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Etude expérimentale et comparative de la myrmécochorie : le cas des fourmis dispersatrices *Lasius niger* et *Myrmica rubra.*

Thèse présentée en vue de l'obtention du
grade académique de Docteur ès Sciences par

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Avant-propos

Cette thèse est divisée en deux parties. La première contient trois chapitres et concerne la partie expérimentale. La deuxième contient deux chapitres et concerne la synthèse bibliographique et l'étude d'écologie comparative. Chaque chapitre est rédigé sous forme d'un article en anglais soumis (ou en voie de soumission) à une revue internationale à comité de lecture.

Partie 1 – Expérimentation

1. Servigne, P. & Detrain, C. (2008). Ant-seed interactions: combined effects of ant and plant species on seed removal patterns. *Insectes Sociaux*, In press.

2. Servigne, P. & Detrain, C. Opening myrmecochory's black box: what happens inside the ant nest? (Manuscrit soumis à *Comptes Rendus Biologies*).

3. Servigne, P. & Detrain, C. Seed desiccation limits removal by ants. (Manuscrit soumis à *Ecological Entomology*).

Partie 2 – Synthèses

4. What features make ants major seed dispersers? (Manuscrit préparé pour *Biological reviews*).

5. Myrmecochory in European mesic habitats: which plant species are involved? (Manuscrit préparé pour *Plant Ecology*).

Les protocoles expérimentaux ont été dessinés avec Claire Detrain. Toutes les acquisitions de données ont été réalisées par moi-même, avec l'aide ponctuelle expérimentale d'Aurélie Motard (chapitre 1), bibliographique de Nicolas Vereecken (chapitre 4), et technique d'Ingolf Kühn (chapitre 5). Les analyses statistiques ont bénéficié des conseils constants de Claire Detrain, et ponctuels de Jesus Millor, Pierre Meerts et Raphael Jeanson. J'ai initié la rédaction des cinq manuscrits et ils ont atteint leur version finale grâce aux efforts collaboratifs répétés de Claire Detrain. Les personnes ayant apporté des conseils ponctuels sont cités à la fin de chaque chapitre. L'introduction et la conclusion en français ont bénéficié des relectures pertinentes et constructives de Claire Detrain, Stéphane Canonge, Nicolas Vereecken et Aina Astudillo Fernandez.

Introduction

Définitions et généralités

L'accomplissement du cycle de vie d'une plante passe par le déplacement de ses structures reproductrices : le pollen pour la fécondation croisée et les graines pour la colonisation de nouveaux espaces (Herrera 2002). Le transport des graines peut être effectué mécaniquement par le vent (anémochorie), l'eau (hydrochorie) ou la gravité (barochorie), mais aussi par les animaux (zoochorie) tels que les oiseaux, les rongeurs, les écureuils, les chauves-souris ou les fourmis.

La dispersion des graines par les fourmis est appelée *myrmécochorie* (Sernander 1906).

Contrairement aux cas de dispersion par les animaux où la graine est transportée dans le tractus digestif après ingestion des graines (endozoochorie) comme c'est le cas par exemple chez la plupart des oiseaux, les graines myrmécochores sont transportées « à l'extérieur » de l'animal (exozoochorie), et plus précisément entre les mandibules. Par ailleurs, dans cette même catégorie, la myrmécochorie se distingue des graines qui sont dispersées de manière *inactive* en s'accrochant à la fourrure des vertébrés. Au contraire, les fourmis prennent les graines de manière *active* (Howe & Smallwood 1982). Enfin, il existe également des cas de transport actif par des animaux granivores (écureuils, rongeurs ou fourmis moissonneuses) qui oublient ou perdent accidentellement une petite partie de leur récolte en route. Ce mode de dispersion est appelé dyszoochorie (Van der Pijl 1972).

Alors que la dispersion des graines par les fourmis au sens large inclut aussi la dispersion accidentelle par les fourmis moissonneuses, nous définissons la myrmécochorie *sensu stricto* comme une exozoochorie active non-dyszoochore, c'est-à-dire qui exclut cette dispersion accidentelle.

L'originalité de la myrmécochorie réside dans le fait que les graines portent un appendice nutritif appelé *élaiosome* qui n'est pas indispensable à la germination de la graine et dont les fourmis se nourrissent. Le terme *élaiosome* (*elaios*, huile ; *soma*, corps) a été introduit pour la première fois par Sernander (1906) pour désigner les appendices charnus sur les diaspores dispersées par les fourmis. Les élaiosomes sont considérés comme des adaptations qui favorisent la dispersion des graines et ont diverses origines morphologiques (arille, caroncule, funicule, etc.) (Gorb & Gorb 2003). Ils sont dès lors un bel exemple de convergence évolutive (Beattie 1985, Mayer et al. 2005, Edwards et al. 2006, Fischer et al. 2008).

Le processus de dispersion est généralement le suivant: les fourmis récoltent les diaspores[1] à la source, les rapportent au nid, consomment l'élaiosome et rejettent les graines dépourvues d'élaiosome (Sernander 1906). Ainsi, la myrmécochorie est habituellement considérée comme un mutualisme, c'est-à-dire une relation qui bénéficie à la fois aux plantes (dispersion) et aux fourmis (apport alimentaire) (Beattie 1985).

A part quelques cas très rares cas de dispersion par les guêpes ou les abeilles (Zettler et al. 2001, Wallace et al. 2007), les fourmis sont les seuls invertébrés à disperser massivement des graines. Jusqu'à présent, plus de 3 000 espèces de plantes sur 270 000 (environ 1,2 %) ont été recensées dans le monde comme myrmécochores (Beattie 1985, Beattie & Hughes 2002, Rico-Gray & Oliveira 2007). Ces plantes appartiennent à 80 familles différentes (Beattie & Hughes 2002, Dunn et al. 2007) et sont présentes dans de nombreux écosystèmes de par le monde (Fig. 1). Parallèlement, des centaines d'espèces de fourmis ont été observées transportant des graines à élaiosome. Dans un même habitat, une plante peut être disséminée par plusieurs espèces de fourmis et inversement, une espèce de fourmi peut disséminer plusieurs espèces végétales (Handel & Beattie 90).

L'évolution de la myrmécochorie

La large distribution géographique et taxonomique des plantes myrmécochores, ainsi que la diversité ontogénique des élaiosomes montre clairement que la myrmécochorie est apparue

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[1] Diaspore : unité de dispersion des plantes (Van der Pijl 1972). Dans le cas de la myrmécochorie, c'est la graine pourvue de son élaiosome.

plusieurs fois indépendamment au cours de l'évolution (Beattie 1985, Dunn et al .2007). Il s'agit désormais de comprendre comment est apparue cette convergence évolutive et sous l'influence de quels facteurs (biotiques ou abiotiques) auraient évolué les élaiosomes et leur fonction de dispersion. Seules deux études phylogénétiques permettent aujourd'hui d'amorcer une réponse.

L'analyse phylogénétique de la famille des Polygalaceae a montré que l'apparition de l'élaiosome dans cette famille était survenue il y a environ 50 millions d'années, longtemps après la diversification des fourmis (60-100 millions d'années), mais au même moment qu'une augmentation globale de la température terrestre, suggérant que les élaiosomes seraient apparus suite à des pressions de sélection abiotiques (température, sécheresse) puis auraient évolué sous la pression de sélection des fourmis (Forest et al. 2007). Une autre étude a montré que l'évolution des élaiosomes des monocotylédones n'étaient pas associée à l'apparition des forêts du crétacé, mais plutôt à l'augmentation de l'abondance écologique des fourmis (fin Eocène) (Dunn et al. 2007).

L'hypothèse la plus communément admise est que la myrmécochorie (mutualisme) semble avoir évolué à partir de la granivorie (antagonisme) (Rissing 1986, Levey & Byrne 1993, Rico-Gray & Oliveira 2007). Mais il est cependant trop tôt pour conclure sur un schéma général de l'évolution de la myrmécochorie ou pour tracer des scénarios évolutifs sur son origine ou sa disparition.

Distribution géographique

A l'échelle mondiale, la myrmécochorie est présente dans de nombreux écosystèmes, tant aux altitudes et latitudes les plus élevées qu'aux plus basses, et même quand les communautés de fourmis sont pauvres en espèces (Beattie 1983). On distingue six principaux écosystèmes où la myrmécochorie est particulièrement présente : les forêts sclerophylles d'Australie, le Fynbos d'Afrique du Sud, les autres climats de type Méditerranéen, les régions semi-arides d'Amérique du Sud, les forêts tropicales et enfin les milieux tempérés de l'hémisphère Nord. Le nombre d'études portant sur la myrmécochorie (Fig. 1) indique que le phénomène est bien présent, mais n'est cependant pas proportionnel au nombre d'espèces impliquées.

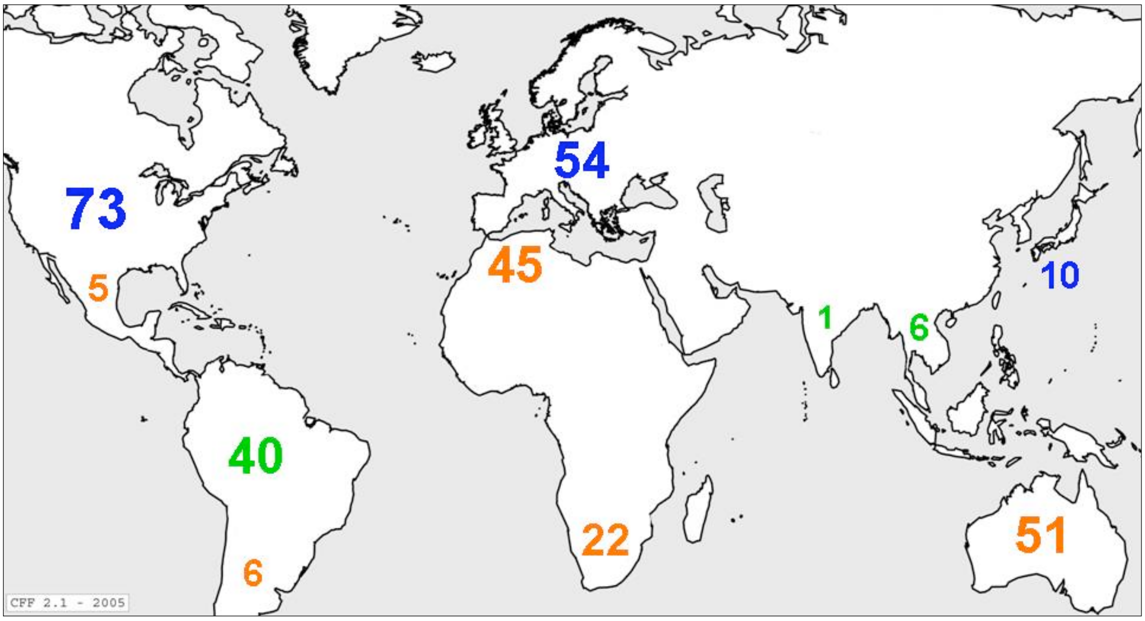

Figure 1. Distribution géographique du nombre d'études sur la myrmécochorie. Bleu : climats tempérés ; Orange : climats secs, savanes et de type méditerranéens ; Vert : climats tropicaux.

Australie et Afrique du Sud

Plus de 95% des 3000 espèces de plantes myrmécochores recensées à ce jour sont originaires d'Australie et d'Afrique du Sud et en particulier présentes dans le Fynbos sud-africain et les bois secs à végétation sclérophylle d'Australie (Berg 1975, Bond et al. 1991, Rico-Gray & Oliveira 2007). Ces deux régions se caractérisent surtout par des sols pauvres et un climat très semblable (Bond & Slingsby 1983).

La flore d'Australie, en particulier de certaines régions arides (par exemple la Province du Sud-Est) sont extrêmement riches en espèces endémiques et en espèces myrmécochores (environ 1500 espèces). On peut compter de 30 à 50 % des espèces végétales présentes dispersées par les fourmis (Westoby et al. 1991). La plupart des plantes myrmécochores sont des arbustes, diplochores (autochore + myrmécochore), à élaiosomes fermes et persistants (Berg 1981). Même si elles représentent une faible proportion de la couverture végétale (15 %), les plantes myrmécochores sont présentes sur tout le territoire et ne seraient pas liées à une structure de végétation particulière (Rice & Westoby 1981). Deux caractéristiques pourraient expliquer la forte présence de myrmécochores en Australie : une proportion importante de sols très pauvres et le passage de feux intenses (Orians & Milewski 2007). Les plantes bénéficient de la dispersion par les fourmis lorsque la profondeur à laquelle les graines sont laissées dans le nid est telle qu'elles reçoivent assez de chaleur pour germer après le passage d'un feu (Majer 1982). Par ailleurs, une corrélation existe entre la pauvreté de sols en nutriments et le mode de dispersion : dans les sols riches, on retrouve plus d'espèces à fruits charnus. Le coût de la production d'élaiosome étant moindre que le coût de production de fruits charnus, la myrmécochorie pourrait être une adaptation des plantes à des sols pauvres (Westoby et al. 1982, Westoby et al. 1991).

En Afrique du Sud, on estime à près de 1300 le nombre d'espèces myrmécochores appartenant à 78 genres et 29 familles, ce qui représente un cinquième de la flore du Cap (Slingsby & Bond 1981, Milewsky & Bond 1982, Bond & Slingsby 1983). Les plantes myrmécochores d'Afrique du Sud sont principalement des grands buissons ligneux, des petits arbustes et des herbes pérennes. On ne trouve que de très rares géophytes[2] et aucune plante annuelle (Bond et al. 1991). Les élaiosomes peuvent être durs et permanents ou mous et perdant rapidement tout pouvoir attractif après dessiccation. Comme en Australie, la diplochorie est fréquente et les plantes myrmécochores sont plus fréquentes dans les végétations subissant des feux fréquents et sur les sols pauvres en nutriments (Bond & Slingsby 1983). Paradoxalement, le nombre d'espèces de fourmis impliquées est plutôt faible (Bond et al. 1991).

Milieux de type méditerranéen

Le climat méditerranéen est caractérisé par des longues périodes chaudes et sèches en été et des précipitations en hiver (frais à froid). Les régions méditerranéennes sont peu pourvues de plantes myrmécochores malgré l'abondance de fourmis potentiellement myrmécochores. En 1938, Molinier & Müller dénombrent 10 espèces, soit 3,6 % de la flore. Aujourd'hui, le nombre exact n'est pas connu, mais il est sans doute plus important. Ces milieux possèdent en revanche beaucoup de fourmis moissonneuses (dyszoochores) (Aronne & Wilcock 1994, Wolff & Debussche 1999). Il est intéressant de constater que le milieu méditerranéen présente les traits du syndrome myrmécochorique australien : végétation sclérophylle, élaiosome dur, diplochorie importante, absence d'autres modes de dispersion, mais que les myrmécochores y sont beaucoup moins présentes (Espadaler & Gomez 1996). La myrmécochorie est plutôt rare (mais présente) dans les climats de type méditerranéen du Chaparral Californien et Mexicain (Berg 1966, Bullock 1989, Boyd 1996, Valiente-Banuet et al. 1998).

Régions semi-arides d'Amérique du Sud et Centrale

En Amérique du Sud, certaines régions semi-arides commencent à être explorées du point de vue de la myrmécochorie (Morrone 2000, Saba 2003, Leal 2007, Christianini et al. *2*007).

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[2] Géophyte : type de plante vivace possédant des organes lui permettant de passer la mauvaise saison enfouis dans le sol. La plante est donc inapparente au cours de quelques mois de son cycle annuel.

Dans la région semi-aride du Caatinga (Nord-Est du Brésil), un quart de la flore ligneuse est dispersée par les fourmis (avec 12,8 % de myrmécochore *sensu stricto*, *i.e.* avec élaiosomes). La famille des Euphorbiaceae semble particulièrement concernée (Leal et al. 2007). Mais l'occurrence des espèces myrmécochores n'est ni liée à la pauvreté des sols, ni à la fréquence des feux (Leal et al. 2007). Récemment, Mendoza & Castaño-Meneses (2007) ont décrit des cas de myrmécochorie (une espèce de fourmi et trois espèces de plantes) dans des régions arides et semi-arides du Mexique, connues pour être riche en biodiversité endémique. Van der Pijl (1955) suggère que les savanes de Java peuvent être particulièrement riches en plantes myrmécochores (*cité par* Beattie 1983). Il est très probable qu'un nombre important d'espèces myrmécochores reste à découvrir dans les milieux secs du globe.

Forêts tropicales

Sous les tropiques et particulierement en forêt tropicale humide, les fourmis sont remarquables par leur diversité et leur nombre (Wilson 1987). Dans les forêts tropicales humides, la myrmécochorie peut être présente sous trois formes particulières : la myrmécochorie classique avec présence d'un élaiosome, la dispersion secondaire de graines (avec ou sans élaiosome) des fruits charnus préalablement dispersés par les oiseaux, ainsi que dans les jardins de fourmis (Pizo et al. 2005, Orivel & Dejean 1999).

Une particularité des milieux tropicaux est le rôle que jouent les grandes fourmis Ponerines (*Odonthomachus* ou *Pachycondyla*, par exemple) dans la dispersion des graines (Horvitz 1981, Horvitz & Beattie 1980, Kaspari 1993). Etant de grandes tailles et terricoles, elles peuvent transporter les graines à plus de 10 m et les placer dans des endroits favorables à la germination, telle que la litière humide. Certaines petites espèces comme *Wasmannia auropunctata*, parmi les dizaines qui sont intéressées par les graines, ne les transportent pas et consomment l'élaiosome *in situ* (Horvitz & Schemske 1986). Les fourmis de la tribu des Attini, connues comme principales défoliatrices des cultures dans le Nouveau Monde, peuvent également être un vecteur de dispersion (Leal & Oliveira 2000, Leal & Oliveira 1998). Les fourmis jouent aussi souvent un rôle dans la dispersion secondaire des graines se trouvant dans les déjections d'oiseaux ou d'autres vertébrés (Böhning-Gaese et al. 1999, Pizo & Oliveira 1999). En forêt néotropicale, environ 90% des espèces d'arbres et d'arbustes sont dispersées par les frugivores. Cependant, entre le dépôt des graines (par les fèces par exemple) et leur germination, les graines sont souvent amenées à rencontrer les fourmis. De plus, le nettoyage du mucilage et de la pulpe des fruits par les fourmis empêche l'attaque de la graine par des champignons et augmente ainsi les chances de survie des graines (Horvitz 1981, Oliveira et al. 1995, Passos & Oliveira 2003).

