameters such as the distance it moves from the source, plume structure and the concentration of the message at any given point within the plume (see Elkinton and Cardé, 1984; Bell et al., 1995 and references therein). The second aspect relates to how variations in wind speed influence the behaviours of both sexes. This may modify the proportion of the individuals emitting pheromone (Kaege and Shorey, 1972) or change the actual behaviour associated with pheromone release (Conner et al. 1985). In the case of the responding sex, wind speed may influence the proportion of individuals reaching the source. This may be related to the actual characteristics of the plume mentioned above, as well as the ability of the insect to sustain flight. There will be an upper wind velocity, above which upwind flight is not possible, that is determined in part by the size of the insect.

In recent years the sex pheromone of several aphid species have been identified (Dawson et al., 1987; Dawson et al., 1989; Campbell et al., 1990; Lilley et al., 1994/1995; Gabryś et al., 1997; Boo et al., 2000, Goldansaz et al., submitted) and there have been a few behavioural studies under controlled laboratory conditions (Eisenbach & Mittler, 1980, 1987; Guldmond and Dixon, 1994; Thieme and Dixon, 1996). However, there has been little work examining the pheromone mediated behaviour of the aphid sexual under field conditions in late summer, early fall when these morphs are present in nature. Virgin female oviparae raise their back legs off the substrate when releasing sex pheromone from the glands on the tibia and Goldansaz and McNeil (in press) recently demonstrated that fluctuating climatic conditions in the field have a marked effect on the expression of this calling behaviour in the potato aphid, *Macrosiphum euphorbiae*. Wind velocity was one of the significant factors,

with calling being inhibited at wind speeds exceeding 4 m/s. Aphids are weak flyers and the speed and direction of flight is usually governed by the wind (Dixon and Howard, 1986). The inhibitory effects of wind speeds $>2\text{m/s}$ on the flight behaviour of parthenogenetic female alate aphids have been documented (Kennedy and Thomas, 1974; Kennedy, 1990; Storer et al., 1999) but despite the fact that wind velocity could have a marked influence on the reproductive success of aphids there are no really detailed studies examining the performance of the sexual morphs. We, therefore, carried out experiments under both laboratory and field conditions to examine the effect of wind speed on the flight behaviour of alate males in the potato aphid how this related to the calling behaviour of wingless oviparae under similar conditions.

Materials and Methods

Insects

All aphids used came from a multiclonal laboratory culture, established and restocked annually using parthenogenetic individuals collected from potato fields around Quebec City, Canada. Colonies were maintained on potato seedlings *Solanum tuberosum* c.v. Norland, at 20 ± 1 °C, $60 \pm 10\%$ RH, under a LD 16:8 h photoperiod, conditions that ensure continuous asexual production. In all experiments aphids were held on four to six leaf stage potato seedlings, and new plants were provided every 3 days. To obtain sexuals, aphids were reared at 20 ± 1 °C, $60 \pm 10\%$ RH, under a LD 10:14 h photoperiod. The gynoparous females (which produce the sexual females) are produced in the first generation and were held in groups on host plants. Their offspring were collected daily to ensure production of known-age oviparae and held on potato seedlings under these conditions until used in experiments.

Wind tunnel bioassays

Experiments were conducted in a laminar airflow wind tunnel (290 cm long, 120 cm wide, and 100 cm high) with a capacity to generate wind speeds from 0 to 120 cm/s. The tunnel was located in an environmental chamber maintained at 18 ± 0.5 ° C with RH 40- 50%. Tunnel lighting was provided by 8 (40 W) fluorescent bulbs uniformly placed above the tunnel ceiling. Light intensity was 150 lux at the point of potato plant with calling females or male release. All assays, using 5-6-day-old virgin females and/or males, were carried out in the 3rd to 6th hours of the photophase as this was the period of maximum calling activity under controlled laboratory conditions (Goldansaz and McNeil, 2003).

The effect of wind on the calling behaviour of oviparae was tested by placing a calling virgin female in the wind tunnel, and classified either “calling” or “non calling” after 30 min at wind speeds of 0, 20, 40, 60, 80, 100, and 120 cm/s. Fifteen oviparae were tested at each wind speed and the entire experiment replicated three times, using a randomised block design.

As wind speed is known to affect the probability of take-off in parthenogenetic alates (see Kennedy, 1990) flight behaviour of males in clean air was also tested over the same range of wind speeds. Each male was placed down wind in a plastic cage (5 cm long and 3 cm in diameter). The time to take-off and flight direction were recorded for 15 males at each wind speed and the entire experiment replicated three times, using a randomised block design.

We then examined the response of males to the presence of 5 calling virgin females on a single potato seedling, 40 cm upwind, at wind speeds of 40, 80, and 120 cm/s, velocities at which >80, <20 and 0% of males initiated flight in the clean air assays above. Despite the presence of a pheromone source any males taking flight, still flew downwind, as they did in clean air. Therefore, we repeated the experiment but this time we provided a bridge, consisting of a single white string connecting the male release site and the plant with the calling females that would allow males to reach the source by walking upwind (Goldansaz et al., submitted). We recorded if males orientated to the pheromone source (facing up wind with rapid antennal movement), as well as the time take to either walk to the source or initiate downwind flight. The experiment was replicated three times with 15 males per replicate at each wind velocity, using a random block design.

A split plot design was used to test the effect of distance of males from the pheromone source (at 40, 70, and 100 cm) at the same three wind speeds, 40, 80, and 120 cm/s. There were three replicates, each testing 12 males at all distance/wind speed combinations.

Males and females, all naïve 5-6 day old individuals, were only used once. All bioassays testing males were terminated after 3 min if the individual aphids did not respond.