Par ailleurs, Ule (1902, 1905, 1906, *cités dans* Rico-Gray & Oliveira 2007) a signalé l'existence de myrmécochorie dans les jardins de fourmis en forêt amazonienne. Chaque espèce de fourmi est attirée et transporte préférentiellement les graines de leur partenaire épiphyte. La reconnaissance spécifique des graines a lieu même lorsque l'élaiosome est enlevé (Orivel & Dejean 1999). Cinq composés chimiques volatiles déclencheurs du comportement de transport des graines ont récemment été identifiés chez le couple fourmi-épiphyte *Camponotus femoratus/Peperomia macrostachya* (Youngstead et al. 2008). Les jardins de fourmis sont à l'heure actuelle les seuls exemples d'interactions spécifiques et obligatoires en myrmécochorie.

Le nombre d'études sur la myrmécochorie en milieu tropical ne cesse d'augmenter vu la biodiversité de ces régions, il ne serait pas étonnant de revoir à la hausse le nombre d'espèces myrmécochores.

Milieux tempérés de l'hémisphère Nord

Même si environ 300 espèces de plantes sont concernées par ce mode de dispersion en milieu tempéré (Beattie 1983), c'est à dire relativement peu, leur importance écologique est considérable en particulier dans les sous-bois et dans certains habitats ouverts. Les fourmis dispersent par exemple plus de 30 % de la flore herbacée des forêts caducifoliées de la côte Est des Etats-Unis (Beattie & Lyons 1975, Culver & Beattie 1978, Handel et al. 1981). En forêt, les espèces myrmécochores herbacées peuvent représenter entre 40% et 60 % des tiges émergente, c'est-à-dire 40 % de la biomasse (Beattie & Culver 1981, Handel et al. 1981). Mais cette guilde est rare dans les forêts jeunes (Mitchell et al. 2002, Flinn & Vellend 2005, Ness & Morin 2008) et dans les lisières (Roy & Blois 2006), en comparaison avec les plantes dispersées par les animaux ou le vent. Les espèces de fourmis observées à ce jour comme dispersatrices de graines ne sont pas nombreuses. Par exemple, on recense 18 espèces en Europe du Nord (non-méditerranéenne) (Tableau 1).

Dans les forêts tempérées, il existe une tendance à la séparation temporelle (saisonnière) entre les espèces de plantes myrmécochores dont les graines mûrissent précocement au printemps, et les espèces dont les graines ornithochores ont tendance à mûrir tardivement (été) (Thompson 1981). Par ailleurs, les saisons de fructification des plantes myrmécochores sont souvent corrélées à des périodes d'activité maximale des fourmis, une période où la nourriture alternative pour ces dernières est relativement peu abondante (Oberrath & Boehning-Gaese 2002).

Tableau 1. Liste des espèces de fourmis d'Europe du Nord ayant été citées comme dispersatrices de graines myrmécochores. Les données concernant les habitats sont issues du livre « The ants of Poland » (Czechowski et al 2002). Références : [1] Sernander 1906, [2] Weiss 1909, [3] Bresinsky 1963, [4] Brian 1977, [5] Handel 1978, [6] Culver & Beattie 1980, [7] Kjellsson 1985a, [8] Kjellsson 1985b, [9] Kjellsson 1985c, [10] Pemberton 1988, [11] Oostermeijer 1989, [12] Baiges et al. 1991, [13] Woodell & King 1991, [14] Gorb & Gorb 1995, [15] Gorb & Gorb 1996, [16] Mark & Olesen 1996, [17], Gorb et al. 1997b, [18] Gorb & Gorb 1998, [19] Gorb & Gorb 1999a, [20] Gorb & Gorb 1999b, [21] Gorb et al. 2000, [22] Oberrath et al. 2002, [23] Peters et al 2003, [24] Dostal 2005, [25] Fischer et al. 2005, [26] Gammans et al. 2005, [27] Gammans et al. 2006, [28] Guitian & Garrido 2006, [29] Delatte & Chabrerie 2007, [30] Fokuhl et al. 2007, [31] Delatte & Chabrerie 2008, [32] Servigne & Detrain 2008.

Espèces	**Nb d'espèces de graines transportées**	**Habitats**	**Références**
Lasius niger	65	Ubiquiste	[1], [4], [6], [22], [23], [32]
Formica rufa	46	Forest de conifères	[1], [2]
Formica exsecta	41	Forest de conifères	[1],
Myrmica ruginodis	32	Lisières, clairières, landes	[6], [7], [8], [9], [16], [22], [23], [24], [26], [27], [31]
Formica polyctena	27	Forest de conifères	[14], [15], [17], [18], [19], [20], [21], [29],
Formica pratensis	21	Habitats secs	[1],
Formica rufibarbis	14	Prairies sèches	[1], [11], [22], [23], [28]
Formica fusca	13	Ubiquiste	[1], [6], [11], [22],
Myrmica rubra	12	Ubiquiste	[1], [20], [22], [23], [25], [30], [32]
Lasius fuliginosus	11	Forêts caducifoliées	[1], [3], [20], [22], [23]
Tetramonium caespitum	5	Habitats secs	[1], [11], [24]
Formica cunicularia	3	Habitats ouverts	[6], [22], [23]
Lasius psammophilus	3	Prairies sèches	[11]
Leptothorax nylanderi	2	Forêts caducifoliées	[20], [22], [23]
Myrmica scabrinodis	2	Habitats humides, forêts	[6], [12]
Leptothorax acervorum	1	Forest de conifères	[1],
Lasius alienus	1	Prairies sèches et forêts	[1], [4], [12]
Myrmica lobicornis	1	Forest de conifères et prairies de montagne	[1],

Dispersion primaire et secondaire

Bien que de nombreuses espèces de graines soient dispersées exclusivement par les fourmis (myrmécochorie stricte), il arrive que d'autres espèces impliquent plusieurs vecteurs. Dès lors, le processus de dispersion est composé de plusieurs étapes séquentielles. On parle de *diplochorie* lorsque le nombre de phases est supérieur ou égal à deux (Vander Wall & Longland 2004) et de dispersion *primaire*, *secondaire*, etc. suivant l'étape. La dispersion secondaire est probablement bien plus courante qu'on ne le pensait car elle a souvent été négligée dans les études sur la dispersion (Vander Wall et al. 2005).

En effet, la myrmécochorie arrive souvent en phase secondaire, précédée par une phase primaire autochore[3] (e.g., Stamp & Lucas 1983, Aronne & Wilcock 1994, Nakanishi 1994, Ohkawara & Higashi 1994, Martins et al. 2006) ou ornithochore (e.g., Gill 1985, Roberts & Heithaus 1986, Clifford & Monteith 1989, Kaufmann et al. 1991, Byrne & Levey 1993, Böhning-Gaese et al. 1999, Passos & Oliveira 2002, Pizo et al. 2005). Après la phase myrmécochore, les graines peuvent être transportées à nouveau par l'eau ou d'autres animaux (Hanzawa et al. 1985, Turnbull & Culver 1983). Si ces derniers sont des prédateurs de graines, on parle alors de *prédation post-dispersion* (Hulme 1998).

En règle générale, la phase primaire offre le plus souvent aux plantes l'avantage de limiter la compétition causée par une densité élevée de plantules ou par la proximité de la plante-mère, ainsi que la possibilité de coloniser de nouveaux milieux. La phase secondaire (myrmécochorie) offre généralement aux graines des microsites favorables à leur germination où la probabilité de germer y est particulièrement élevée (i.e. *« dispersion dirigée »*) (Vander Wall & Longland 2004)

Il a été montré que les fourmis préféraient les graines des plantes myrmécochores strictes à des diplochores, au sein de certains groupes intra-génériques (Sernander 1906, Beattie & Lyons 1975) et inter-génériques (Oostermeijer 1989). Par exemple, les taux de prises des graines étaient significativement supérieurs pour l'espèce myrmécochore stricte *Polygala vulgaris* comparée à deux autres espèces diplochores, *Luzula campestris* et *Viola curtisii* (Oostermeijer 1989).

Les étapes du processus myrmécochorique

Le fait que les fourmis soient des « central-place foragers » (i.e. qui centralisent la nourriture récoltée au nid), trois étapes de dispersion peuvent être mises en évidence, chacune se

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[3] ou « barochore », c'est à dire dispersées par la gravité.

différenciant des autres par son mécanisme, son résultat sur le devenir des graines, ainsi que par les caractéristiques de chaque partenaire impliqué -plantes ou fourmis.

Etape 1 : le retour au nid

Dans un premier temps, les fourmis collectent les graines à la source et les ramènent au nid dans un mouvement centripète. Du point de vue de la graine, deux problèmes se posent: la rapidité avec laquelle les graines sont prises et la distance de dispersion. La rapidité des prises dépend de traits de la fourmi tels que la taille (Hughes & Westoby 1992), le régime alimentaire (Hughes et al. 1994), la capacité d'apprentissage (Smith et al 1989) ou la phénologie (Boulay et al. 2007). Plus des graines seront prises rapidement par les fourmis, moins elles risquent d'être prises par des prédateurs tels que les rongeurs, les oiseaux ou les coléoptères (Heithaus 1981, Bond & Breytenbach 1985, Ohkawara et al. 1997). La distance de dispersion à partir de la plante-mère dépend de traits tels que la taille de la fourmi (Ness et al. 2004) et la structure de la communauté de fourmis (Gomez & Espadaler 1998a). L'enjeu est alors l'évitement de la compétition des plantules avec les plantes-mères (Handel 1976, 1978).

Etape 2 : dans le nid

La deuxième étape a lieu dans le nid, là où les élaiosomes sont généralement consommés. Bien que le séjour dans le nid soit une étape cruciale et probablement l'endroit où les contacts entre les fourmis et les graines sont les plus longs, nous n'avons que très peu de données sur cette étape.

Etape 3 : le rejet du nid

Une fois les élaiosomes enlevés, les graines demeurent toujours viables mais n'intéressent plus les fourmis. En tant que déchet, les graines peuvent alors être abandonnées dans le nid ou être rejetées hors du nid dans un mouvement centrifuge. Dans ce dernier cas, le problème pour les graines est encore une fois la distance et la qualité des sites finaux. Ceci dépend de traits de la fourmi tels que la taille de son territoire et le taux de perte accidentel en chemin (Gorb & Gorb 2003). Les destinations finales des graines peuvent être les bordures de territoire ou l'entrée du nid (Dlussky 1967, *cité par* Gorb & Gorb 2003). La qualité de ces sites pour la germination des graines est très variable (Beattie & Culver 1982, Levey & Byrne 1993).

Les avantages sélectifs pour les plantes

Les avantages sélectifs de la myrmécochorie pour les plantes ont été étudiés à travers plusieurs hypothèses (Beattie 1985, Bennett & Krebs 1987, Giladi 2006, Rico-Gray & Oliveira 2007). Chaque hypothèse, testée plusieurs fois, a été validée ou invalidée selon les cas. Ainsi, il est difficile de tirer une conclusion ou de dresser un scénario général de l'évolution de la myrmécochorie (Bronstein et al. 2006).

Eviter la prédation

Les graines qui sont ramenées et abandonnées à l'intérieur du nid restent hors de portée des prédateurs de graines, tels que les rongeurs, fourmis moissonneuses ou oiseaux. Si la prédation est présente dans un habitat, les graines qui ne sont pas prises par les fourmis à la source finissent par être prises par les prédateurs (O'Dowd & Hay 1980, Heithaus 1981, Smith et al. 1989, Nakanishi 1994, Ohkawara et al. 1997, Pizo & Oliveira 1998). Sur 27 études qui ont testé cette hypothèse, 22 l'ont confirmée, une l'a infirmée et 4 n'ont pas été concluantes (Giladi 2006).

Réduction de la compétition intra- et inter-spécifique

La dispersion par les fourmis peut entrainer les graines loin des plante-mères, réduisant ainsi la compétition entre plantules (Westoby & Rice 1981, Boyd 1996, Kalisz et al. 1999). Les fourmis peuvent aussi amener les graines vers des sites où la compétition avec d'autres espèces est réduite. C'est le cas par exemple de *Carex pedunculata* qui ne pousse pas bien en présence de *C. platyphylla* et *C. plantaginea*. Les fourmis transportent les graines vers des lieux où ces dernières sont absentes (Handel 1976, 1978, Boyd 1996).

La distance de dispersion

En règle générale, les fourmis sont considérées comme des vecteurs de dispersion à courte distance, avec une distance moyenne d'environ un mètre (Gomez & Espadaler 1998b) (Fig. 2).

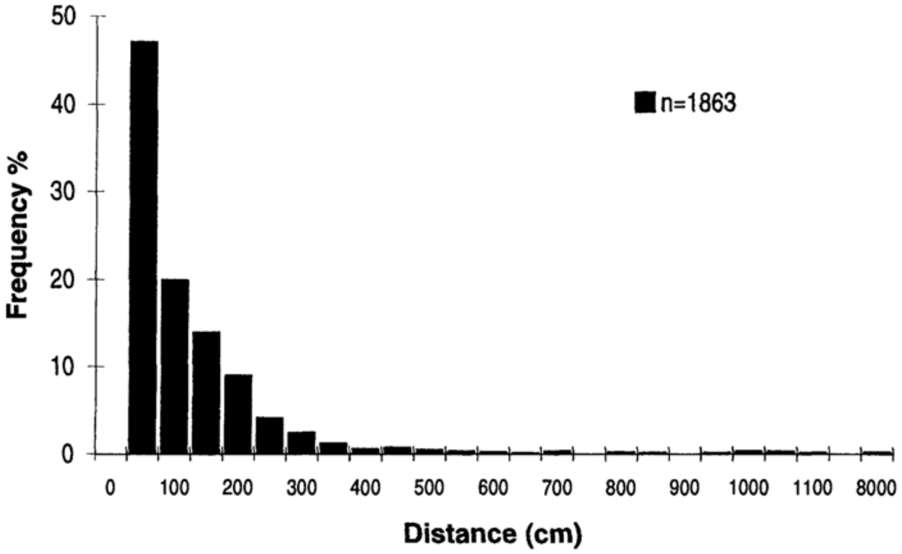

Figure 2. Courbe de dispersion par les fourmis regroupant toutes les données disponibles, tous écosystèmes confondus (Gomez & Espadaler 1998b)

Les fourmis de grande taille transportent les graines à plus grande distance que les fourmis de petite taille (Pudlo et al. 1980, Hughes & Westoby 1992, Gomez & Espadaler 1998a, Ness et al. 2004). La distance maximale de dispersion jamais mesurée est de 180 m en Australie et concerne les grandes fourmis carnivores du genre *Iridomyrmex* (Whitney 2002). Mais le plus souvent, les distances sont de l'ordre de quelques mètres (Winkler & Heinken 2007). Les fourmis peuvent néanmoins représenter un avantage en termes de distances de dispersion par rapport à la simple dispersion par gravité (e. g. Andersen 1988), facilitant la colonisation de nouveaux milieux. Sur 17 études, 13 études ont confirmé cette hypothèse, une l'a infirmée, trois n'ont pas été concluantes (Giladi 2006).

La protection contre le feu

Les graines qui sont transportées par les fourmis dans leurs nids survivent aux incendies. Ce phénomène a été montré surtout en Australie et en Afrique du Sud où les feux sont fréquents. En outre, les graines subissent alors dans le sol des températures qui faciliteront le déclenchement de la germination (Berg 1981, Majer 1982, Bond 1983).

Dépôt dans des microsites enrichis en nutriments

Même si les fourmis ont tendance à nettoyer les graines qui germent dans leur nid et autour de l'entrée du nid (Beattie 1985), les sols des nids de fourmis sont souvent enrichis par rapport au substrat où les nids se trouvent (Oostermeijer 1989, Dostal et al. 2005). De la même manière, les piles de déchets à l'extérieur des nids sont aussi des microsites enrichis en nutriments. En abandonnant les graines dans ces sols enrichis, les fourmis favorisent donc la germination et la croissance des plantules (Culver & Beattie 1980, Davidson & Morton 1981, Beattie & Culver 1983, Hanzawa et al. 1988, Passos & Oliveira 2002). Mais cette hypothèse a été infirmée dans de nombreux cas (Horvitz & Schemske 1986a, Mossop 1989, Higashi 1989, Bond & Stock 1989).

Favoriser la germination

Le traitement des graines par les fourmis (scarification et arrachage de l'élaiosome) peut dans certains cas lever une dormance (Horvitz 1981, Pacini 1990, Ohkawara 2005) ou augmenter le taux de germination (Culver & Beattie, 1980, Gibson 1993, Horvitz & Schemske 1994, Gomez et al. 2003). Cependant, dans certains cas, ces scarifications peuvent n'avoir aucun effet sur la germination (Kaufmann et al. 1991, Lobstein & Rockwood 1993, Boyd 2001) ou même avoir un effet négatif, c'est-à-dire une baisse du taux de germination (Bond & Slingsby 1984, Dalling & Wirth 1998, Zettler et al. *2*001a).

En résumé, les fourmis ne transportent pas les graines sur de grandes distances, mais peuvent les emmener dans des endroits favorables (hors des prédateurs ou du feu, dans des microsites enrichis, etc.). Il est à noter que les pressions de sélection sont très différentes pour chaque habitat et d'une année à l'autre (Kalisz et al. 1999). L'effet de la dispersion par les fourmis pour les plantes peut donc être très variable. Pour certains cas, la dispersion par les fourmis peut même s'avérer négative en termes de distance, de germination ou de survie.

Les avantages pour les fourmis

Les études portant sur les avantages que tirent les fourmis de la myrmécochorie sont peu nombreuses. Comme c'est souvent le cas dans les recherches sur les mutualismes animaux-plantes, il y a une asymétrie entre la connaissance que nous avons de l'écologie évolutive de chaque partenaire ; les plantes sont toujours beaucoup plus étudiées que les partenaires animaux (Cushman & Beattie 1991, Bronstein 1994). Alors que plus de 200 articles nous renseignent sur les avantages pour les plantes, seules six études ont tenté de mesurer les bénéfices pour les fourmis, mais les résultats sont loin d'être clairs (Morales & Heithaus 1998, Bono & Heithaus 2002, Fischer et al. 2005, Gammans et al. 2005, Marussich 2006, Fokuhl et al. 2007). Par exemple, l'apport alimentaire d'élaiosome peut biaiser le sex-ratio de la fourmi *Aphaenogaster rudis* en faveur des femelles en laboratoire mais en faveur des mâles sur le terrain (Bono & Heithaus 2002), alors qu'ils n'ont aucun effet sur le sex-ratio de la fourmi *Myrmica ruginodis* (Gammans et al. 2005).