Field experiments

As the distinct behavioural patterns observed under constant laboratory regimes may be less evident under variable field conditions (Goldansaz and McNeil, in press) we examined the effect of wind on female calling and male searching behaviours under field conditions on the campus of Laval University, Ste-Foy, Quebec the 15th and 27th September,

2001, that were clear days with temperatures varying between 14-17 and 12-14°C, respectively, during observation period. On both days the calling behaviour of 30 oviparae from the laboratory culture, each held on an individual potato seedling placed on a large table around 10:00, was recorded every 30 min from 11:00 to 15:00. Over the same time period the behaviour of males was also tested hourly on the two days. Each day 100 males were held in individual release cages placed on the table (described above) and each hour two cohorts of 10 individuals were tested. We removed the covers from the release cages and the behaviours of the 20 males recorded, noting if they remained immobile, walked (around the cage and/or the release platform site), or took flight over a 3 min period. At each observation period the wind speed were monitored for 1 min. As it were variable the conditions obviously fluctuated they were grouped in categories 0-X m/s, where X was the maximum wind speed observed during a give recording interval.

The behaviour of individual males in presence of 5 calling females on a potato seedling was examined under field conditions on the 11th (N=29) and 24th (N=17) October 2001. Both were clear days and the air temperatures varied between 17-15 and 16-13 °C respectively, during the bioassays carried out between 14:00 and 16:00. As in the controlled wind tunnel experiments the females were placed 40 cm upwind of the male, and a string bridge connected the platforms holding the potato plant and the release cage. The cover of the release cage was removed and the behaviours of the male recorded during 3 min.

Statistics

The proportion of male taking flight in the presence or absence of calling females as a function of wind speed was compared with an analysis of contrast, using the GENMOD procedure (SAS, 1999). Categorical responses (orient/not orient; reach the source/not reach the source) of males exposed to calling females were compared with a logit model using the GENMOD procedure of SAS (SAS, 1999) while the time to reach the source as a function of wind speed was compared with an analysis of variance using GLM procedure of SAS. The proportion of males taking flight, orientating to and/or reaching the source at different wind speeds and distances were compared with a logit model.

Results

Laboratory

There was no change in the calling behaviour of oviparae under the range of velocities tested in the wind tunnel (Fig. 1). In contrast, the propensity of males taking flight in clean air varied significantly with wind speed ($df = 5$, $\chi^2 = 126.89$, $p < 0.0001$: as winds of 120 cm/s totally inhibited flight we excluded this treatment from the analysis). The proportion taking flight increased with increasing wind speeds up to 40 cm/s and then declined at higher wind speeds, with practically 100% inhibition at >80 cm/s. (Fig. 1).

When a pheromone source was present the majority of males oriented towards the source at all wind speeds ($>80\%$), however significantly fewer males took flight in winds of 40 cm/s ($df = 1$, $\chi^2 = 14.63$, $p < 0.0001$; Fig. 2) but not at 80 and 120 cm/s ($df = 1$, $\chi^2 = 0.11$, $p = 0.7431$; $df = 1$, $\chi^2 = 1.40$, $p = 0.2367$; Fig. 2). In all cases males taking flight flew downwind and not towards the source. However, when there was a bridge between the release cage and calling females, males are able to reach the source. Wind speed had a significant effect on the proportion of males' orienting toward ($df = 2$, $\chi^2 = 11.11$, $p = 0.0039$; Fig. 3a), and walking to the source ($df = 2$, $\chi^2 = 16.78$, $p = 0.0002$; Fig. 3b), both of which was higher at the higher wind velocities. The time taken to reach the source was also influenced by wind speed ($df = 2$, $F = 8.63$, $p = 0.0354$), taking significantly longer at 120cm/s than at the lower wind speeds (Fig. 3c). Varying the distance from the source did not significantly modify the effect of wind speed on the proportion of males orienting to the source, initiating downwind flight or reaching the source (Tables 1, 2). As one would expect, at any given wind speed there was an obvious effect of distance on the time to reach the source (Table 1). There was a significant wind speed*distance interaction (Table 2), undoubtedly due to the fact that at wind speeds of 40 and 80cm/s males took more time to reach a source 40cm away than one at 70cm (Table 1).

Females maintained calling at variable wind speeds up to 3m/s but above this level it was rarely observed under variable conditions in the field (Fig. 4a, b). A proportion of males

exhibit downwind flight behaviour if the variable wind speeds did not exceed 2 m/s, but above this velocity flight is inhibited (Fig. 4a, b). However, they exhibited some level of active walking behaviour at wind speeds up to 4m/s, the same range of velocities at which females sustain their calling behaviour (Fig. 4a,b). When males were tested in the presence of calling females in the field the behaviors observed differed somewhat from those under laboratory conditions. While 52% of the 46 males tested still walked to the source, about 30% actually flew upwind to the calling females, with very few actually flying downwind (Fig. 5). Detailed studies of male behaviours during the assay carried out on 11 October (Fig. 6) showed that males walked upwind when there were wind currents but if the wind dropped while they were approaching the females they profited from the lull to take off and fly to the source. We were able to verify this behaviour experimentally in the laboratory by cutting off the wind when the male was half way across the bridge: within seconds the males took flight and moved in the “upwind” direction towards the calling females.

Discussion

Variable climate conditions have a significant effect on the calling behaviour of *M. euphorbiae* (Goldansaz and McNeil, 2003) and the results presented here show that the wind speeds prevailing during late summer and fall will have a significant effect on the reproductive success of *M. euphorbiae*, especially with respect to the males ability to reach receptive females. Whether sex pheromone is present or not male flight is inhibited at constant wind speeds $>1\text{m/s}$ in the laboratory and under variable field conditions if the maximum velocity exceed 2m/s . This is not surprisingly, as many small insects stop flying at relatively low wind speeds (Juillet, 1964; Kring, 1972; Nealis, 1986; Kennedy, 1990; Fink and Volk, 1995; Messing et al., 1997; Weisser et al., 1997; Marchand and McNeil, 2000), and the values observed for *M. euphorbiae* male are similar those reported for flight by *Aphis fabae* virginoparous alates (Kennedy, 1990). Furthermore, the importance of oriented male upwind flight in the mating biology of *M. euphorbiae* is somewhat enigmatic for, at least under conditions of constant wind speeds, all individuals taking flight moved downwind rather than towards the source.