Quels traits des graines influencent le comportement des fourmis ?

Même si cela semble évident, la présence d'un élaiosome est le principal facteur expliquant la prise de graines par les fourmis (Auld 1986, O'Dowd & Hay 1980, Mesler & Lu 1983, Hanzawa et al. 1985, Kjellsson 1985a, Ohara & Higashi 1987, Pemberton 1988, Oostermeijer, 1989, Hughes & Westoby 1992, Gibson 1993, Espadaler & Gomez 1997, Auld & Denham 1999, Carney et al. 2003). Cependant, les diaspores n'ont pas toutes le même pouvoir attractif. Plusieurs facteurs sont à l'origine de ces différences. Nous les évoquons brièvement dans cette section.

Caractéristiques physiques des graines et des élaiosomes

De manière générale, les graines de plus de 100 mg ont tendance à être adaptées à la dispersion par les vertébrés et les graines de moins de 0.1mg ont tendance être dispersées par le vent ou l'eau (Hughes et al. 1994b). Entre 0.1 et 100 mg (la majorité des graines), tous les modes de dispersion se retrouvent.

Il est logique de penser que si les fourmis recherchent un apport nutritionnel, elles devraient préférer les graines avec un élaiosome plus grand (ou lourd). Mais le transport ayant un coût pour les fourmis, l'avantage serait donné aux graines avec une plus petite diaspore. Il a été effectivement montré que les taux de prises augmentent avec le ratio entre l'élaiosome et la taille de la graine (Gunther & Lanza, 1989, Hughes & Westoby, 1992b, Bas el al., 2007, Rey & Manzaneda 2007). Mais d'autres études ont trouvé que les taux de prises augmentaient simplement avec la taille de la graine (Gorb & Gorb, 1995, 2000) ou la taille de l'élaiosome (Oostermeijer, 1989, Mark & Olesen, 1996).

Dans l'hémisphère nord, les élaiosomes sont typiquement mous et perdent leur attractivité quelques jours après l'ouverture des capsules (Hanzawa et al. 1985, Kjellsson 1985, Smith et al. 1986, Mark & Olesen 1996, Guitián et al. 2003). Ce phénomène est très probablement dû à la dessiccation des graines et à l'évaporation des composés chimiques attractifs.

L'élaiosome joue également le rôle d'une « poignée » de transport. Les fourmis ont des difficultés à prendre les graines sans élaiosome, sans poils, sans prises (O'Dowd & Hay 1980, Horvitz 1981, Oostermeijer 1989, Byrne & Levey 1993, Gomez et al. 2005). Enfin, de manière anecdotique, sur des graines d'*Acacia ligulata*, les fourmis ne font pas la différence de couleur entre les graines, alors que les oiseaux la font (Whitney 2005).

La composition chimique

La première étude à analyser la composition chimique des élaiosomes (Szemes 1943 avec *Chelidonium majus*) montre que la fonction d'attracteur ne provient pas seulement des matières grasses, mais aussi d'autres tissus contenant de l'amidon ou des protéines (Szemes 1943). Plus tard, Bresinsky (1963) confirme que les élaiosomes sont des aliments très complets pour les fourmis. Ils contiennent des sucres, des protéines, des acides aminés, des lipides et des vitamines, dont les proportions varient suivant les espèces. Il affirme que l'acide ricinoléique est le composé responsable de l'attractivité des élaiosomes, ce qui a été démenti à plusieurs reprises par la suite (Marshall et al. 1979, Skidmore & Heithaus 1988, Kusmenoglu et al. 1989).

Plusieurs études s'accordent à dire que les comportements de prises des fourmis sont pour la plupart déclenchés par trois familles de lipides (acides gras libres, diglycérides et triglycérides) (Marshall et al. 1979, Skidmore & Heithaus 1981, Brew et al. 1989, Hughes et al. 1994, Boulay et al. 2006), et plus précisément sous la forme de diglycéride 1,2 diolein[4] (*Viola odorata,* Marshall et al. 1979, *Hepatica americana*, Skidmore & Heithaus 1988, *Jeffersonia diphylla*, Kusmenoglu et al. 1989, *Acacia myrtifolia* & *Tetratheca stenocarpa*, Brew et al. 1989). Cependant, malgré les preuves de l'efficacité de certains composés sur certaines espèces de fourmis, il est difficile d'établir une règle universelle simple. La base chimique de cette

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[4] Le diglycéride est composé de deux acides gras simples. Dans notre cas, l' 1,2 diolein est composé de deux acides oléique (2 acide oléique + glycérol □ > 1,2 diolein + H_2O)

attraction n'est pas seulement due aux teneurs en acide oléique, mais à un large spectre de composés (Midgley & Bond 1995). En réalité, les élaiosomes varient considérablement de composition chimique (Skidmore & Heithaus 1988, Kusmenoglu et al. 1989, Boulay et al. 2006). Certaines espèces sympatriques possèdent des taux différents d'acides aminés ou de lipides (Beattie 1985, Brew et al. 1989, Lanza et al. 1992). Il peut même y avoir des variations géographiques de la teneur en acide oléique au sein d'une même espèce, ce qui induit des comportements différents (Boulay et al. 2007). Parfois, les acides aminés peuvent être attractifs (Brew et al. 1989). Mais le plus souvent, c'est la partie lipidique des élaiosomes qui est responsable de la prise, et sur certaines espèces, les élaiosomes contiennent jusqu'à 34 % de lipides, (dont 60% d'acide oléique) (Hocking & Kortt 1987).

Par ailleurs, certains composés sont essentiels à l'alimentation d'une fourmi car leur métabolisme ne permet pas de les synthétiser. L'acide oléique est l'acide gras le plus abondant dans les tissus végétaux et animaux et c'est aussi le précurseur de la synthèse de l'acide linoleique et linolénique (Christie 2005, *cité par* Fischer et al. 2008). Ces deux derniers composés sont des nutriments essentiels qui ne peuvent pas être synthétisés par les hyménoptères (Hagen et al. 1984, Barbehenn et al. 1999, *cités par* Fischer et al. 2008). C'est par exemple le cas des stérols (Canavoso et al. 2001) ou de l'acide linolénique (Soukup & Holman 1987 ; Lanza et al. 1992). Il a été montré que les élaiosomes de deux espèces d'*Ulex* sp. contenaient des stérols (Gammans et al. 2006) et que neuf espèces du genre *Trillium* sp. contenaient de l'acide linolénique, lui-même attractif (Soukup & Holman 1987). Par contre, Brew et collègues excluent cette hypothèse car dans leurs expériences, la fraction la plus attractive (acides gras insaturés) n'a rien à voir avec ces composés essentiels (Brew et al. 1989).

La phénologie

Dans les régions méditerranéennes, certaines plantes myrmécochores mettent leurs graines à disposition des fourmis le matin, lorsque l'activité des fourmis est au plus haut (Espadaler & Gomez 1996, Narbona et al. 2005). Ce phénomène a été observé également dans d'autres régions tempérées. Par exemple la capsule contenant les graines de la plante *Viola nuttallii* s'ouvre le matin, ce qui correspond au pic d'activité des fourmis dispersatrices *Myrmica discontinua* et *Formica podzolica*, et qui donne le temps aux fourmis de prendre les graines avant que les rongeurs ou les coléoptères nocturnes ne le fassent (Turnbull & Culver 1983). Ainsi, la synchronisation entre la disponibilité des graines et l'activité des agents dispersateurs constituerait une pré-adaptation essentielle pour l'évolution des mutualismes plantes-animaux (Gibson 1993, Ruhren & Dudash 1996, Ness & Bressmer 2005, Ohara & Kawano 2006, Boulay et al. 2007). Cependant, il y a encore peu de preuves que cette corrélation phénologique soit issue d'une pression de sélection. Il se pourrait que les deux partenaires aient subi une pression de sélection abiotique indépendante (Boulay et al. 2007).

Par ailleurs, on observe que la majorité des plantes myrmécochores libèrent leurs graines plus précocement dans la saison que les autres plantes. Plusieurs facteurs peuvent expliquer cette précocité : les graines précoces sortent à une période où les fourmis ne disposent pas de beaucoup de ressources alimentaires alternatives (printemps) et où les besoins de la colonie sont élevés (sortie de l'hiver) (Thompson 1981, Kjellsson 1985a, Ohkawara et al. 1997, Oberrath & Boehning-Gaese 2002, Guitián & Garrido 2006). Cependant, cette précocité n'est pas une règle stricte et certaines plantes myrmécochores fructifient encore en été et même en automne.

La structure des communautés de plantes et densité des graines

Les taux de prises peuvent aussi être influencés par la taille de la population de plantes myrmécochores. Par exemple, les fourmis préfèrent les graines de *Sanguinaria canadensis* à celles de *Asarum canadense* lorsqu'elles sont placées dans une population d'*Asarum* (Heithaus 1986); les graines d'*Asarum canadense* sont préférées dans les sites à population dense de *Jeffersonia diphylla*, et inversément (Smith et al. 1989b); et enfin, les taux de prises des graines d'*Hepatica acutiloba* et *Trillium nivale* sont plus faibles dans des population denses que dans des populations éparses (Smith et al. 1989a).

Le regroupement des graines peut influencer les taux de prises. Une graine augmente sa probabilité d'être déplacée lorsqu'elle se trouve à proximité d'une autre graine (Bulow-Olsen 1984, Gorb & Gorb 2000). Mais le résultat inverse a été observé en Australie (Andersen & Ashton 1985), et, une autre étude n'a pas montré d'influence significative de la densité des graines à la source sur les taux de prises (Smith et al. 1986).

Objectifs et méthodes

Ce travail de recherche est divisé en deux parties. La première est une approche expérimentale où nous nous sommes intéressés au détail des comportements des fourmis à toutes les étapes du processus de dispersion : à la source, dans le nid, et après le passage des graines dans le nid. La deuxième partie est un travail de synthèse comprenant une partie bibliographique offrant un point de vue original sur le sujet et d'autre part une analyse comparative de données écologiques des principales espèces de plantes d'Europe du Nord impliquées dans la myrmécochorie.

Partie 1 - Expérimentation

Au sein d'un même habitat, le réseau d'interactions entre graines et fourmis est complexe. Comme signalé plus haut dans le texte, une espèce de fourmis peut disperser des graines de plusieurs espèces de plantes et inversement, une espèce de plante peut être dispersée par plusieurs espèces de fourmis. Mais ces relations ne sont pas toutes égales : certaines fourmis préfèrent les graines de telle ou telle plante (Hughes & Westoby 1992, Gove et al. 2007, Ness & Morin 2008). Ceci entraine d'importantes différences dans les taux de prises et finalement dans le devenir de la guilde des plantes myrmécochores. Un des principaux défis de l'étude de la dispersion des graines est de comprendre pourquoi et comment les différentes espèces de plantes reçoivent des traitements variables par les fourmis. Par ailleurs, des travaux récents ont mis en évidence les variabilités temporelles et géographiques des interactions myrmécochoriques (Garrido et al 2002, Boulay et al. 2007, Manzaneda et al. 2007). Enfin, pour ajouter une dimension à la complexité du tableau, les résultats de chaque interaction graine-fourmi en termes d'avantages sélectifs pour les deux partenaires sont compris dans un continuum entre antagonisme et mutualisme (i.e. la relation n'est pas toujours avantageuse pour chaque partenaire). La recherche des conditions favorables à un bénéfice mutuel des partenaires est sans aucun doute l'un des défis majeurs de l'étude de l'évolution des réseaux d'interactions mutualistes, tels que la dispersion des graines ou la pollinisation (Thompson 1994, Rico-Gray & Oliveira 2007).

Ainsi, la myrmécochorie est un système plante-fourmi complexe et multidimensionnel où les sources de variabilité sont nombreuses. Jusqu'à présent, une imposante majorité des travaux portant sur la myrmécochorie ont pris comme modèle d'étude une espèce de fourmi ou de plante, interagissant avec un ou plusieurs partenaires. Des récents travaux (Giladi 2006, Boulay et al. 2007) incitent à entreprendre des études sur plusieurs espèces *simultanément*, voire sur des guildes entières, afin de comprendre les tendances et les schémas généraux de ces réseaux complexes d'interactions. Dans cette première partie, nous avons donc travaillé avec quatre espèces simultanément : deux espèces de fourmis (*Lasius niger* et *Myrmica rubra*) auxquelles on a présenté des graines de deux espèces de plantes myrmécochores (*Viola odorata* et *Chelidonium majus*). La combinaison des quatre espèces a donné quatre « paires » qui permettent de mesurer l'influence de chaque partenaire sur le résultat de l'interaction.

Afin de contrôler un maximum de paramètres abiotiques (température, humidité, nourriture alternative) et biotiques (taille et âge des colonies, interactions avec d'autres espèces, âge et dessiccation des graines), nous avons choisi de travailler au laboratoire. Le fait de ne pas travailler en conditions naturelles peut être pénalisant pour certaines questions écologiques et il est nécessaire d'interpréter les données avec précaution. Néanmoins, cette approche en laboratoire d'un phénomène écologique (qui a été rarement entreprise pour l'étude de la myrmécochorie) permet une plus grande précision des mesures de comportement, et offre la possibilité d'observer l'intérieur du nid. Enfin, elle permet l'étude de paramètres très difficilement contrôlables sur le terrain tels que la dessiccation des graines.

Pour résumer, la combinaison d'une approche multi-spécifique en laboratoire permet la mesure précise de l'influence de chaque partenaire (fourmi ou plante) sur les résultats des interactions.

Les questions

A. Le comportement des fourmis à la source de graines

Q1 : Les fourmis sont-elles attirées à distance par les graines ? **(Chap. 1)**

Q2 : Comment la présence de l'élaiosome influence les comportements des fourmis à la source ? **(Chap. 1)**

Q3 : Quelle est l'influence de chaque partenaire sur les dynamiques de prises ? **(Chap. 1)**

Q4 : Quelle est l'effet de la dessiccation et de la réhydratation des diaspores sur les taux de prises ? **(Chap. 3)**

B. Le comportement des fourmis dans le nid

Q5 : Quelles sont les dynamiques de rejet des graines et d'arrachage de l'élaiosome ? **(Chap. 2)**

Q6 : Les graines sont-elles localisées près du couvain ? **(Chap. 2)**

Q7 : Quelle est l'influence de l'élaiosome sur le rejet des diaspores ? **(Chap. 2)**

Q8 : Quelle est l'influence de chaque partenaire sur les interactions ? **(Chap. 2)**

C. Le devenir des graines après le nid

Q9 : Quelle est la composition des rejets (présence/absence d'élaiosome) ? **(Chap. 1 et 2)**

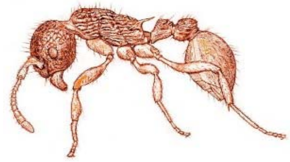
Q10 : Quel est le lien entre la composition des rejets et la dynamique de traitement des graines dans le nid ? **(Chap. 2)**

Chaque chapitre est un article soumis à une revue internationale à comité de lecture. Ils contiennent donc chacun une section « matériels et méthodes », des résultats et une discussion. Les dispositifs expérimentaux des trois articles ont des points communs décrits ci-dessous, mais tous les détails méthodologiques sont décrits en détail dans le corps de chaque chapitre.

Les fourmis

Les deux espèces de fourmis *Lasius niger* et *Myrmica rubra* ne sont pas granivores. Elles ont été par ailleurs observées comme dispersatrices de graines myrmécochores (Sernander 1906, Bülow-Olsen 1984, Gorb & Gorb 2000, Oberrath & Böhning-Gaese 2002). Elles sont même parmi les espèces les plus utilisées dans l'étude, ce qui leur confère le statut d'espèce standard pour les futures études de la myrmécochorie des régions tempérées d'Europe. Les deux fourmis sont omnivores, mais diffèrent cependant par leurs préférences alimentaires, à tendance plus carnivore pour *Myrmica rubra* et éleveuse de pucerons pour *Lasius niger*.

Lasius niger (L.)



Myrmica rubra L.

Les colonies de la fourmi noire des jardins *Lasius niger* (L.) sont monogynes et peuvent contenir jusqu'à 13000 ouvrières (Stradling 1970). Ces ouvrières mesurent de 3 à 4 mm de longueur et se nourrissent principalement de miellat de pucerons (Pontin 1958). Elle peut cependant être considérée comme omnivore car elle mange occasionnellement ces mêmes pucerons (Pontin 1958, Offenberg 2001), des insectes morts ou vivants (Pontin 1961) ou des élaiosomes (Sernander 1906, Ohkawara & Higashi 1994). On la trouve à la fois dans les habitats ouverts et les forêts (Oberrath & Böhning-Gaese 2002).

La fourmi rouge *Myrmica rubra* L. mesure de 4 à 6 mm de longueur, forme des colonies polygynes qui peuvent atteindre jusqu'à 6000 ouvrières et plus de 50 reines (Elmes 1973). C'est une fourmi ubiquiste et prédatrice de petites proies au corps mou, qui récolte également les petites proies mortes (Le Roux et al. 2002). Bien qu'elle soit considérée comme carnivore, elle peut aussi occasionnellement consommer des solutions sucrées (miellat de pucerons, nectar) (Cammaerts 1977) ou des élaiosomes (Bülow-Olsen 1984, Gorb & Gorb 2000).

Collecte et élevage

Les colonies ont été collectées à Bruxelles (B-1050) et La Gorgue (F-59). Au laboratoire, les colonies ont été placées dans des nids en plâtre (Type Janet, 0,5 cm d'épaisseur). Les parois des bacs en plastique contenant la colonie (50 x 38 x 10 cm) sont tapissées de Fluon pour éviter que les fourmis ne s'enfuient. Les nids étaient régulièrement humidifiés, gardés à 21 ± 2 °C, 35 ± 5 % d'humidité relative et une photopériode constante de 12h par jour. Les nids ont été recouverts d'une plaque de verre recouverte d'un film de plastique transparent rouge à travers laquelle les fourmis pouvaient être observées. Toutes les colonies contenaient entre 1000 et 1500 ouvrières et approximativement la même quantité de couvain (environ 10-15% de la surface du nid). Les colonies de *Lasius niger* étaient sans reine et les colonies de *Myrmica rubra* contenaient de 5 à 10 reines. Les fourmis disposaient d'eau et d'une solution sucrée 1M *ad libitum*, de blattes (*Periplaneta americana*) et de vers de farine (*Tenebrio molitor*) deux fois par semaine et d'une gelée artificielle contenant protéines, sucres et vitamines (Bathkar & Withcomb, 1970).