Even though initiating (Dixon and Mercer, 1983) and terminating (Thomas et al., 1977) flight are active decisions, once on the wing aphids are rather clumsy fliers and movement is generally governed by prevailing wind direction (Dixon and Howard, 1986). Thus, under windy conditions oriented flight towards a pheromone source may be ineffective and certainly energetically costly (see Keller, 1990). However, prevailing environmental conditions fashion how animals search (Bell, 1991) and walking towards a pheromone source may have evolved as a major component of mating in the potato aphid as the inhibition of flight by strong winds could have a marked impact on a small insect's overall reproductive success (Weisser et al., 1997). Results obtained in both laboratory and field experiments would support this hypothesis. Firstly, both the female calling and male ambulatory behaviours continue at winds approaching 4m/s , well above the threshold that inhibits male flight behaviour. Secondly, at lower wind speeds the proportion of males initiating downwind flight decreased significantly when a pheromone source was present, which certainly would

not be expected if flight was the principal means of locating calling females. Thus, we propose that *M. euphorbiae* males use flight to locate habitats where the probability of receptive females is high. It is possible that they do so by integrating olfactory cues such as primary host plant volatiles and the female sex pheromone, as suggested for other aphid species (Campbell et al., 1990, Hardie et al., 1994) and phytophagous insects (Landolt and Phillips, 1997). Once on the host plants males would generally walk towards any pheromone source it detected but would deploy a “point and shoot” flight strategy during lull periods in the wind velocity, as this should reduce overall searching time. Kennedy (1990) reported an acceleration in the incidence of “taking off” behaviour in black bean aphid parthenogenetic alates following a period when flight was inhibited by winds of 2m/s. A similar behavioral post-inhibitory rebound by males could play an important role in mate location and certainly merits further investigation. The combined use of ambulatory and flight movement in response to varying environmental conditions has been demonstrated in host plant location in herbivores, such as the Colorado potato beetle (Boiteau, 2001) and parasitic wasps (e.g. Weisser et al., 1997), as well as male moth responses to pheromone sources (e.g. Willis and Baker, 1987).

Clearly, species-specific differences may exist. Under semi natural conditions male hop aphids exhibited upwind flight to a pheromone source (Hardie et al., Pers. comm.), yet in the present study potato aphid males were only fly towards the source during periods when the wind drops. To exploit lull periods only mechanoreceptors would be necessary to gauge the instantaneous wind direction and determine flight orientation, while the sensory inputs for a oriented upwind flight would be more complex (Elkinton and Cardé, 1984; Murlis et al., 1992; Justus and Cardé, 2002). Therefore, considerably more comparative research on the behaviours associated with mate location of aphids will be required to clarify any species specific differences and to establish any similarities within this entire group within a meaningful ecological framework. This should include detailed studies on the interactions between wind speed and distance from the source affecting male movement, for the relationships certainly are not straightforward (Tables 1, 2). In addition needs to examine plume structure within the complex and variable habitats occupied by aphid sexuals. Such experiments would not only provide information on what chemical cues actual reach males

and how they react to these infochemicals, but could also provide insight into the evolution of mate location strategies in weak flying insects.

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Table 1: Proportion of *Macrosiphum euphorbiae* males responding to sexual females as a function of distance to the source at different wind speeds in a wind tunnel at $18 \pm 0.5^\circ \text{C}$, 40- 50 % RH.

Wind speed (cm/s)	Distance (cm)	Orientation	Fly down wind	Walk to source	Time to reach source (s)
40	40	80.5 ± 2.8 a*	52.8 ± 2.8 a	41.7 ± 4.9 a	126.5 ± 6.6 a
	70	83.3 ± 8.5 a	61.1 ± 5.7 a	38.9 ± 5.7 a	204.4 ± 7.9 b
	100	80.5 ± 2.8 a	66.7 ± 4.9 a	30.1 ± 5.7 a	266.5 ± 9.2 c
80	40	94.4 ± 2.8 a	11.1 ± 2.8 a	77.8 ± 2.8 a	112.2 ± 1.6 a
	70	94.4 ± 2.8 a	13.9 ± 2.8 a	80.6 ± 2.8 a	148.1 ± 6.0 b
	100	88.9 ± 5.7 a	11.1 ± 5.7 a	72.2 ± 2.8 a	240.4 ± 10.2 c
120	40	100 ± 0.0 a	0 ± 0.0 a	83.3 ± 8.5 a	159.6 ± 11.2 a
	70	96.1 ± 2.8 a	2.8 ± 2.8 a	69.4 ± 2.8 a	331.0 ± 5.0 b
	100	88.9 ± 7.5 a	0 ± 0.0 a	72.2 ± 2.8 a	396.1 ± 6.0 c

*For each wind speed, proportions with the same letter are not significantly different ($p < 0.05$).

Table 2: Two-way ANOVA results summarizing the effect of distances from source within a given wind speed on *Macrosiphum euphorbiae* males' behaviour in a wind tunnel at $18 \pm 0.5^\circ \text{C}$, 40- 50 % RH.