Les plantes

Les deux espèces de plantes *Viola odorata* et *Chelidonium majus* sont communes dans nos régions et sont considérées comme myrmécochores. Les graines diffèrent par leur taille, couleur et probablement leur composition chimique (cf Annexe).

Viola odorata L.

Chelidonium majus L.

Viola odorata L. est une plante pérenne des forêts tempérées, haies et habitats ouverts et rudéraux (Lambinon et al. 1992), très répandue en Belgique, et capable de reproduction végétative par stolons. Elle a une floraison précoce au printemps (Février-Mars) et quelque fois en été (Aout-Septembre). Les graines peuvent être trouvées de mai à octobre. Les tiges des infrutescences se penchent jusqu'au sol et les capsules s'ouvrent sans exploser, montrant une adaptation à la dispersion par les fourmis (Beattie & Culver 1981). Cette plante est considérée comme une plante myrmécochore stricte, c'est-à-dire dont les graines sont dispersées uniquement par les fourmis (sans phase d'autochorie). Les graines sont jaune-brun, mesurent 3.34 ± 0.21mm de longueur et 2.24 ± 0.14 mm de large (n=10). Les élaiosomes sont blancs, de forme cônique, plus petits que les graines : ils mesurent 2.01 ± 0.25mm de longueur et 1.65 ± 0.16mm de large (n=10) et proviennent principalement du tissu du raphe (Sernander 1906, Bresinsky 1963). Le poids moyen des graines est de 4.231 mg (n=10) et de l'élaiosome est de 0.772 mg (n=10), ce qui donne un rapport élaiosome/graine de 0.18. Les élaiosomes sont classés dans le premier groupe (« mou avec des larges cellules ») parmi les trois groupes de la classification de Mayer et al. (2005)[5].

Chelidonium majus L. est une plante pérenne d'habitats ouverts et rudéraux comme des déblais, des bords de route ou des vieux murs (Lambinon et al. 1992), très répandue en Belgique. Elle est incapable de reproduction végétative et a une floraison tardive (Mai-

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[5] Les trois groupes sont: (1) « *mou avec des larges cellules* » qui contiennent beaucoup d'eau, se dessèchent rapidement en perdant leur forme originale ; (2) « *mous avec des petites ou moyennes cellules* » qui contiennent peu d'eau et ne perdent pas leur forme originale après dessiccation; (3) « *durs* » avec une proéminente cuticule qui protège une fine couche de cellules qui ne perdent pas leur forme originale (Mayer et al. 2005).

Novembre). Sa période de fructification est très étendue et sa production de graines constante (Juin-Novembre). C'est une plante diplochore, c'est à dire comprenant deux phases : la première autochore (ballistique) où les graines sont éparpillées autour de la plante-mère et la deuxième est myrmécochore. Les graines sont brunes foncées et petites (1.95 ± 0.06mm de longueur et 1.43 ± 0.11mm de large, n=10). Les élaiosomes sont blancs et mous, ils ont la forme d'une larve et mesurent 1.66 ± 0.06mm de longueur et 0.81 ± 0.12mm de large (n=10) et provenant du tissu du raphe (Sernander 1906, Bresinsky 1963). Le poids moyen des graines est de 0.985 mg (n=10) et de l'élaiosome est de 0.309 mg (n=10), ce qui donne un rapport élaiosome/graine de 0.31.

La partie expérimentale est constituée des chapitres 1, 2 & 3 du manuscrit, chacun contenant une introduction spécifique, une méthodologie détaillée, les résultats et une discussion et une bibliographie spécifique. Dans la section qui suit, nous dressons les objectifs de la deuxième partie de la thèse, c'est-à-dire une étude comparative rassemblant une synthèse bibliographique originale et une analyse d'écologie comparative.

Partie 2 – Synthèse

Les fourmis : un agent dispersateur efficace ? (Chapitre 4)

Depuis l'imposante monographie de Sernander (1906), considérée comme le point de départ de l'étude scientifique de la myrmécochorie, près de 300 articles de recherche ont été publiés sur le sujet (Fig. 3), ainsi qu'une dizaine de synthèses traitant exclusivement du sujet. L'étude moderne de la myrmécochorie a démarré grâce aux travaux pionniers du botaniste Andrew Beattie à la fin des années 70, et en particulier grâce à deux synthèses (Beattie 1983, 1985), très citées par la suite. Ces synthèses, centrées sur la myrmécochorie en milieux tempérés, résument pour la première fois les différents avantages sélectifs de la myrmécochorie pour la plante (cf. p. 9), dressent le bilan des principaux genres de fourmis et de plantes impliquées, et enfin décrivent les caractéristiques des élaiosomes et la diversité géographique et taxonomique (au niveau générique) des plantes myrmécochores (Tableau 2).

La vision de botaniste d'Andrew Beattie (centrée sur la plante) a considérablement influencé les questions abordées dans les études et synthèses ultérieures (e. g., Bennett and Krebs 1987, Mayer et al. 2005, Giladi 2006, Rico-Gray & Oliveira 2007) qui ont prioritairement testé les hypothèses concernant les avantages sélectifs des plantes (plus d'une centaine d'articles) plutôt que mesurer les bénéfices pour les fourmis (six articles). On peut également souligner que certaines synthèses ont abordé d'autres aspects de la myrmécochorie, en particulier l'évolution de la myrmécochorie ou l'importance de la myrmécochorie en tant que dispersion secondaire en forêt tropicale (Tableau 2). Enfin, d'autres synthèses ont fait le point sur des habitats particuliers, comme l'Australie (Berg 1981), l'Afrique du Sud (Bond et al. 1991), les forêts brésiliennes (Pizo 2005) et les régions tempérées d'Europe du Nord (Mayer et al. 2005).

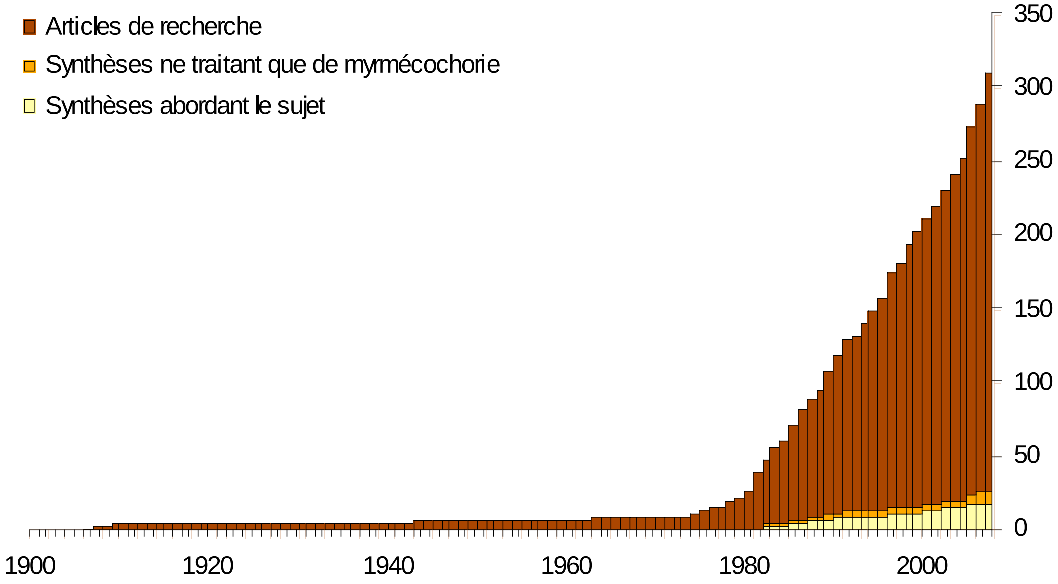

Figure 3. Nombre cumulé de travaux publiés sur la myrmécochorie depuis un siècle.

Du point de vue des fourmis, la synthèse pionnière de Beattie (1983) a recensé les principaux genres impliqués et souligné que le régime alimentaire des fourmis dispersatrices pouvait être omnivore, carnivore ou granivore. Depuis, seul un article a tenté une analyse globale en recensant tous les genres de fourmis concernés (47 genres et 200 espèces) mais sans trouver de trait taxonomique ou géographique caractéristique (Gomez & Espadaler 1997, en catalan).

Très récemment, une imposante mise à jour de l'ouvrage majeur de Beattie (1985) sur l'écologie et l'évolution des interactions plantes-fourmis est parue (Rico-Gray & Oliveira 2007). Outre la mise à jour des thèmes récurrents, les auteurs apportent une contribution originale en soulignant l'importance de la myrmécochorie en tant que dispersion secondaire en forêt tropicale, et tentent pour la première fois de placer la myrmécochorie dans le cadre conceptuel de la Mosaïque Géographique de la Coévolution (Thompson 1982, 1994, 2005). En perspective, à la fin du chapitre sur la myrmécochorie (p 62), ils suggèrent que « *des futures recherches devraient se concentrer sur l'étude des composantes qualitatives des fourmis en tant que dispersatrices* », introduisant l'idée que « *la présence d'organisations coloniales particulières, de traits morphologiques ou comportementaux est clairement nécessaire à une espèce de fourmis pour être considérée comme une dispersatrice effective* ». La recherche de ces traits caractéristiques des fourmis, et de leur contribution à la dispersion des graines, est le but de ce chapitre. De ce fait, nous tenterons de comprendre pourquoi les fourmis sont les seuls insectes (ou presque) à disperser des graines.

Tableau 2. Thèmes abordés dans les synthèses sur la myrmécochorie.

Thèmes abordés dans les synthèses	Références
Avantages sélectifs pour les plantes	Beattie 1983, Beattie 1985, Bennett and Krebs 1987, Beattie & Hughes 2002, Mayer et al. 2005; Giladi 2006, Rico-Gray & Oliveira 2007
Distribution géographique mondiale	Beattie 1983, Beattie 1985, Beattie & Hughes 2002, Rico-Gray & Oliveira 2007
Diversité taxonomique des plantes	Beattie 1983, Beattie 1985, Beattie & Hughes 2002
Diversité taxonomique des fourmis	Beattie 1983, Gomez & Espadaler 1997
Caractéristiques des élaiosomes	Beattie 1983, Beattie 1985, Beattie & Hughes 2002, Gorb & Gorb 2003, Mayer et al. 2005
Evolution de la myrmécochorie	Beattie 1985, Giladi 2006, Bronstein et al. 2006, Rico-Gray & Oliveira 2007
L'importance de la myrmécochorie en tant que dispersion secondaire en forêt tropicale	Pizo 2005, Rico-Gray & Oliveira 2007

La recherche bibliographique a demandé un effort considérable. Alors que le moteur de recherche ISI Web of Science recense 152 articles à l'entrée « myrmecochory » (entre 1987 et 2008), j'ai trouvé 308 articles de recherche (dont 228 entre 1987 et 2008) et 9 articles de synthèses traitant exclusivement du sujet, ainsi que 3 livres majeurs. Une analyse de cette bibliographie a permis dans un premier temps de trier les articles contenant des données pertinentes sur les fourmis dispersatrices et dans un deuxième temps de définir les principaux traits des fourmis (i.e. la structure des chapitres), qui ont ensuite fait l'objet d'une recherche bibliographique élargie.

Quelles espèces de plantes sont impliquées dans la myrmécochorie en Europe tempérée ? (Chapitre 5)

Même si l'Europe a été la première zone d'étude de la myrmécochorie (Sernander 1906) et si les habitats tempérés (Europe du Nord et centrale) ont fait l'objet d'un nombre d'études non négligeable (54), il n'existe pas de liste d'espèces de plantes ni de fourmis impliquées dans ce phénomène. Cependant, quelques espèces de plantes et de fourmis ont souvent servi de modèles biologiques. Ainsi, pour les fourmis, les genres *Lasius*, *Myrmica* et *Formica* sont les plus représentés (Beattie 1983) et parmi eux les espèces *Lasius niger, L. fuliginosus, Myrmica rubra, M. ruginodis, Formica rufa, F. excecta* et *F. polyctena* sont les plus souvent utilisées

(Sernander 1906, Beattie 1983, Peters et al. 2003, Gorb & Gorb 2003, Fischer et al 2005). Pour les plantes, certaines espèces sont souvent utilisées, comme *Viola odorata* probablement à cause de la taille de son élaiosome et des travaux fondateurs de Beattie (Beattie & Lyons 1975, Culver & Beattie 1978, Beattie 1978, 1979). La chélidoine a aussi été prise comme modèle de référence probablement en raison de l'abondance de ses graines et de l'attractivité remarquable qu'elle exerce sur les fourmis (Oberrath & Boehning-Gaese 2002, Peters et al. 2003, Gorb & Gorb 2003, Prinzing et al. 2007, 2008).

Le premier but de ce chapitre est donc de dresser une liste des espèces de plantes impliquées dans la myrmécochorie des régions d'Europe tempérée. Ensuite, à cette liste seront associées des données qualitatives et quantitatives sur ces espèces pour tenter de dégager des tendances ou des traits caractéristiques des plantes myrmécochores par rapport à la flore totale de ces régions.

La liste des espèces de plantes myrmécochores provient de la compilation des deux bases de données Biolflor[6] © et Grime et al. (2005)[7], ainsi que la monographie de Sernander (1906) et certains articles parmi les 54 articles ayant étudié la myrmécochorie dans nos régions. Une fois la liste dressée, des données sur la taille des graines, le mode de reproduction, le stock grainier, etc. issues de la base de données *Biolflor* ont été analysées.

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[6] Klotz *et al.*, 2002. '*BiolFlor', a database on biological and ecological traits of the vascular flora of Germany*. UFZ, Centre for Environmental Research Leipzig-Halle, Dept. Community Ecology, Version 1.1 (http://www.ufz.de/biolflor/index.jsp). Cette flore couvre la majorité des espèces d'Europe Centrale (3660 espèces).

[7] Grime JP, Hodgson JG, and Hunt R. 2005. *Comparative plant ecology. A Functional Approach to Common British Plant Species*. Castlepoint Press.

Chapitre 1

Ant-seed interactions: combined effects of ant and plant species on seed removal patterns

Abstract

Seed dispersal by ants (i.e. myrmecochory) is usually considered as a mutualism: ants feed on nutritive bodies, called elaiosomes, before rejecting and dispersing seeds in their nest surroundings. While mechanisms of plant dispersal in the field are well documented, the behaviour of the ant partner was rarely investigated in details. Here, we compared in laboratory conditions the foraging behaviour of two ant species, the omnivorous *Lasius niger* and the insectivorous *Myrmica rubra* to which was given seeds of two European myrmecochorous plants (*Chelidonium majus* and *Viola odorata*). Ant colonies were simultaneously presented three types of items: entire seeds with elaiosome (SE), seeds without elaiosome (S) and detached elaiosomes (E). The presence of elaiosomes on seeds did not attract workers from a distance since ants first contact equally each type of items. Although ants are mass-recruiting species, we never observed any recruitment nor trail-laying behaviour towards seeds. For ants having contacted seed items, their antennation, manipulation and seed retrieval behaviour strongly varied depending on the species of each partner. Antennation behaviour, followed by a loss of contact, was the most frequent ant-seed interaction and can be considered as a "hesitation" clue. For both plant species, insectivorous *Myrmica* ants removed items in larger number and at higher speed than *Lasius*. This fits with the hypothesis of a convergence between odours of elaiosomes and insect preys. For both ant species, the small *Chelidonium* seeds were retrieved in higher proportion than *Viola* ones, confirming the hypothesis that ants prefer the higher elaiosome/diaspore-ratio. Thus, in these crossed experiments, the ant-plant pair *Myrmica/Chelidonium* was the most effective as ants removed quickly almost all items after a few antennations. The presence of an elaiosome body increased the seed removal by ants excepting for *Myrmica* which retrieved all *Chelidonium* seeds, even those deprived of their elaiosome. After 24h, all the retrieved seeds were rejected out of the nest to the refuse piles. In at least half of these rejected items, the elaiosome was discarded by ants. Species-specific patterns and behavioural differences in the dynamics of myrmecochory are discussed at the light of ant ecology.

Keywords: Myrmecochory, elaiosome, *Chelidonium majus*, *Viola odorata*, *Myrmica rubra*, *Lasius niger*.

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Introduction

Myrmecochory, or seed dispersal by ants, is a worldwide mechanism involving more than 3,000 plant species (Beattie and Hughes, 2002) and hundreds of ant species across many ecosystems such as dry sclerophyll woodlands of Australia (Berg, 1975), sclerophyll shrubs in south Africa (Bond and Slingsby, 1983), tropical regions (Horvitz and Beattie, 1980) or temperate deciduous forests of Europe (Sernander, 1906) and North America (Culver and Beattie, 1978). Generally, ants remove intact diaspores, bring them back to the nest, eat the nutrient-body appendage called elaiosome and reject seeds within or outside the nest.

Depending on each ecosystem, many benefits are listed for plants and can be multiple: directed dispersal towards nutrient-enriched microsites (Hanzawa et al., 1988; Passos and Oliveira, 2002), reduction of parent-offspring or sibling competition (Higashi et al 1989; Boyd, 2001), predator avoidance (Heithaus, 1981; Ohkawara et al., 1997) or fire avoidance as seeds are quickly retrieved within the ant nest (Berg, 1981; Bond and Slingsby, 1983). Myrmecochory can also facilitate germination due to elaiosome removal or seed testa scarification by ants (Culver and Beattie, 1980; Gomez and Espadaler, 1997). Some negative effects of myrmecochory on plant fitness were also reported such as increased competition due to a clumping of seeds in the refuse piles around ant nests (Culver and Beattie, 1980; Davidson and Morton, 1981) as well as a decreased germination in a few plant species due to elaiosome removal or due to seed scarification (Imbert, 2006).

As it is often the case in research dealing with animal-plant mutualisms, there is an imbalance in our current knowledge about the evolutionary ecology of each partner (Cushman and Beattie, 1991; Bronstein, 1994). Indeed, benefits due to myrmecochory are far less known for ants. While more than two hundred papers tell us about plant benefits, only seven studies have shown so far that ants can benefit from elaiosomes by using them to feed their larvae (Handel, 1976; Morales and Heithaus, 1998; Bono and Heithaus, 2002; Fischer et al., 2005; Gammans et al., 2005; Marussich, 2006; Fokuhl et al., 2007) what may increase gynes production in some ant species (Morales and Heithaus, 1998) or increase larval weight (Gammans et al., 2005).