Sources	Orientation			Fly down wind			Reach the source			Time to source (s)		
	df	F	P	df	F	P	df	F	P	df	F	P
<i>Replicate</i>	2,4	0.04	0.9626	2,4	0.27	0.7784	2,4	2.46	0.2013	2,4	1.85	0.2696
Wind speed	2,4	5.56	0.0699	2,4	121.32	0.0003	2,4	60.75	0.0010	2,4	111.45	0.0003
Distance from source	2,12	1.77	0.2114	2,12	1.03	0.3875	2,12	3.25	0.0745	2,12	176.93	< 0.0001
W x D	4,12	1.25	0.3408	4,12	0.63	0.6497	4,12	1.11	0.3953	4,12	11.88	0.0004

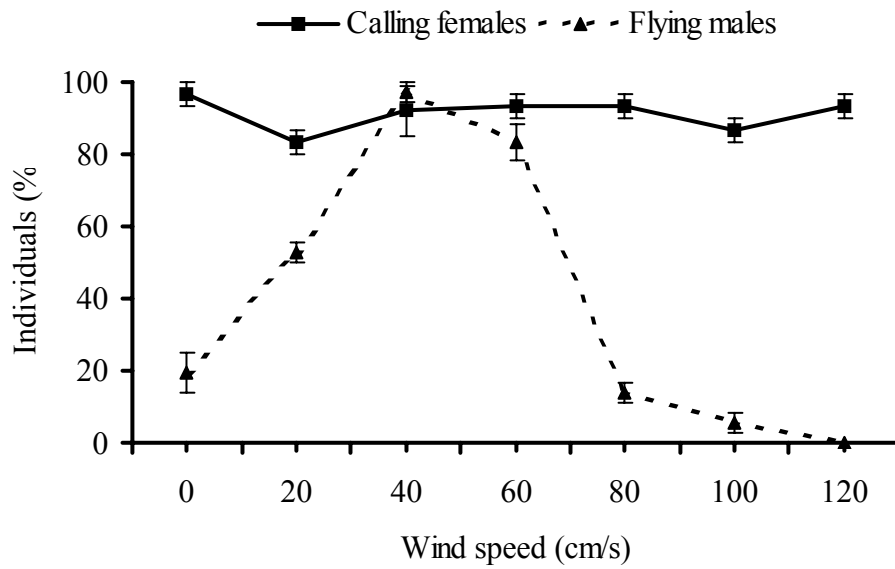


Figure 1: Effect of wind speed on the proportion of *Macrosiphum euphorbiae* oviparae calling and males taking flight ($\bar{x} \pm \text{SEM}$; 3 replicates of 15 individuals at each wind speed) in a wind tunnel at $18 \pm 0.5^\circ \text{C}$, 40- 50 % RH.

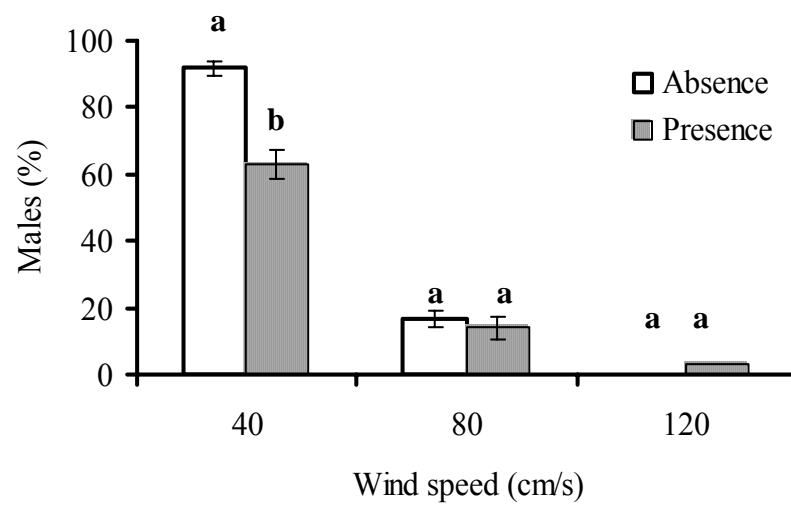


Figure 2: Effect of wind speed on the proportion of *Macrosiphum euphorbiae* males ($\bar{x} \pm \text{SEM}$; 3 replicates of 15 individuals) taking flight in the presence or absence of calling females sexuals in a wind tunnel at $18 \pm 0.5^\circ \text{C}$, 40- 50 % RH.

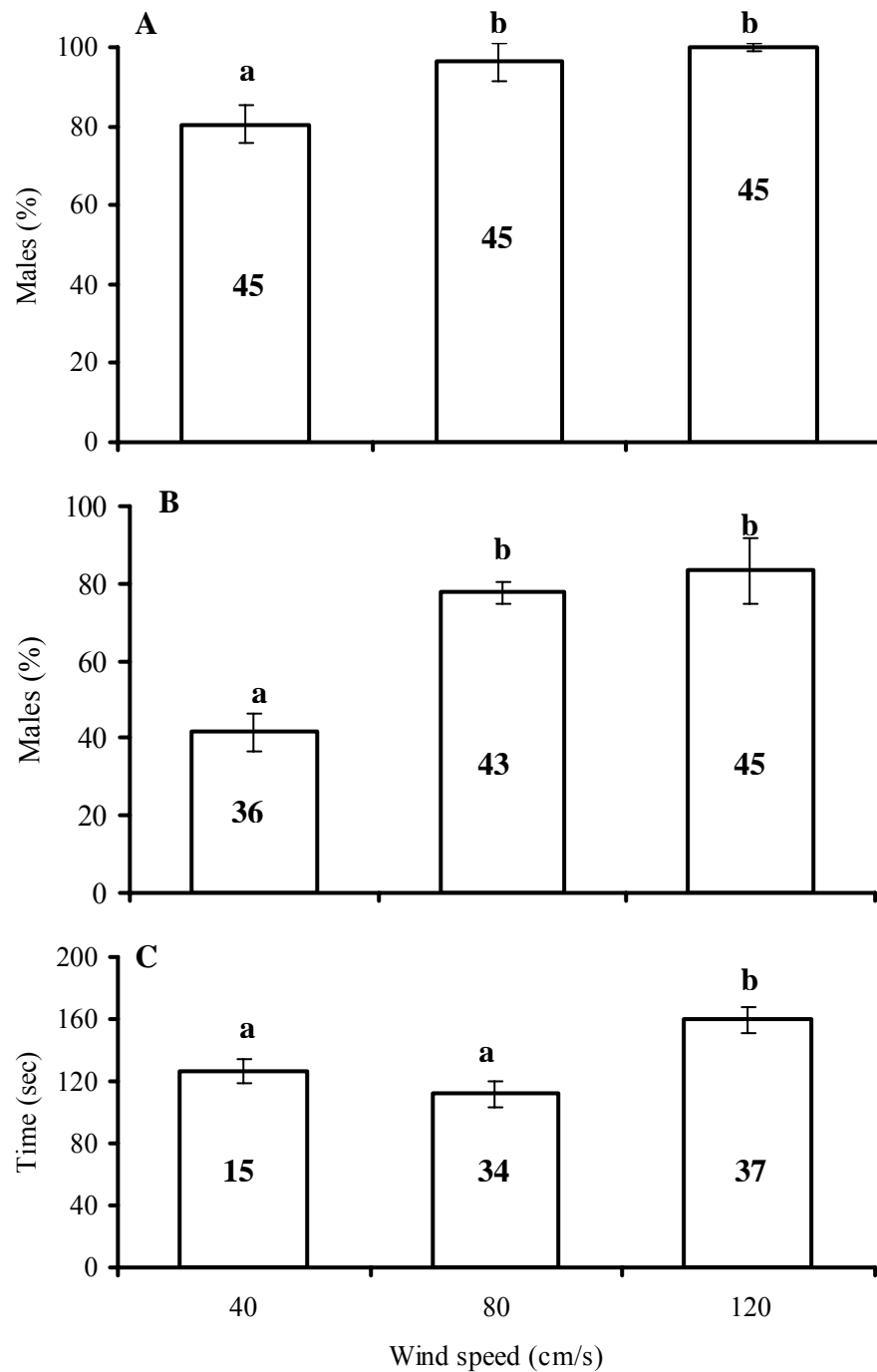


Figure 3: Effect of wind speed on the proportion of *Macroisiphum euphorbiae* males ($\bar{x} \pm \text{SEM}$) (a) orientating and (b) walking the source, as well as (c) the time to reach the source in a wind tunnel at $18 \pm 0.5^\circ \text{C}$, 40- 50 % RH. The source was 5 calling oviparae 40 cm upwind of the release site. Number of males tested are indicated within each column.

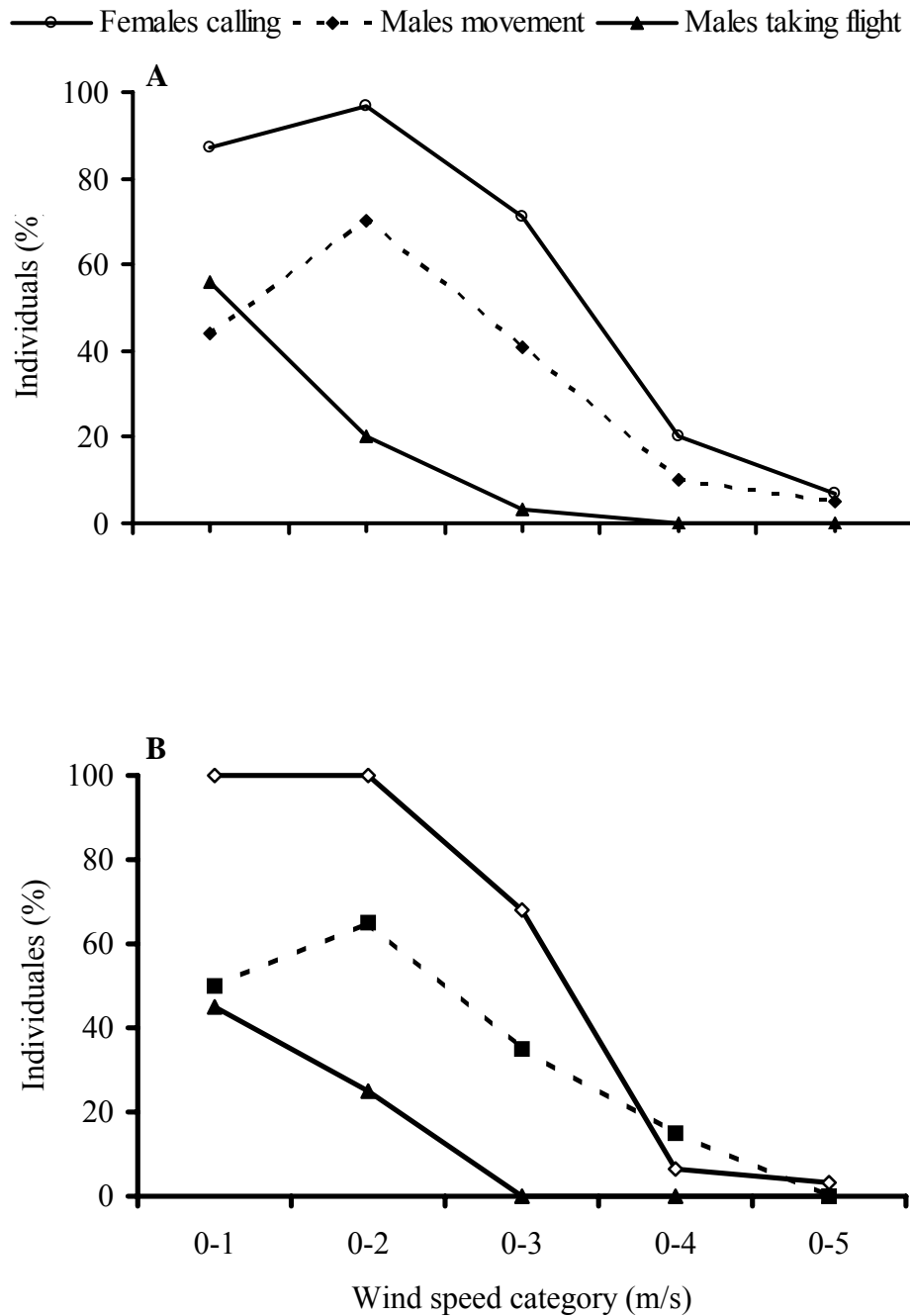


Figure 4: Effect of wind speed on the proportion of *Macrosiphum euphorbiae* oviparae calling and males taking flight ($\bar{x} \pm \text{SEM}$) under variable wind conditions in the field on (a) 15/9/01 and (b) 27/9/01. 30 females and 20 males were observed at each observation period.