Myrmecochory involves a variety of taxa, often a guild of ants and a guild of plants, and is thus considered as a diffuse mutualism (Beattie and Culver, 1981; Giladi, 2006). This does not mean that all ant-plant pairs are equivalent since as argued by Giladi (2006), "*the overall fitness gain for plants resulting from myrmecochory significantly depends on the identity and behaviour of the seed disperser*" (p 482). In this respect, species-specific quantitative differences, even small ones, can have major consequences on the overall seed dispersal when multiplied by the number of ant-seed interactions occurring during a fructification season.

The aim of this paper is to dig into some aspects of ant behaviour likely to influence seed dispersal and thus to be a possible source of variability of myrmecochorous ant-plant interactions. In controlled conditions we shall see how ant traits such as diet and foraging behaviour may influence seed retrieval and elaiosome removal, and conversely how seed type may alter ants' response.

Therefore, we compared the foraging behaviour of the aphid-tending ant *Lasius niger* to that of the more insectivorous species *Myrmica rubra*. These ant species were given two different types of seeds quite common in their natural biotopes: *Viola odorata* seeds and smaller *Chelidonium majus* seeds. We assessed how seed species as well as the presence of an elaiosome influence the ants' behaviour by following up all steps from seed retrieval to seed dispersal out of the nest.

Materials and methods

Ant species: collection and rearing of colonies

Mature colonies of the black garden ant *Lasius niger* (L.) are monogynous and contain up to 13,000 workers (Stradling, 1970). These workers are 3 to 4 mm in length and mainly feed on honeydew (Pontin, 1958). This aphid-tending ant species can however be considered as omnivorous because it occasionally eat aphids (Pontin, 1958; Offenberg, 2001), dead or small alive insects (Pontin, 1961) as well as seed elaiosomes (Sernander, 1906; Oberrath and Böhning-Gaese, 2002).

The red ant *Myrmica rubra* L. is 4 to 6 mm in length, forming polygynous colonies which can reach 6,000 workers and more than 50 queens (Elmes, 1973). It is a common predator of small and soft-bodied animals, also scavenging upon large dead prey items (Le Roux et al., 2002). Whereas *Myrmica rubra* is considered as a carnivorous species, it can also consume sugared solutions (Cammaerts, 1977) or elaiosomes (Bülow-Olsen, 1984; Gorb and Gorb, 2000).

Both ant species are not granivorous and are becoming reference species for the study of myrmecochory in temperate regions (see e.g. Oberrath and Böhning-Gaese, 2002; Prinzing et al., 2007). They are found in open and forested habitats. We collected eight colonies of *Lasius niger* and eight colonies of *Myrmica rubra* from earth banks in Brussels (Belgium) and La Gorgue (France). In the laboratory, colonies were reared in plaster nests (Janet type, 20 x 20 x 0,5 cm) and placed in arenas (50 x 38 x 10 cm) with Fluon-coated borders to prevent ants' escape. Nests were regularly moistened and kept at 21±2°C, 35±5% relative humidity and a constant photoperiod of 12h per day. We covered the nest chamber with a red glass plate through which the ants could be observed. All colonies contained 1000-1500 workers. Nests with brood contained similar numbers of larvae, which covered 10-15% of the nest area. *Myrmica rubra* colonies contained 5-10 gynes. *Lasius niger* colonies were queenless.

Foraging activity of queenless and queenright colonies may differ or not depending on the ant species (see e.g. Vienne et al., 1998; Della Lucia et al., 2003; Brightwell and Silverman, 2007). Not too much weight should however be given to the presence/absence of a queen in the present research. Indeed, we simply investigate the basic responses of ant individuals facing food items without addressing any development or social regulation issues. Moreover, in the case of *Lasius niger*, using queenless colonies allowed us to make reliable comparisons with previous studies on foraging towards different food types (sucrose solution or prey: see e.g. Portha et al., 2004; Devigne and Detrain, 2006). We supplied ants *ad libitum* with water and sucrose solution (1M), and twice a week with cockroaches (*Periplaneta americana*) and an artificial diet with proteins, sugars and vitamins enriched (Bathkar and Withcomb, 1970). Colonies were collected in September and experiments were carried out in laboratory between October and January. As for previous studies with *Lasius niger* (e.g. Portha et al., 2004) or *Myrmica rubra* (e.g. Cammaerts, 1978, personal communication) that were carried out in winter, our tested colonies kept a high foraging activity towards sugary food sources (21 to 25 workers of *Lasius niger* and 17 to 20 workers of *Myrmica rubra* present after 15 min at a source of 1M sucrose solution). Hence, the absence of colony overwintering does not seem to significantly lower down the foraging response of ant workers.

Plant species and seed storage

Viola odorata L. is a perennial plant of temperate forests, edge bushes, open or ruderal habitats (Lambinon et al., 1992), widespread in Belgium, capable of vegetative reproduction. Plants flower from February to September with a peak in early spring and produce seeds from May to October (Oberrath and Böhning-Gaese, 2002; pers. obs.). Inflorescence stems bend down to the litter layer and capsules open barely without exploding. The peduncle carrying the diaspores is weak and prostrated towards the litter level, thus considered as an adaptation for ant removal (Beattie and Culver, 1981). Seeds are then clustered within the capsule and available for ants. It is considered as an obligate myrmecochorous plant. Seeds are yellow-brown, measured 3.34±0.21mm length and 2.24±0.14 mm width (n=10) with white-coloured and soft cone-like basal elaiosomes, smaller than the seed as they measured 2.01±0.25mm length and 1.65±0.16mm width (n=10). Seed mean weight (4.231 mg; n=10) and elaiosome mean weight (0.772 mg, n=10) gives a elaiosome/seed ratio of 0.18.

Chelidonium majus L. is a perennial plant present in ruderal habitats, edges or small ruderal forests (Lambinon et al., 1992). It is common in Belgium, flowers from April to October and produces seeds from May until November in a more important and continuous way (without peak) than *Viola odorata*. It is considered as a diplochorous plant, of which seed dispersal involves two steps: after fruit dehiscence, seeds are scattered around the parent plant on the ground (autochory) and are then removed by ants from the soil surface (myrmecochory). Seeds are dark brown and small: 1.95±0.06mm length and 1.43±0.11mm width (n=10). Elaiosomes are white-coloured, soft, larvae-like and measured 1.66±0.06mm length and 0.81±0.12mm width (n=10). Seed mean weight (0.985 mg; n=10) and elaiosome mean weight (0.309 mg; n=10) gives a elaiosome/seed ratio of 0.31.

Mature seeds were collected and stored at -18°C before being used in behavioural experiments. Nevertheless, we checked in preliminary experiments whether this preservation mode might have altered seed attractivity and potential for removal by ants. Therefore, for each plant (*Chelidonium* and *Viola*), three colonies of *Lasius* and three colonies of *Myrmica* were presented with simultaneously six fresh seeds and six seed stored at -18°C during 6 months. We compared the number of contacts, the removal rates for 45 minutes as well as the mean time before removal of fresh seeds to those of frozen items. On average, the number of contacts were not significantly different between frozen and fresh seeds (*Lasius/Viola*: P=0.7436; *Lasius/Chelidonium*: P=0.4212; *Myrmica/Viola*: P=0.0833; *Myrmica/Chelidonium*: P=0.7819; NS for □ =0.05; n=18, Wilcoxon paired test). After 45 minutes, all *Chelidonium* seeds were removed by ants and the mean time before removal were not significantly different for fresh and cold preserved seeds (*Lasius/Chelidonium*: P=0.0887; *Myrmica/Chelidonium*: P=0.4683; NS for □ =0.05; n=18, Wilcoxon paired test). Concerning *V. odorata* seeds, nearly all of them (94.4 %, n=72) were removed by both ant species except two seeds (one fresh and one frozen). Furthermore, on average, the time before removal did not significantly differ as both fresh and frozen seeds were as likely to be removed (*Lasius/Viola*: P=0.6441; *Myrmica/Viola*: P=0.1089; NS for □ =0.05; n=18, Wilcoxon paired test). All these results strongly suggest that the seed attractivity to the ants was not altered by our method of seed storage.

Experimental procedure

Most of the previous studies carried out on at least one of the four tested species were done in the field and looked at myrmecochory from a global point of view (such as global rate of seed retrieval, cafeteria experiments; see e.g. Beattie and Lyons, 1975; Gorb and Gorb, 2000; Peters et al. 2003). Here, for the first time, we detail ant-seed interactions in a standardized way by comparing the influence of seed/ant traits on myrmecochory in a 2 x 2 species combination.

In order to highlight species-specific differences in the myrmecochorous process, we investigated the possible four pairs of ant-plant interactions: Lasius/Chelidonium (LC), Lasius/Viola (LV), Myrmica/Chelidonium (MC) and Myrmica/Viola (MV). We carried out 24 experiments for each ant-plant pair: eight colonies with three replicates per tested colony. A colony was tested weekly and starved four days before each experiment. One day before, the colony was connected by a bridge to a foraging arena (50 x 38 x 10 cm) with Fluon-coated borders. The seed source was a square plastic film (3 x 4 cm) placed in the centre of the arena and used once. We presented to ants three types of items: entire seeds (i.e. with elaiosome) (SE), seeds without elaiosome (S) and detached elaiosomes (E).

Elaiosomes were cut off with a scalpel under a binocular microscope. *V. odorata* elaiosomes were discarded easily contrarily to *C. majus* ones, for which we took care to keep their turgescent cells unburst. Moreover, we checked that no elaiosome amounts (white tissue) were left attached at the insertion point and that only *C. majus* seed embryo (grey, foamy tissue) appeared at the tiny cutting wound (wound size of around 0.4mm x 0.2 mm). Items were placed on the source following the pattern shown in Figure 1. Each experiment started with the first contact between an ant and a seed. The ants' behaviours at the source were video-recorded for 45 minutes (magnification 10 xs).

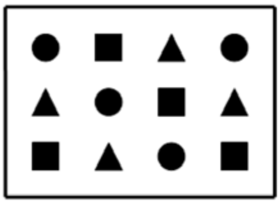

Figure 1. Spatial distribution of items at the food source. Entire seed (SE) (circle), seeds without elaiosome (S) (square) and elaiosome detached (E) (triangle).

Recruitment

We counted the number of ants at the seed source every 15 minutes after the start of the experiment. We noted possible trail-marking behaviour by directly observing ants going from and to the food source. Mean numbers of ants exploring the foraging area before seed introduction were compared between ant species with a Mann-Whitney Test (n=24, unpaired data). In order to detect any increase or decrease over time in the number of foraging ants, we compared measures done at 0, 15, 30, and 45 min by using the Page test for ordered alternatives (k=4, n=24).

Seed attractivity

The attractivity of an item from a distance was given by its probability to be contacted by the first ant reaching the seed source. Besides, as long as no item was removed, we observed whether each food item had equal chances to be contacted by any approaching ant. This was done by comparing the relative frequencies of contacts directed to each item type. Statistical tests used were Chi-square Goodness-of-fit, comparing our three probabilities with random (0.33).

Behavioural parameters

For each contacted seed item, we quantified the following three types of ants' behaviours:

1. *Antennation*: the ant merely touched the item with antennas, and then continued to explore the foraging area.

2. *Manipulation*: the ant contacted the item with antennas, held it between mandibles, bit it and then released it, without any removal.

3. *Removal*: after having antennated and manipulated an item, the ant took it away out of the camera field (1cm around the seed source).

These behaviours can be summarized in the following sequence (Fig. 2).

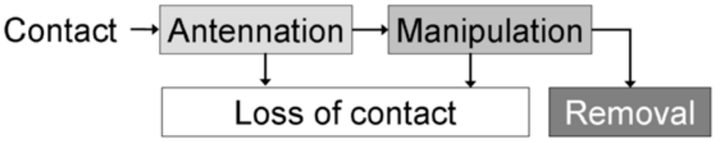

Figure 2. Sequence of behaviours.

In order to compare behavioural means between the four ant-plant pairs, we took into account only scores of entire seeds (SE) and used a Kruskall-Wallis Test followed by a Dunn Post-hoc test.

For each ant-plant pair, we compared the mean number (n=24) of behaviours (antennations, manipulations, removals) displayed by ants after contacting the three types of items (SE, S and E). This was done by using Friedman Test followed by a Post-hoc Test.

The removal dynamics were compared for each type of seed item. Slopes of the regression lines (calculated on Log transformed data) were compared with a Test of Comparison of several Slopes followed by a Post-hoc Test (q).

Seed fate

Twenty-four hours later the experiment, we noted the location of seeds in the whole setup as well as the presence (or absence) of elaiosomes on rejected seeds (SE or S). No elaiosome-item (E) was found out neither in the nest nor in the refuse piles, either because we overlooked them due to their minute size or because they were eaten.

Results

Recruitment

We never observed any recruitment towards seed sources. At the beginning of the experiment, the numbers of exploring ants were low and similar whatever the seed species for *Myrmica rubra* (*Viola*: 2.2±1.73; *Chelidonium*: 1.58±0.91; N.S., p= 0.2855, Mann-Whitney test, n=24) and for *Lasius niger* (*Viola*: 1.33±1.07; *Chelidonium*: 0.87±1.33; N.S., p=0.0677, Mann-Whitney test, n=24). Every 15 min, we counted the number of ants at the source and did not detect any increase in ant population even after 45 minutes (Table 1). On the contrary, there was a decrease in the number of foraging ants, this trend being significant for all pairs but the L/C pair (MV p=0.0455; MC p=0.0202; LV p=0.0307; LC p=0.2090, N.S.; Page test for ordered alternatives, k=4, n=24). For both *Lasius* and *Myrmica*, no foraging ant was seen laying a chemical trail neither along its homeward nor its outward journey. Even successful individuals that were transporting seeds were never seen laying a trail on their way back to the nest.

Table 1. Mean number of ants (± S.D., n=24) on the source square at the start of the experiment, after 15 min, 30 min and at the end (45 min).

	Start	15 min	30 min	End
Lasius/Viola	1.3 ± 1.1	1.3 ± 1.8	1.0 ± 1.3	0.6 ± 0.9
Lasius/Chelidonium	0.9 ± 1.3	0.6 ± 1.2	0.5 ± 0.8	0.6 ± 0.7
Myrmica/Viola	2.2 ± 1.7	1.8 ± 1.9	1.6 ± 1.7	1.1 ± 1.0
Myrmica/Chelidonium	1.6 ± 0.9	1.1 ± 1.2	0.8 ± 0.9	1.0 ± 1.0

Seed attractivity

For all pairs, each type of item (SE, S and E) was as likely to be contacted by the first ant approaching the food source. Indeed, approaching ants showed a probability to first contact one of the three item types that did not significantly differ from a random choice (0.33) (Table 2) (N.S.; Chi-square goodness-of-fit test; □ =0.05, n=24). Likewise, as long as no food item was removed, each item remained as likely to attract foraging ants from a distance with probabilities to be contacted not different from random (N.S.; Chi-square goodness-of-fit test; □ =0.05). However, while food items were not selected by ants from a distance, their first removal strongly depended on whether an elaiosome was present or not (Table 2). Seeds without elaiosomes (S) were less frequently first removed, this trend being significant for all pairs but the M/C pair (LV, p<0.01; LC, p<0.01; MV, p<0.05; MC, N.S.; Chi-square goodness-of-fit test; □ =0.05). This demonstrates that the perception of elaiosome chemicals plays a key role in the triggering of seed removal. Furthermore, we noticed that for *Viola*, two thirds of items first removed were elaiosomes alone (E) (0.64

for *Lasius*; 0.58 for *Myrmica*). This suggests that, in addition to the elaiosome chemicals, the small size of one item is another factor that may enhance removal rates by the ants (comparison of SE versus E results). Hence, the choice of removing seeds was done only after the ants had contacted the item and was driven by the perception of elaiosome chemicals and to a lesser extent by the food item size.

Table 2. Proportions of each food item (SE: seed with elaiosome; S: seed without elaiosome; E: elaiosome alone) to be first contacted or first removed by ants. The proportion of contacts with each food item was also calculated before any seed removal as an indicator of the relative attractivity of these items. These proportions were compared to the expected values (0.33 * n) when items are assumed to be equally attractive (Chi-square test goodness to fit, □ = 0.05). □ ² theorical = 5.991)

Pair		SE	S	E	n	□ ²	p
Lasius/Viola	First contacted	0.33	0.17	0.5	24	4	N.S.
	Contacted before any seed removal	0.32	0.35	0.33	440	0.88	N.S.
	First removed	0.27	0.09	0.64	24	10.2	**
Lasius/Chelidonium	First contacted	0.29	0.42	0.29	24	0.75	N.S.
	Contacted before any seed removal	0.42	0.29	0.29	150	5.08	N.S.
	First removed	0.5	0.04	0.46	24	9.25	**
Myrmica/Viola	First contacted	0.29	0.29	0.42	24	0.75	N.S.
	Contacted before any seed removal	0.33	0.29	0.38	157	1.62	N.S.
	First removed	0.33	0.08	0.58	24	9	*
Myrmica/Chelidonium	First contacted	0.25	0.46	0.29	24	1.75	N.S.
	Contacted before any seed removal	0.29	0.45	0.26	42	2.71	N.S.
	First removed	0.42	0.21	0.38	24	1.75	N.S.

Ant behaviour at the seed source

The species effect

For the entire seeds (SE), the number of contacts, antennations and manipulations during the whole experiment differed greatly among pairs (Figs 3 and 4) (all p values <0.0001, Kruskall-Wallis Test, n=24).

Viola seeds were three times more contacted than *Chelidonium* ones by both ant species (Figure 3) (MV *vs.* MC, p<0.001; LV *vs.* LC, p<0.01; Dunn Post-Hoc Test). As regards ants, *Lasius* usually performed more contacts than *Myrmica*, although this trend was only significant for *Chelidonium* (LC *vs.* MC, p<0.01; LV *vs.* MV, N.S.; Dunn Post-Hoc Test).