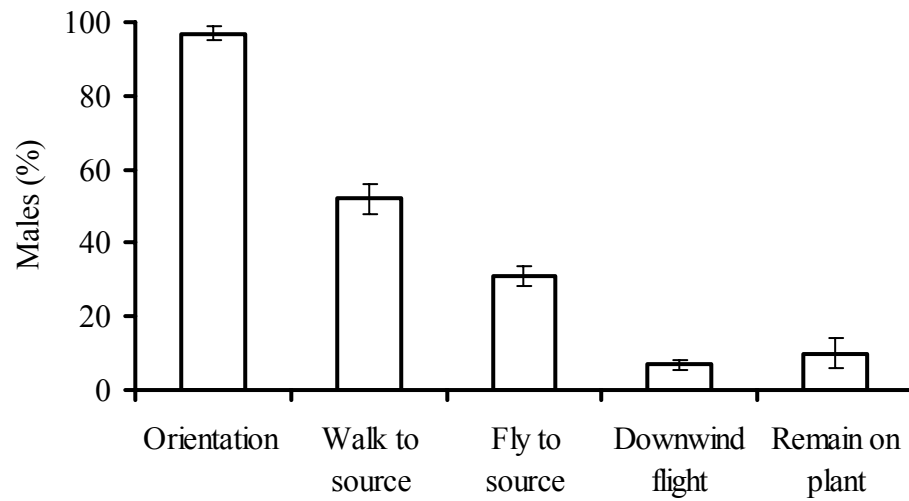


Figure 5: Response of *Macrocephalus euphorbiae* males ($N = 46$; $\bar{x} \pm \text{SEM}$) to calling females located 40 cm upwind under variable wind conditions in the field assays carried out on 11 and 24 October, 2001. Males could either fly or walk to the source.

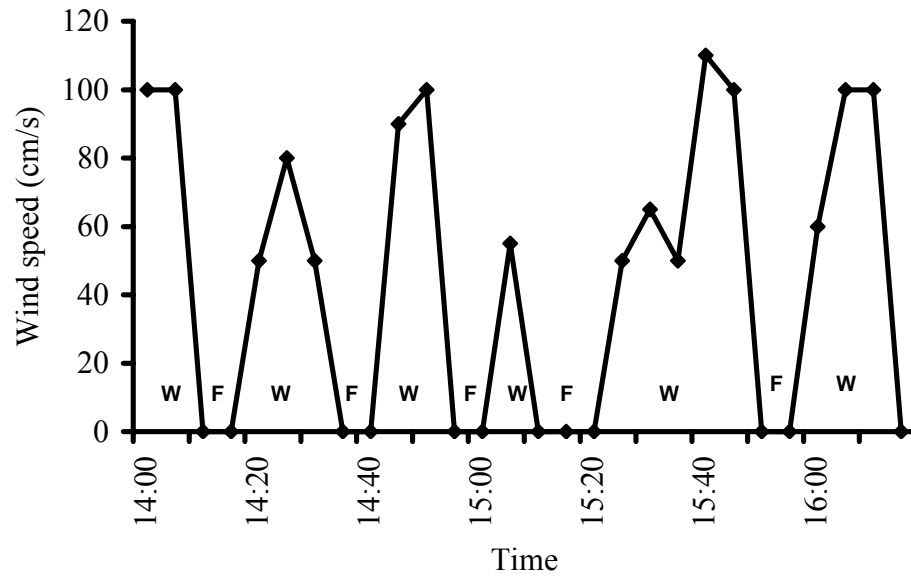


Figure 6: Temporal pattern of wind velocity during the assay carried out on 11 October, 2001 to test the response of *Macrosiphum euphorbiae* males to calling females located 40 cm upwind under variable wind conditions in the field. The periods identified as W and F represent the conditions males either walked or flew to the source.

Chapitre V

Conclusion

Mayr (1977) a défini une espèce comme étant un groupe de populations naturellement interfécondes et isolés reproductivement des autres populations de la sorte. Même si un certain débat existe au sujet de la façon l'isolement reproductif a évolué (Paterson, 1978), généralement, les espèces bien établies sont isolées les unes des autres par un ou plusieurs facteurs d'ordre comportemental, écologique et/ou ayant trait à la communication dans le temps et/ou dans l'espace (McLellan et al., 1991; Via, 1999; Dres and Mallet, 2002).

Le degré auquel les voies de communication phéromonale spécifiques à chaque espèce agissent comme des mécanismes efficaces d'isolement reproductif a été étudié chez de nombreuses espèces (McLellan et al., 1991; Mazor and Dunkelblum, 1992; Thomas et al., 2003). De toute évidence, l'hybridation semble très peu probable lorsque des espèces, sur une base saisonnière, sont temporellement distinctes, comme ce fut noté pour plusieurs espèces géographiquement sympatriques de sesies (Lepidoptera: Sessiidae) (Greenfield et Karandinos, 1979), même si elles se servent de mélanges phéromonaux très semblables. L'échelle temporelle peut être raffinée au point de jouer un rôle à l'intérieur d'une même journée, tel que ce fut rapporté pour deux espèces de *Platyptilia carduidactyla* et *P. williamsii* (Lepidoptera: Pterophoridae) (Haynes et Birch, 1986). Les mâles répondaient au même appât sexuel, le (Z)-11-hexadecenal, mais les femelles de *P. carduidactyla* libéraient des phéromones pendant la première moitié de la nuit et celles de *P. williamsii* appelaient pendant la seconde moitié. Toutefois, malgré la grande importance potentielle de la séparation temporelle de l'émission des phéromones dans l'isolement reproductif, cet aspect n'a reçu que très peu d'attention, tout particulièrement en conditions naturelles. Étant donné que la fenêtre quotidienne d'appel d'une espèce peut se déplacer et qu'elle dépend d'un grand nombre de facteurs biotiques et abiotiques, cet aspect est d'une très grande importance (voir McNeil, 1991 et références citées).

Guldmond et Dixon (1994) ont démontré que trois espèces de *Cryptomyzus* différaient dans leur cycle journalier de libération de phéromones sexuelles en laboratoire: *C. galeopsidis* débutait ses appels immédiatement après l'ouverture des lumières; *C. ribis* appelle surtout de 5 à 7 heures après l'ouverture des lumières, et *C. maudamanti* appelait surtout en après-midi. À la lumière de ces résultats, les auteurs ont suggéré que les différences interspécifiques dans la fenêtre d'appel pouvaient jouer un rôle premier plan dans la reconnaissance des partenaires et dans l'isolement reproductif chez les espèces sympatriques de pucerons. Toutefois, nos résultats présentés dans le second chapitre, démontrent clairement que même si la période d'appel est très bien définie en conditions contrôlées de laboratoire, cela n'est vraiment pas le cas dans la nature. Le moment des appels et le temps passé à appeler variaient grandement d'un jour à l'autre selon les changements de certains facteurs tels que la température, la vitesse du vent et les précipitations. Si ce que nous avons observé pour *M. euphorbiae* se tient pour d'autres espèces de pucerons, il est clair qu'on rejete l'hypothèse suggérant que les fenêtres d'appel pourraient jouer un rôle important en tant que mécanisme d'isolement reproductif. À moins que les espèces sympatriques répondent aux changements environnementaux d'une manière semblable et que, par conséquent, elles conservent une séparation temporelle dans la périodicité de la communication phéromonale. Manifestement, des études de terrain détaillées sont nécessaires pour savoir jusqu'à quel point les fenêtres d'appel temporelles spécifiques réduisent la probabilité d'accouplement interspécifique chez les pucerons en conditions naturelles.

Deux espèces sympatriques qui ne possèdent pas de fenêtres temporelles d'appel différentes clairement définies peuvent tout de même minimiser leur hybridation si elles se servent de mélanges phéromonaux différents (e.g. Roelofs et Cardé, 1974; McLellan et al., 1991; Thomas et al., 2003). Habituellement, les espèces partageant un composé phéromonal sexuel majeur émettent également de composés mineurs uniques pour s'assurer d'avoir un signal spécifique. Ivanova et al. (1988) ont rapporté que des variations dans la composition des phéromones de sept espèces sympatriques de *Archips* (Lepidoptera: Tortricidae) assuraient leur isolement reproductif. De manière semblable, Löfstedt et Van Der Pers (1985) ont démontré que quatre espèces européennes de lépidoptères (Yponomeutidae) partageaient la Z11-14:OAc comme composé phéromonal sexuel

primaire, mais qu'elles étaient isolées reproductivement par une proportion variable de l'isomère *E*.

Les phéromones sexuelles de plusieurs espèces de pucerons étudiées jusqu'à maintenant contenaient toutes deux monoterpénoides, le nepetalactol (I) et le nepetalactone (II). Il y a des différences spécifiques dans les rapports I:II émis par les femelles et auxquels les mâles répondent. En principe, ceci peut mener à un isolement reproductif. Toutefois, tel que démontré dans la présente étude (Chapitre III), le rapport peut varier en fonction de l'âge de la femelle et d'autres espèces sympatriques peuvent utiliser des rapports semblables, mettant en doute que seul un ratio fixe des deux composants peut être un signal propre et fiable à une espèce. D'autres données tirées de la littérature suggèrent qu'il serait peu probable que la spécificité puisse être atteinte seulement par les rapports changeants du nepetalactol et du nepetalactone. Des études ont ainsi démontré que, chez certaines espèces, les mâles pouvaient répondre à un éventail relativement large de rapports, comprenant ceux d'autres espèces (Hardie et al., 1990), ou encore ne répondaient pas aux rapports trouvés chez les femelles conspécifiques (Hardie et al., 1990; Guldmond et al., 1993). Ainsi, un besoin pour d'autres recherches portant sur le mélange phéromonal utilisé par les diverses espèces se fait sentir. Tel que suggéré pour certaines espèces (Guldmond et al., 1993), il serait important de clairement déterminer si des composés mineurs procurent une spécificité chez les pucerons. D'autre part, il serait aussi important de voir si la spécificité des espèces peut être induite par des différences de chiralité et de stéréochimie des deux composés phéromonaux (voir Hardie et al., 1997), tel que mentionné ci-haut pour des espèces de papillons de nuit. De plus, des études sur le comportement d'accouplement sera nécessaire. Si les femelles vierges s'accouplent dès l'émergence, l'effet du changement de ratio de deux composantes de la phéromone de pucerons en fonction de l'âge des femelles sera annulé. De telles études devraient aussi comprendre la description du comportement de cour d'individus rapprochés, puisque ce dernier pourrait varier selon les espèces et ainsi réduire l'hybridation même lorsque la composition phéromonale est similaire (Steffan, 1990). Par ailleurs, une attention devrait être portée à l'existence possible de phéromones mâles, puisqu'il est très évident chez les Lépidoptères que celles-ci jouent un rôle appréciable dans le choix de la femelle à courte distance (e.g., Phelan et al., 1987; Schlaepfer et McNeil, 2000; Torres-Vila et McNeil, 2001).