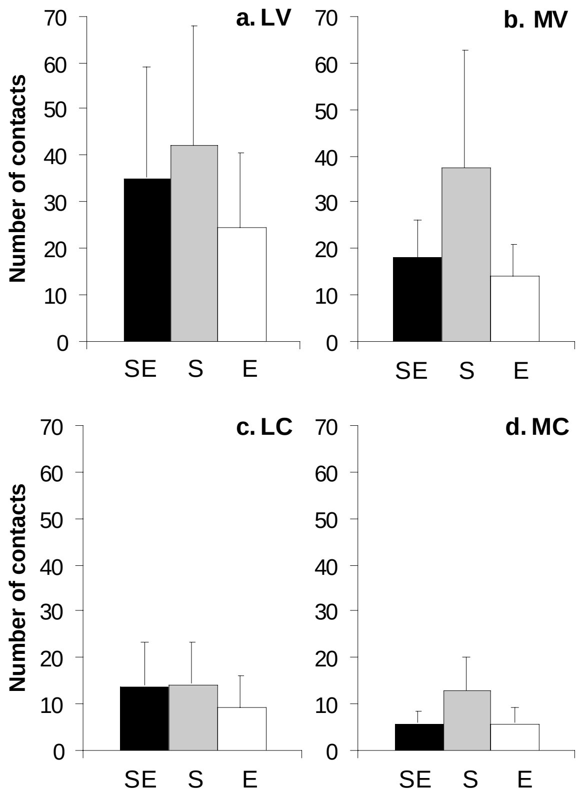

Figure 3. Mean number of contacts (±S.D., n=24 experiments per pair) for entire seeds (SE, black), seeds without elaiosome (S, grey) and elaiosomes (E, white). a. *Lasius niger/ Viola odorata.* b. *Myrmica rubra / Viola odorata.* c. *Lasius niger/Chelidonium majus.* d. *Myrmica rubra/Chelidonium majus.*

Any ant contacting a seed item always started to antennate it. In most cases, ants just antennated the food item and then left it to further explore the foraging area. Indeed, "antennation" behaviour (followed by a loss of contact) accounted for 49% to 63% of all the observed contacts for the three following ant/seed pairs (LV, LC and MV). Only *Myrmica* foragers had lower antennation scores (11% to 14%) when they encountered *Chelidonium* seeds (Fig. 5). As previously reported for contacts, *Viola* seeds were always more antennated than *Chelidonium* ones (Fig. 4) (MV *vs.* MC, $p<0.001$; LV *vs.* LC, $p<0.01$; Dunn Post-Hoc Test). Likewise, we found out the same trend of *Lasius* workers to antennate seeds more frequently than *Myrmica*, especially when faced with *Chelidonium* seeds (LC *vs.* MC, $p<0.001$; LV *vs.* MV, N.S.; Dunn Post-Hoc Test). One should notice that a low number of contacts or antennations does not reflect a lack of interest for a seed item but, on the contrary, results from their shorter lasting availability due to their quick removal by ants (see below).

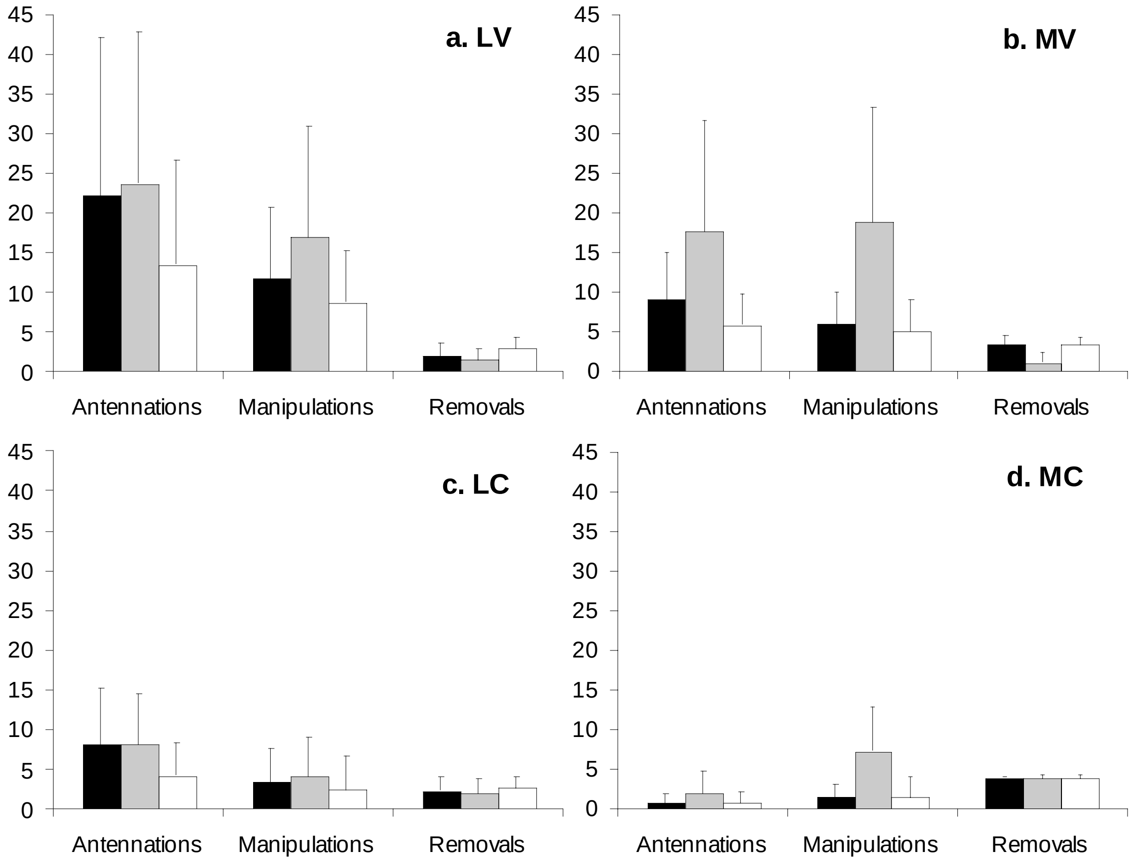

Figure 4. Mean number of behaviours (±S.D., n=24 experiments per pair) for entire seeds (SE, black), seeds without elaiosome (S, grey) and elaiosomes (E, white). a. *Lasius*/*Viola*. b. *Myrmica*/*Viola*. c. *Lasius*/*Chelidonium*. d. *Myrmica*/*Chelidonium*.

Some ants that discovered a seed item went further than a simple antennation as they manipulated the seed by biting it, rolling it up or seizing it up in their mandibles for a while. Manipulation behaviours accounted in all pairs for a similar proportion (between 23.3% and 32.3% of all contacts) (Fig. 5). But the mean number of manipulations was different for plant species: *Viola* seeds were three times more manipulated than *Chelidonium* ones (Fig. 4) (MV *vs.* MC, $p<0.001$; LV *vs.* LC, $p<0.001$; Dunn Post-Hoc Test). However, there was no significant difference between ant species (LC *vs.* MC, N.S.; LV *vs.* MV, N.S.; Dunn Post-Hoc Test). This shows that seed inspection and/or difficulty to handle a seed is dependent on plant species, but not on the manipulating ant.

The elaiosome effect

The tearing out of the elaiosome may deeply influence the behaviour of ants contacting a seed item. Indeed, as regards *Myrmica* ants (Figs 4b,d), items without elaiosomes were always more frequently antennated than items with elaiosomes (*Viola*: $p=0.0005$, Post-hoc test: SE *vs.* S, $P<0.05$; *Chelidonium*: $p<0.001$, Post-hoc test: SE *vs.* S, $P<0.05$; Friedman test, n=24). The discarding of the elaiosome had the same effect on manipulations by *Myrmica* ants (Figs 4b,d) (*Viola*: p=0.0002, Post-hoc test: SE *vs.* S, p<0.01; *Chelidonium*: p<0.0001, Post-hoc test: SE *vs.* S, p<0.001; Friedman test, n=24).

Lasius antennations and manipulations displayed almost the same trends. The only difference was that they antennated more frequently large items (SE and S) than smaller ones such as elaiosomes (E) (Figs 4a,c) (*Viola*: p=0.0038, Post-hoc test: SE *vs.* E, P<0.01; S *vs.* E, P<0.05; *Chelidonium*: p=0.0009, Post-hoc test: SE *vs.* E, P<0.05; S *vs.* E, P<0.01; Friedman test, n=24). Furthermore, they manipulated these large items more frequently, especially *Viola* ones (Figs 4a,c) (*Viola*: p=0.0020, Post-hoc test: S *vs.* E, p<0.05; *Chelidonium*: p=0.2686, N.S.; Friedman test, n=24).

The high number of pre-removal behaviours (antennations and manipulations) showed that ants were interested in items but could not go further in the removal process when they did not find an elaiosome attached to the seed. Three non-exclusive explanations can be evoked: the lack of a grasping point facilitating the items' transport, the large size of the food items (SE and S of *Viola*) or possibly some minute remains of elaiosome chemicals that elicit a prolonged inspection of the seeds by the ants.

Seed removal

The species effect

We also found differences among pairs in seed removal patterns. Proportions of seeds (SE) removed were higher for *Myrmica* ants and higher for *Chelidonium* seeds (Fig. 5). When looking at the mean number of removals (Fig. 4), global differences still occurred (p<0.0001, Kruskall-Wallis Test, n=24), but the plant-effect was not significant (MV *vs.* MC, N.S.; LV *vs.* LC, N.S.; Dunn Post-Hoc Test). Only the ant-effect was confirmed, showing that seed removal depend strongly on the ant species: entire seeds (SE) were more removed by *Myrmica* than *Lasius* (LC *vs.* MC, p<0.01; LV *vs.* MV, p<0.05.; Dunn Post-Hoc Test). Indeed, *Myrmica* ants removed more than 90 % of items in 54 % of experiments, with a mean of 9.7±2.9 items removed per experiment (n=48, both pairs pooled), whereas *Lasius* removed more than 90% of items in only 27 % of experiments, with a mean of 6.9±4.3 items removed per experiment (n=48). This ant-effect is also clear in removal dynamics (Fig. 6). Each ant species had a different removal-curve pattern, in which *Myrmica* removed more seeds (SE) after 45 min. All these curves had an exponential shape, which means that the probabilities of seed removal per minute were constant values given by the slopes of the regression lines (calculated on Log transformed data). Then, *Myrmica* tended to remove *Chelidonium* seeds (SE) five times faster than *Lasius* did (*Myrmica, p*=0.109; *Lasius, p*=0.023), and *Viola* seeds three times faster (*Myrmica, p*=0,049; *Lasius, p*=0.017).

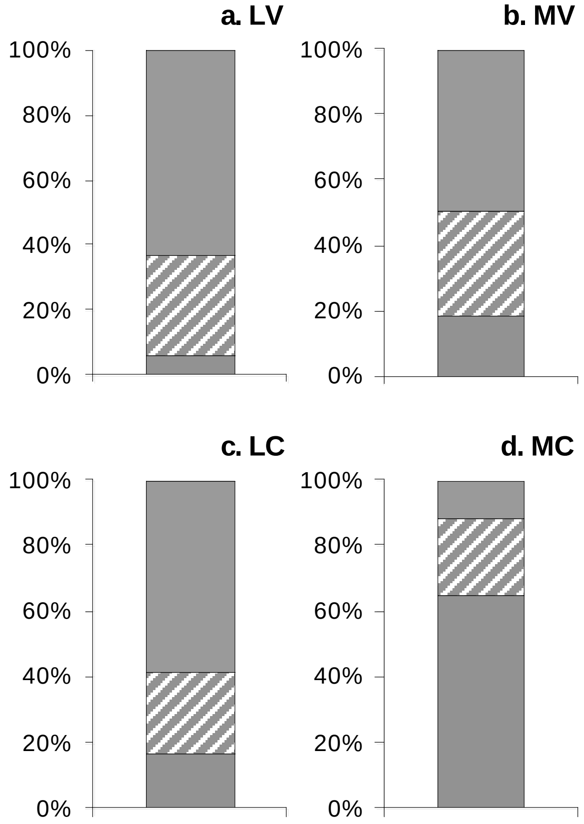

Figure 5. Proportions of antennations (doted), manipulations (hatched), and removals (full) displayed by ants contacting entire seeds (SE).

The elaiosome effect

Besides the species effect, the presence of elaiosomes also influenced seed removal patterns. Firstly, *Lasius* ants removed more items with elaiosomes (SE and E) than seeds without elaiosomes (S) (Figs 4a,c) (*Viola*: $p<0.0001$, Post-hoc test: S *vs.* E, $p<0.0001$; *Chelidonium*: $p=0.0092$, Post-hoc test: S *vs.* E, $p<0.05$; Friedman test, $n=24$). Comparing seed-removal dynamics, *Lasius* ants removed 25 % of *Viola* seeds with elaiosomes (SE) after only 15 min, whereas it took around 33 min to remove the same percentage of seeds when they were deprived of their elaiosome (S) (Fig. 6a). This was confirmed by slopes of the regression lines of seed-removal curves that differed among items (Test from comparison of several slopes: $F_{0.05,2,46}=405.9$, $P<0.0001$; Test post-hoc: $q_{GE\text{-}G}=10.79$, $p<0.001$; $q_{G\text{-}E}=39.01$, $p<0.001$; $q_{GE\text{-}E}=28.22$, $p<0.001$). Indeed, seeds with elaiosomes (SE) had a twice higher probability ($p=0.017$) to be removed per minute than seeds without elaiosomes ($p=0.010$). We also noticed that the size of the item could play a role as small elaiosome items (E) were removed faster than large items (SE and S). For *Chelidonium* (Fig. 6c), we found out a similar enhancing effect of the elaiosome on the seed removal as slopes of seed-removal curves were quite different (Test from comparison of several slopes:

$F_{0.05,2,46}=45.96$, $P<0.0001$; Test post-hoc: $q_{GE-G}=7.85$, $p<0.001$; $q_{G-E}=13.50$, $p<0.0001$; $q_{GE-E}=5.64$, $p<0.001$). This effect was however smaller than for *Viola*.

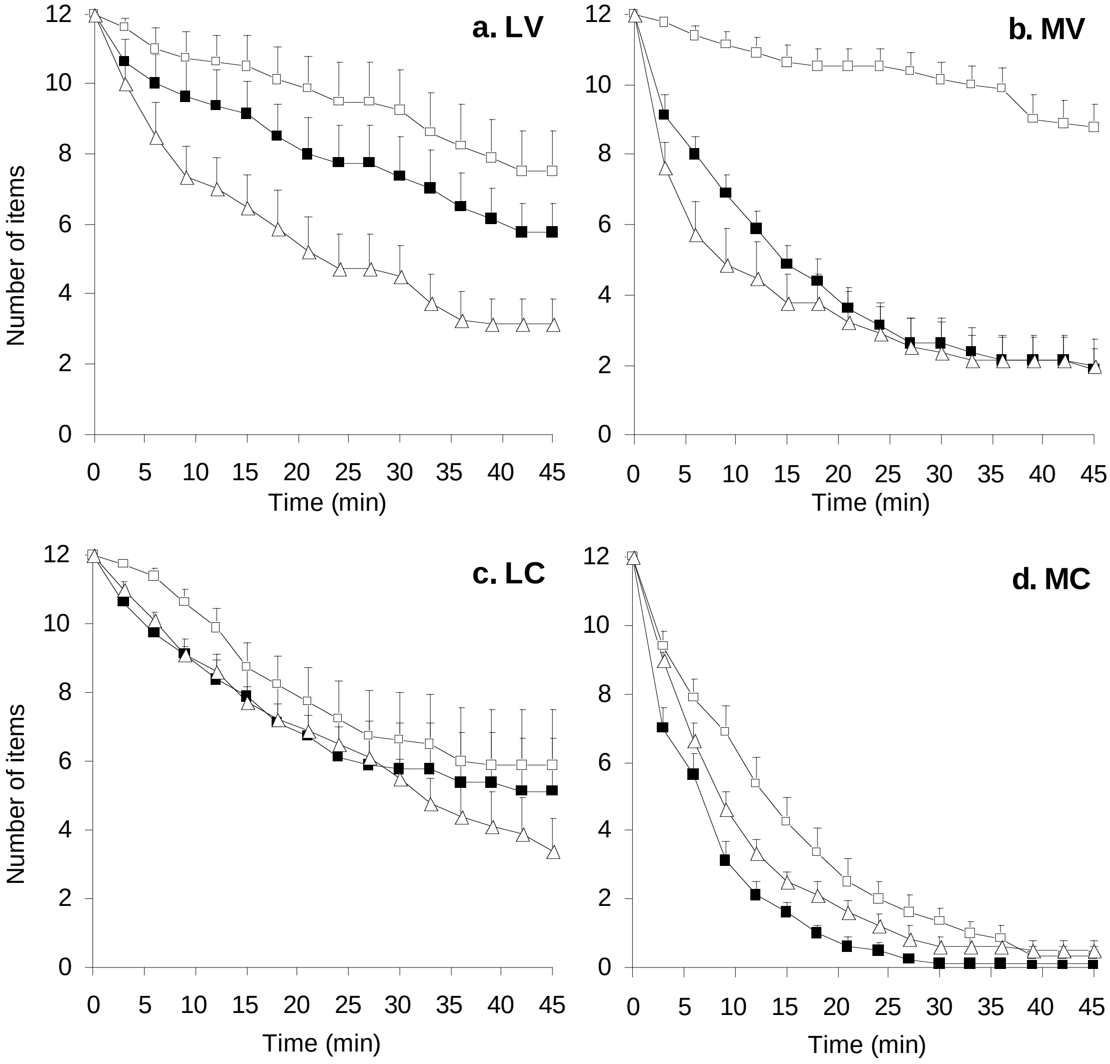

Figure 6. Removal dynamics of the three items: seed with elaiosome (SE, full square), seed without elaiosome (S, open square) and elaiosome alone (E, triangle), during the experiment (45 min). The three replications were pooled for each colony (i.e. 12 items at the beginning of the experiment). Each point corresponds to a mean (±S.E.) of the number of colonies (n=8). a. *Lasius*/*Viola*. b. *Myrmica*/*Viola*. c. *Lasius*/*Chelidonium*. d. *Myrmica*/*Chelidonium*.

Concerning *Myrmica* ants, they showed the same choice pattern on *Viola* items than *Lasius*, removing significantly more items with elaiosomes (SE and E) (Fig. 4b) (p<0.0001, Post-hoc test: SE *vs.* S, p<0.0001; S *vs.* E, p<0.0001; Friedman test, n=24). The effect on removal dynamics was also very clear as they removed 25 % of *Viola* SE seeds after only 3 min, compared with 45 min when they were deprived of their elaiosome (S) (Fig. 6b). This is confirmed by slopes of the regression lines (Test of comparison of several slopes $F_{0.05,2,46}=147.33$; $P<0.0001$; Test post-hoc: $q_{GE-G}=20.86$, $p<0.001$; $q_{G-E}=21.18$, $p<0.001$; $q_{GE-E}=0.33$, NS), giving a probability per minute to be removed seven times higher for SE ($p=0.05$) than for S ($p=0.007$).

Curiously, this pattern is totally absent on *Chelidonium* items (Fig. 4d) ($p=0.4437$, N.S.; Friedman test, $n=24$). Indeed, nearly 100% of all *Chelidonium* items –even those deprived of elaiosome– were removed after 45 min. As a corollary, slopes of seed-removal curves were not significantly different between items (Test from comparison of several slopes; N.S.), and probabilities of being taken per minute were always high, independently of the presence or not of an elaiosome (SE, $p=0.1$; S, $p=0.08$) (Fig. 6d).

Seed fate

After 24h, for all tested ant/plant pairs, no difference was found out since all seed items that bore or not an elaoisome were removed and recovered outside the nest in the refuse piles. Approximately half of elaiosomes from SE items were discarded by ants except for the pair *M/C* for which 100% of elaiosomes were removed.

Discussion

Recruitment

Ant recruitments are rarely reported in the whole myrmecochory literature (see Bond et al., 1991; Gorb and Gorb, 1999). In our case, we never observed any recruitment nor any significant increase of the foragers' population, which confirms the field results obtained with the pair *Myrmica rubra / Viola odorata* (Gorb and Gorb, 1999). Mass-recruiting ants such as *Lasius* and *Myrmica* species can nevertheless display all intermediate strategies between solitary foraging and collective food exploitation through trail recruitment (Hölldobler and Wilson, 1990). The lack of recruitment towards elaiosome-bearing seeds can thus be explained by the two following characteristics of food source. Firstly, the number of food items was not enough to trigger recruitment. Secondly, elaiosomes could be considered by foragers as small dead-insect preys (Carroll and Janzen, 1973) and as such, did not elicit recruitment as they could be easily individually retrieved, as shown in *Myrmica rubra* (Cammaerts, 1978) and *Pheidole pallidula* (Detrain and Deneubourg, 1997).

The elaiosome effect

As expected, the presence of an elaiosome influenced positively seed removal of *Viola odorata* for both ant species. The obligate myrmecochorous plant *Viola odorata*, which produces a few dozens of seeds during a season, blooms in the early spring like most of the other European myrmecochorous plant species (Oberrath and Böhning-Gaese, 2002). This pattern differs from *Chelidonium majus* which flowers later and produces thousands of seeds during a season. In this species, the presence of an elaiosome on *Chelidonium majus* seeds does not seem to enhance its removal since the seed itself, even deprived of its elaiosome (E), remains very attractive to ant workers, especially *Myrmica* ones. Differences -even slight ones- in removal dynamics between pairs or between items can have major consequences on plant fitness at the time scale of the whole season. This is especially true for *Chelidonium majus*, since one single medium-sized plant produces more than 75,000 seeds per year (Servigne, unpubl. data). Hence, by "flooding the market" and being very appreciated by ants, seeds of *Chelidonium majus* will increase their chances to be scattered everywhere, what may compensate the lack of vegetative reproduction. As a result, *Chelidonium majus* is very efficient in colonizing ruderal places in which ants are used to forage such as pavements or cracks in walls.

The attractivity of a seed deprived of its elaiosome (S) has already been reported for non-granivorous ants (Weiss, 1909; Bond and Breytenbach, 1985; Pemberton, 1988; Oostermeijer, 1989; Espadaler and Gomez, 1997; Orivel and Dejean, 1999). However, these studies never reached the unexpected level of our pair *Myrmica/Chelidonium* (97 %). In our case, it is likely that the testa itself was appreciated by workers. An additional explanation may be that ants were interested in the embryo tissue that can be reached by workers at the tiny wound (around 0.4 mm x 0.2 mm) due to the cutting of the elaiosome (as also reported for *Aphaenogaster iberica*; Boulay et al., 2005). Another consideration could be that, as *Chelidonium majus* seeds are mainly present during summer, they have to compete with insect preys which are abundant at this time and need to be more attractive than early spring seeds such as *Viola odorata* seeds. Further investigations will focus on what could make it attractive and how its attractivity varies in the field with different food spectrum, like Boulay et al. (2005) did it with *Helleborus foetidus* in a Mediterranean ecosystem.

The fact that every seed item has the same probability to be first contacted means that ant orientation is not influenced by the presence of an elaiosome. One ant has thus to come into direct contact (antennating, licking, chewing) before taking any removal decision. This confirms that ants either do not perceive elaiosome volatile compounds, or that elaiosomes have no volatile compounds. EAG experiments also showed that other ant species do not perceive elaiosomes from a distance, but only after antennations (Sheridan et al., 1996 but see also Giliomee, 1986).

The ant effect

Our two ant species are undoubtedly seed dispersers as they retrieve seeds of *Chelidonium majus* and *Viola odorata*, bring them back to the nest, remove the elaiosomes and reject (*i.e.* disperse) them. Nevertheless, seed removal dynamics and elaiosome discarding rates differed depending on partner species and more precisely on ant behaviour, as shown in other species (Hughes and Westoby, 1992b; Gorb and Gorb, 2003; Cuautle et al., 2005). Such behavioural differences may have been an important selective force in the evolution of myrmecochory (Beattie and Hughes, 2002). Here, *Myrmica* ants removed seeds faster and in higher proportions than *Lasius*. Their response were also more sensitive to the presence of an elaiosome, as the differences of removal rates between seeds with (SE) and without (S) elaiosomes were more clear-cut than for *Lasius niger*. Finally, *Myrmica* detached *Chelidonium* elaiosomes more efficiently than the black garden ant. Because both ant species have approximately the same size, we can assume that their different seed removal responses were mainly due to their diet. Following the hypothesis of a convergence between odours of elaiosomes and insect preys (Carroll and Janzen, 1973; Hughes et al., 1994), one can explain why insectivorous and predatory species such as *Myrmica* ants are more involved in the myrmecochory process.

In our experiments, antennations were the main behaviour displayed by ants contacting seeds since they accounted for 41% to 63% of all contacts in three following pairs: M/V, L/V and L/C. Conversely, in the field, one can find out markedly lower rates of "antennations" ranging from 1% to 33% for ten ant species and seeds of *Sanguinaria canadensis* (Pudlo et al., 1980). These values are closer to those obtained in our study for the M/C pair that less antennate seeds and remove them more quickly than the others. We interpret "antennations" as an examination process without further interest towards seeds. For *Myrmica rubra*, the different levels of "antennation" between the seeds of two plant species indicate that ants have a clear preference for *Chelidonium majus* and that foragers can be very efficient in seed removal. It is different for *Lasius niger*, that typically "antennated" both seed species at the same rate. Levels of "antennations" could thus be a measure of the appropriateness of a seed to an ant.

As regards the "manipulation" behaviour, it accounts for an increased interest towards seeds. But since contact is finally lost after manipulation, scouts could be not "satisfied" with the nutritional compounds (protein, sugars, etc.) of the seed. Moreover, as "manipulations" were more frequent for seeds without elaiosomes (S), ants may have difficulties to handle the seed. The handle function of the elaiosome has already been highlighted and could be an important evolutive force in these ant-seed interactions (O'Dowd and Hay, 1980; Horvitz, 1981; Byrne and Levey, 1993; Gomez et al., 2005).

The seed size effect

We found out that the removal rates by both *Lasius niger* and *Myrmica rubra* were higher for the smaller seed (*Chelidonium majus*) than for the larger one (*Viola odorata*). Conversely, *Viola odorata* seeds were more quickly removed than *Chelidonium majus* when foraged by a larger ant species *Formica polyctena* (Gorb and Gorb, 2000). One could evoke a *size-matching* effect to account for this difference: small ant species (*Lasius* and *Myrmica*) transported preferentially smaller seeds than the large ant *Formica polyctena*. This seed-size dependent selectivity is well known for harvester ants, which remove the largest seeds as possible. Below their seed-size scale, the ant does not detect the seed as a potential food, and above, seeds are too large to be removed (Davidson, 1978; Retana and Cerdá, 1994; Kaspari, 1996; Willott et al., 2000; Heredia and Detrain, 2005).

Currently, the seed-size effect on ants' removal is quite controversial. Some studies showed that removal rates were seed-size dependent, not elaiosome-size dependent (Gorb and Gorb, 1995, 2000): for an ant species, the bigger the seed, the higher its removal rate. However, other studies found that removal rates were elaiosome-size dependent (Oostermeijer, 1989, Mark and Olesen, 1996) or dependent on the ratio between elaiosome and diaspore sizes (Gunther and Lanza, 1989, Hughes and Westoby, 1992b, Bas el al., 2007). Our results corroborate this latter elaiosome/diaspore-ratio hypothesis: *Chelidonium majus* has smaller seeds, smaller elaiosomes, but a higher elaiosome/diaspore ratio than *Viola odorata* (*Viola*: 0.18; *Chelidonium*: 0.31). Seed retrieval by myrmecochorous ants may obey to more complex decision criteria. While the reward of harvester ants is the entire seed, for seed-dispersing ants, it is the elaiosome. A large diaspore could then benefit to harvester ants (more transport and more food) whereas it could cost to myrmecochorous ants (more transport without any supplementary food) (Schoener, 1971; Hughes and Westoby, 1992a). Selective forces of myrmecochory should favour increasing elaiosome size or elaiosome/seed size ratio. Hence, the efficiency of myrmecochorous ant-plant interactions may depend on two factors: the seed-retrieving ability of ants depending on seed-size matching, and within their seed-size scale, ants choose those bearing the largest elaiosome or showing the highest elaiosome/diaspore ratio.

In addition to these size effects, seed selectivity may also depend on differences in seed-surface compounds as well as ant sensitivity to these chemicals (Gunther and Lanza, 1989). Finally, removal rates are dependent on ant's historical constraints (Peters et al., 2003). By historical constraints, we mean the seed-specific satiation level of ants (Heithaus et al., 2005), their individual foraging experience coupled to the availability of alternative resources (Boulay et al., 2005). As regards the effect of individual experience, it has been shown that ants can learn how to handle some available seeds and thus increase their removal rates (Johnson, 1991; Gorb and Gorb, 1999). These historical constraints are an important source of variability in field experiments and can be better controlled in laboratory experiments.

This work has evidenced differences in the interaction patterns between four crossed ant-plant pairs in controlled conditions. In those conditions, *Myrmica rubra* appears to be the best potential dispersal vector and *Chelidonium majus* seems to be very attractive to this ant. But this mutualism is not exclusive as other plants or ants may also interact with different removal dynamics. In natural communities, those differences in plant-ant interactions should be added to changing relative abundances and species combinations in a diversity of landscapes. This may create local spatial shifts in ant-plant interaction intensity and change the outcome across space and time. Traits of interacting species will be well matched in some communities and mismatch in others, forming a geographical mosaic of coevolution (Thompson, 1999, 2005; Garrido et al. 2002).

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Résultats annexes

Production de graines myrmécochores et activité de récolte des fourmis en milieu naturel

Parmi les traits de plantes myrmécochores qui peuvent influencer les interactions avec les fourmis, la capacité de production des graines n'a, à notre connaissance, jamais été mesurée. Comme nous venons de le voir, les graines de la plante *Chelidonium majus* suscitent des taux de prises plus importants que les graines de *Viola odorata* chez les fourmis *Lasius niger* et *Myrmica rubra*. Dans ces expériences, la fourmi *Myrmica rubra* a même enlevé la totalité des élaiosomes de *Chelidonium majus*, alors qu'elles n'enlèvent que la moitié des élaiosomes de V*iola odorata*. Il est raisonnable de penser qu'un manque d'affinité pour les graines est compensé sur le terrain par une plus forte production de graines. Nous avons mesuré les productions respectives de graines des deux plantes concernées dans leur biotope.

Par ailleurs, nous avons voulu voir comment les différences de production de graines influencent l'activité de fourragement des fourmis. Nous avons donc suivi l'activité des fourmis au cours d'une saison sur une station de *Viola odorata* et sur une station de *Chelidonium majus*.

Matériel et méthodes

La production de graines au cours d'une saison (de Mai à Octobre 2004) a été mesurée en comptant une fois par semaine le nombre de capsules ouvertes sur la plante (*Chelidonium majus*), ainsi que trois fois dans la saison le nombre de capsules ouvertes (*Viola odorata*). Après comptage, les capsules ont été enlevées. Afin de comparer la production de plantes de taille différentes, nous avons mesuré la production d'une plante de *Chelidonium majus* et d'une petite population de 70 plantes de *Viola odorata*, ce qui correspond pour les deux cas à une surface comparable au sol (une cercle d'environ 0.25 m^2).

L'activité des fourmis a été mesurée toutes les semaines, à la fois autour des plantes et à l'entrée de la fourmilière, dans une station de *Chelidonium majus* (terrain abandonné à Ixelles) et de *Viola odorata* (campus de La Plaine, ULB), toutes les deux contenant des populations de *Lasius niger*. L'activité autour des plantes a été mesurée en comptant le nombre de fourmis sur 3 quadrats (50 x 50 cm) contenant les plantes concernées. L'activité des fourmis à l'entrée du nid a été mesurée en comptant le nombre de sorties et d'entrées d'individus avec et sans graines (4 x 5 min à 1h d'intervalle), entre 14 et 17h, de début Juin à fin août 2004.

Résultats et discussion

L'estimation de la production de graines est reprise au Tableau A. Les résultats indiquent que la production de capsules hebdomadaire est en moyenne 20 fois plus importante chez une plante de *Chelidonium majus* qu'une petite population de *Viola odorata*. De plus, le nombre moyen de graines par capsules est 3,2 fois plus important chez *Chelidonium majus*. Il en résulte qu'à la fin d'une saison, le nombre total de graines produites sur une surface de 0.25 m² serait d'approximativement 1200 graines pour *Viola odorata* et 80000 graines pour *Chelidonium majus*, soit un rapport d'1/67. La chélidoine étant diplochore (autochore + myrmécochore), la majorité des graines produites se retrouve dispersée au sol autour de la plante. A l'opposé, la violette est myrmécochore stricte (unique phase de dispersion). Les graines restent dans la caspide entrouverte et ne sont dispersées que par les fourmis. Dans sa capacité à coloniser de nouveaux espaces, la violette semble désavantagée par rapport à la chélidoine. Cependant, la moindre production de graines de *Viola odorata* est compensée par sa capacité à se reproduire par stolons. Par ailleurs, l'importante quantité de graines produites par la chélidoine et mise à disposition des fourmis tout au long d'une saison devrait donc augmenter la probabilité qu'une fourmi a de rencontrer une graine, mais pourrait être contrebalancée par un effet d'habituation des fourmis (cf. Chapitre 4, section VI). Une perspective de recherche serait de montrer si la chélidoine provoque un tel phénomène d'habituation chez les fourmis les plus abondantes de leur biotope, comme cela a déjà été montré avec d'autres espèces dans les climats tempérés d'Amérique du Nord (Heithaus 1986, Smith et al 1986, 1989a, 1989b).

Tableau A. Production de graines au cours de l'année 2004.

	Début de la fructification	Fin de la fructification	Nb moyen de capsules produites par semaine	Capsules produites	Nombre moyen de graines par capsules
Viola odorata	mi-avril	mi-sept	4.5 ± 4 (estimation)	94	13,1 ± 3.9 (n=17)
Chelidonium majus	mi-mai	mi-octobre	92.5 ± 46	1855	42,8 ± 6.4 (n=10)

La présence des fourmis *Lasius niger* au cours de la saison dans les quadrats à *Viola odorata* diffère de la présence dans des quadrats *Chelidonium majus* (Fig. A). Nous observons une légère baisse d'activité de fourragement entre la mi-juin (25$^{\text{ième}}$ semaine) et la fin-juillet (31$^{\text{ième}}$ semaine) chez *Viola odorata*. De même, une chute marquée de l'activité de fourragement a lieu après la 28$^{\text{ième}}$ semaine dans la population à *Chelidonium majus*. Il est intéressant de noter que cette baisse intervient juste après l'essaimage, qui a eu lieu à la 28$^{\text{ième}}$ semaine. Cette baisse drastique d'activité pourrait être due à la disparition des principaux puits de protéines (larves de sexués) du nid. La différence entre les deux espèces pourrait s'expliquer par le fait que les élaiosomes de chélidoine ne serviraient de nourriture qu'aux larves des fourmis *Lasius niger* alors que les élaiosomes de violette seraient également destinés aux ouvrières. Il est à noter également que l'activité de fourragement des fourmis redevient en automne comparable à celle de la fin du printemps pour les parcelles à *Viola odorata*. Aussi, nous avons superposé la production de graines de chélidoine (courbe orange) à l'activité des fourmis. Ceci indique que la baisse drastique d'activité des fourmis en été ne semble pas liée à la chute de la production de graines. Cependant, ces résultats sont à interpréter avec prudence car les sites de mesure de production des graines et d'activité des fourmis ne sont pas les mêmes.