Considérant nos résultats, le vent est un facteur important et limitant pour rechercher une partenaire sexuelle chez les mâles du puceron de la pomme de terre. En tunnel de vol, avec le vent constant, les mâles ne décollent pas quand le vent est à $>1\text{m/sec}$, et une situation semblable a été observée sur le terrain. Au laboratoire, à $<1\text{ m/sec}$ les mâles volent, mais toujours dans la direction du vent, même en présence de la phéromone sexuelle. En effet, contrairement à notre hypothèse voulant que les mâles volent vers l'amont du vent pour localiser la source, comme décrite pour les Lépidoptères, ils se dirigent vers la source en marchant. Étant donné la petite taille des pucerons, ces résultats ne sont pas vraiment surprenant, la marche peut être importante pour divers petits insectes comme les parasitoïdes (Weisser et al., 1997 et références citées). Cependant, sous des conditions de vents variables sur le terrain, les mâles peuvent parfois, voler à la source; quelque chose que nous n'avons jamais vu sous des conditions de vent constant au laboratoire. Clairement, les mâles marchent vers la source s'il y a du vent, mais lors d'une diminution temporaire de la vitesse (à $<25\text{cm/sec}$), ils volent dans la direction de la source. Des études supplémentaires sont nécessaires afin de déterminer l'importance relative des deux stratégies (marcher versus « point and shoot ») dans la localisation de la femelle. Certaines questions restent à résoudre : (i) est-ce qu'il y a un seuil minimum pour décoller?, (ii) est-ce que les mâles atterissent immédiatement quand la vitesse du vent augmente?, et (iii) est-ce que la distance entre les mâles et la source de phéromone, ainsi que la structure du micro-habitat, affectent la probabilité que le mâle puisse voler plus que marcher vers la source?

Il y a eu plusieurs cas bien documentés de spéciation sympatrique associée à l'utilisation différentielle des plantes hôtes, comprenant celui de la mouche de la pomme et celui de la mite du yucca (i.e., yucca moth; Feder et al., 1994; Groman et Pellmyr, 2000). Des plantes hôtes semblables joueraient un rôle dans l'isolement reproductif de races d'hôtes sympatriques de la même espèce, comme c'est le cas pour le puceron de la poire, *Acyrtosiphon pisum* (Homoptera: Aphididae) (Via, 1999), et la tordeuse grise du mélèze, *Zeiraphera diniana* (Lepidoptera: Tortricidae) (Emelianov et al., 2003). Étant donné que plusieurs espèces de pucerons alternent d'un hôte à un autre, les caractéristiques de la plante hôte, particulièrement celles de l'hôte primaire, lieu des accouplements, peuvent jouer un rôle important dans l'isolement reproductif d'espèces sympatriques. La littérature portant

sur les substances volatiles des plantes hôtes et sur la reproduction des pucerons est peu abondante. Les études sur le sujet sont parfois contradictoires, certains suggérant que les particularités sont importantes (Hardie et al., 1994) et d'autres l'inverse (Guldmond et al., 1993; Thieme et Dixon, 1996). Toutefois, très peu d'études ont été réalisées en conditions naturelles et, bien souvent, elles impliquent de petites plantes en pot comme hôte, alors que, sur le terrain, les pucerons sont trouvés sur des arbres matures. Des études comparatives se penchant sur divers aspects du système de communication phéromonale, comprenant l'effet des substances volatiles des plantes hôtes sur le comportement d'appel des femelles et sur la réponse des mâles envers les phéromones sexuelles, sont nécessaires (e.g., McNeil et Delisle, 1989; Landolt et Phillips, 1997; Yan et al., 1999). Les pucerons sont capable de décoller et d'atterrir activement (Thomas et al., 1977; Dixon et Mercer, 1983), mais dû à leur petite taille, ils ne sont pas souvent capable de faire des vols dirigés et en conséquence ils sont emporter par le vent. Nous proposons l'hypothèse que les mâles quittent l'hôte secondaire activement, puis sont emporters passivement par les vents, mais vont activement atterrir en réponse aux indices physiques et/ou olfactifs des plantes hôtes. Ensuite, ils vont généralement marcher vers les femelles sexuées en appel, avec de courtes périodes de vol si les conditions de vent sont propices. Ainsi, il y aura besoin d'études approfondies sur le terrain afin de déterminer l'impact de plantes hôtes et de phéromones sexuelles sur la recherche des partenaires sexuels chez des pucerons.

Le mieux serait de réaliser des études comparatives visant à explorer différents aspects de l'écologie phéromonale des pucerons mentionnés ci-haut en utilisant divers groupements sympatriques d'espèces de pucerons. Ceci comprendrait la comparaison d'espèces qui démontrent ou non une alternance saisonnière d'une plante hôte vers une autre, de même que ceux de divers habitats (tempérés versus subtropicaux et tropicaux) où les variations dans les conditions écologiques doivent influencer grandement l'évolution des mécanismes assurant l'isolement reproductif. Par ailleurs, les données obtenues à partir de telles recherches procureraient une base contextuelle sur laquelle quelqu'un pourrait s'appuyer pour déterminer si, tel que suggéré par certains chercheurs, les phéromones sexuelles des pucerons peuvent être utilisées efficacement en lutte intégrée des espèces nuisibles (Pickett et al., 1994). Les résultats de la présente étude sur *M. euphorbiae*, suggérant que la marche est une composante comportementale majeure dans la localisation

des partenaires par les pucerons mâles, soulèveraient des doutes quant à l'utilité de pièges à phéromones sexuelles comme outil de contrôle ou pour la dissémination de pathogènes. Ainsi, avant de pouvoir répondre à de telles questions, une banque de données beaucoup plus vaste est nécessaire.

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