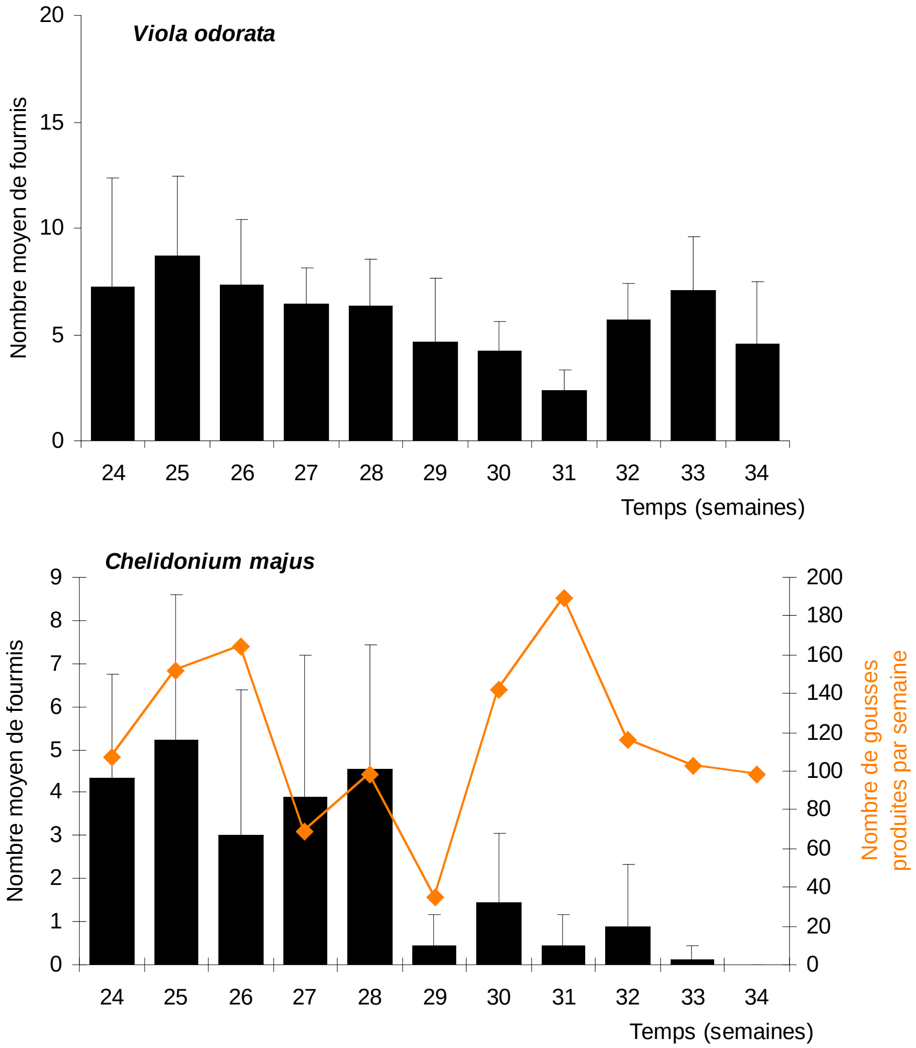

Figure A. Activité de *Lasius niger* sur le site à *Viola odorata* (haut) et *Chelidonium majus* (bas) en juin (24-27), juillet (28-31) et août (32-34) 2004. Les moyennes et écart-types sont calculés sur base de 3 mesures effectuées sur 3 quadrats (n=9).

Par ailleurs, les flux de graines de *C. majus* entrant et sortant du nid de *Lasius niger* sont loin d'être négligeables (Fig. B). De début Juin à mi-Juillet, près de la moitié des fourmis qui rentrent au nid transportent une graine de chélidoine. Ainsi, par extrapolation, la quantité de graines récoltées au cours d'une saison chez ce couple fourmi-plante pourrait s'avérer d'une importance écologique considérable tant au niveau de la dispersion des graines de chélidoine que pour l'alimentation de la colonie de fourmis.

Le taux d'entrées des graines dans le nid chute brutalement à la 29$^{\text{ième}}$ semaine, juste après l'essaimage, de la même manière que chute l'activité des fourrageuses dans les populations de chélidoine (cf. ci-dessus). En août, notre temps d'échantillonnage ne nous a pas permis d'observer d'entrée ni de sorties de fourmis transportant des graines. La proportion de fourmis transportant une graine parmi les fourmis sortant du nid est toujours plus faible qu'en entrant, et est curieusement toujours égale à environ 30 % de la proportion entrante (sauf pour la semaine 26). Il est raisonnable de penser que cette différence entre les graines entrées et sorties peut correspondre aux graines abandonnées dans le nid après la consommation de l'élaiosome.

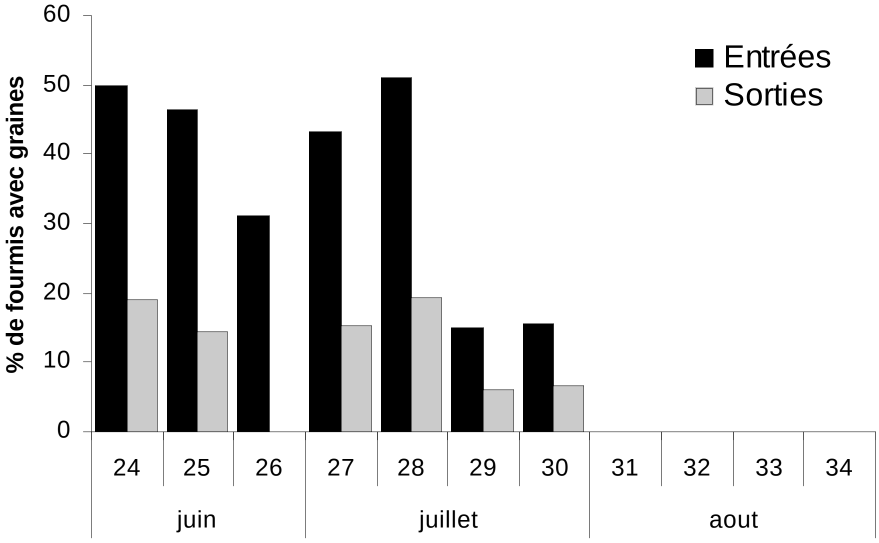

Figure B. Pourcentages de fourmis *Lasius niger* transportant des graines de *Chelidonium majus* dans les flux d'entrées et de sorties du nid de fourmis.

Les mesures de terrain indiquent, à l'instar des résultats de laboratoire, que la relation myrmécochorique dépend fortement des espèces de plantes et de fourmis. Par ailleurs, nous confirmons que la chélidoine peut jouer un rôle écologique majeur tant par son ubiquité (présence même en ville), que par la quantité de graines produites ou encore l'affinité que les fourmis ont pour ses graines.

Chapitre 2

Opening myrmecochory's black box: what happens inside the ant nest?

Abstract

In seed dispersal by ants, workers bring diaspores back to their nest, eat the elaiosome and abandon viable seeds outside or inside the nest. Here, we investigated what happens inside the nest in laboratory experiments for two myrmecochorous plant species and two seed-disperser ant species. We showed that the stay duration of seeds inside the nest were ant-specific and was shorter-lasting with the scavenger ant *Myrmica rubra* than with the aphid-tender *Lasius niger* species. Other issues like the number of seeds in direct contact with larvae and the rates of elaiosome removal depended on both ant and seed partners. Elaiosome removal did not automatically elicit seed rejection: seeds could stay for a while within the nest even if they had no elaiosome left, and conversely, they could be rejected with an elaiosome still attached. Such variability between-species in the impact of elaiosome removal on seed dispersal is unexpected and confirms the complexity of the ecological network of ant-seed interactions.

Keywords: seed dispersal, ant nest, elaiosome, *Myrmica rubra*, *Lasius niger*, *Chelidonium majus*, *Viola odorata.*

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Introduction

To achieve their reproductive cycle, plants require the movement of pollen and seeds across space, which is often provided by animals [1]. In pollination systems involving animal vectors, movements have distinctive and predictable targets (stigma of conspecific flowers) with incentives provided by the target (pollen, nectar) that control animal movements. On the other hand, seed dispersal systems have spatially unpredictable targets (germination sites) with no incentives, involving many disperser species that are seldom seed-specialized [1, 2]. The fate of animal-dispersed seeds is then highly variable and depends strongly on vector traits like diet, morphology and behaviour.

In the case of seed dispersal by ants (myrmecochory), seeds are transported by many different disperser ants that are not interested in the seed itself but in the nutritive lipid-rich appendage called elaiosome. Because disperser ants are central place foragers, three distinct stages can be recognized in the myrmecochory process: first, ants collect seeds near the parent plant and bring them back to the nest in a centripetal movement to a central place (stage I). The second stage takes place inside the ant nest where the elaiosome is usually eaten (stage II). Once elaiosomes are discarded, seeds remain generally viable but do not interest ants anymore. As refused items, seeds can be abandoned inside the nest or actively rejected outward in a centrifugal movement (stage III). There are considerable amount of data in the literature about the first and the third stage, but although the nest is as a place where ants take crucial decisions for seed fate, the ant-nest stage (II) is poorly known and remains a black box.

In each of these stages, the issues as well as the outcomes are quite different for both partners –i.e. the ant and the plant species. From the seed perspective, two issues are primordial in the first stage: their speed of removal and their dispersal distances. The speed of seed removal depends on ant traits such as body size [3], diet [4], learning [5] or phenology [6] and permits seeds to avoid predation by rodents, birds or beetles [7, 8]. The dispersal distance from the mother plant, that depends mainly on the body size [9] and nest community structure of ants [10], permits seeds to avoid competition with parent plants [11, 12]. In the case of the rejection stage (stage III), the main issues for seeds are the distances and quality of germination sites at which seeds are secondarily relocated. The resulting various patterns of seed relocation [13-15] can depend on the territorial range of the ant colony and the dropping rates during seed transport [16]. Concurrently, the outcome of the third stage also depends on the capacity of ants to place seeds in nutrient-rich sites (e.g., refuse piles) that can enhance seedling survival [16-18].

Since data are lacking on the second stage of the myrmecochory process, the present study investigates how seeds are managed by inner-nest workers by following the fate and the movements of seeds within the nest. By carrying out laboratory experiments in controlled conditions, we could observe ant behaviour directly inside the nest and answer the following questions: How fast are elaiosome removed and seeds rejected? Where are seeds located? How are they managed by ants inside the nest?

As a corollary, we aimed at determining whether seed management varies depending on the ant or on the seed species. For this purpose, we carried out a crossed experimental design involving two ant species (*Myrmica rubra* and *Lasius niger*) and two seed species (*Chelidonium majus* and *Viola odorata*). A previous study has shown that the omnivorous and carnivorous ant species *Myrmica rubra* removes both *Chelidonium majus* and *Viola odorata* seeds in larger number and more quickly than the aphid-tending species *Lasius niger* [19]. One may wonder whether this quicker seed harvest is related to a shorter or a longer stay of seeds inside the nest. Moreover, we know that seeds were all rejected outwards in the refuse piles with only half of their elaiosomes being discarded by ants. The only exception was the pair *Myrmica rubra/Chelidonium majus* since all the elaiosomes of rejected seeds were removed by ants [19]. We shall investigate how those between-species differences in seed fate take origin inside the ant nest. In particular, we will relate the dynamics, the location and the duration of seed stay within the nest to the rates of elaiosome removal among rejected seeds. Finally we will discuss the possible ant and seed traits that may affect the outcome of ant-seed interactions within the nest.

Materials and methods

Ant species, collecting and rearing

The black garden ant *Lasius niger* (L.) is 3 to 4 mm in length, very common in European temperate regions, and forming monogynous colonies which can contain up to 13,000 workers [20]. This species mainly feeds on honeydew [21], but can occasionally eat aphids [21], dead or small alive insects [22] as well as elaiosomes [23, 24]. The red ant *Myrmica rubra* L. is 4 to 6 mm in length, also common in European temperate regions, and forming polygynous colonies which can reach 6,000 workers [25]. Its diet is mainly insectivorous, composed by dead or alive prey items, but has also been reported as consuming sugars or elaiosomes [26-29].

We collected six colonies of *Lasius niger* from earth banks in Brussels (Belgium) and six colonies of *Myrmica rubra* in La Gorgue (France). In the laboratory, colonies were reared in plaster nests covered by a glass plate through which the ants could be observed (Janet type, 10 x 10 x 0,4 cm) and placed in arenas (50 x 38 x 10 cm) with Fluon-coated borders to prevent ants' escape. Nests were regularly moistened and kept at 21±1°C, 35±5% relative humidity and a constant photoperiod of 12h per day. All colonies contained 200-300 workers with brood covering approximately 10% of the nest surface. *Lasius niger* colonies were queenless and *Myrmica rubra* ones contained 5 to 10 gynes. We supplied ants *ad libitum* with water and sucrose solution (1M), and twice a week with cockroaches (*Periplaneta americana*) and an artificial diet with proteins, sugars and vitamins [30].

Plant species and seed storage

Viola odorata L. is a perennial plant of temperate forests, edges, bushes, shrubbery and ruderal habitats [31], widespread in Belgium, capable of vegetative reproduction. Seeds are yellow-brown (approx. 3 mm length) with white-coloured and soft cone-like basal elaiosomes (approx. 2 mm length). *Chelidonium majus* L. is a perennial plant, common in Belgium and growing in ruderal habitats, old walls, hedges or small groves [31]. Seeds are dark brown and small (approx. 2 mm length) with white and fleshy elaiosomes (approx. 1.5 mm length). After collection, seeds were stored at -18°C, as it does not alter significantly seed attractivity to the ants [19].

Experimental procedure

Four ant-plant pair combinations were compared: *Lasius/Chelidonium* (LC), *Lasius/Viola* (LV), *Myrmica/Chelidonium* (MC) and *Myrmica/Viola* (MV). We used six colonies of *Myrmica rubra* and six colonies of *Lasius niger*. Each of the six colonies has been tested three times per seed species (i.e. 18 experiments for each ant-plant pair). For each experiment, we presented 15 seeds at 10 cm from the nest entrance and we let ants take 10 seeds into the nest during at most 10 minutes. Inside the nest, we noted the seed location, whether they were laid down in contact with larvae, how many workers were in contact with them and the presence of the elaiosome, every 30 minutes during six hours.

The nest (10 x 10 cm) was divided by a grid of 100 cm^2. At the beginning of each experiment, we noted the presence or absence of workers and brood in each square centimetre. The grid was divided in three areas: the brood areas (all squares containing at least one larvae or pupae), the worker areas (squares in which at least one worker stay at least 10 seconds and containing no brood) and empty areas (squares containing no brood nor workers staying for at least 10 seconds).

We counted the number of ants in contact with seeds –meaning every worker that either antennated, manipulated or displaced a seed. A seed was considered as being in contact with brood as soon as it was deposited by workers over the larvae or at least within a grid square containing at least one larva or pupa.

Results

Dynamics of elaiosome removal and seed rejection

Seeds stayed inside the nest for a while before being rejected with or without their elaiosome gradually from the first 30 minutes until the end of the experiment (Fig 1).

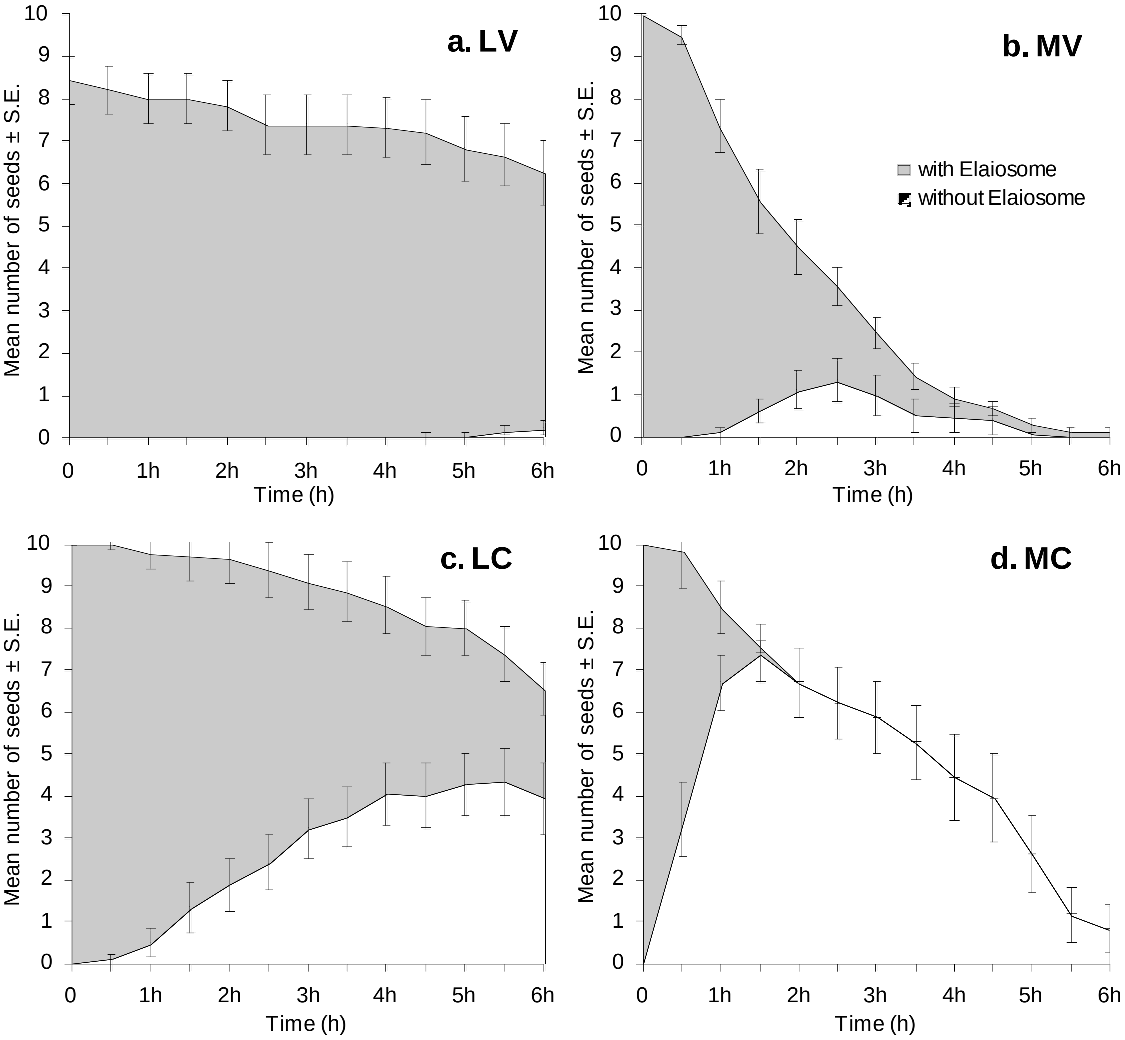

Figure 1. Dynamics of the mean number (± S.E., n=18) of seeds with their elaiosome (full) and without elaiosome (hatched) within the nest.. a. *Lasius/Viola*. b. *Myrmica/Viola*. c. *Lasius/Chelidonium*. d. *Myrmica/Chelidonium*.

Exponential fittings on the dynamics of global number of items in the nest, calculated on Ln transformed data (Fig. 2), indicated that the stay duration of seed items within the nest was highly variable, mostly ant-specific and unexpectedly depended weakly on plant species. *Lasius* ants rejected items of both plant species slowly at a constant rate. These rates were given by slopes of the regression lines with a probability of 2.10^{-4} for an item to be rejected per minute for *Chelidonium majus* and of 10^{-4} for *Viola odorata*. By contrast, *Myrmica* ants rejected both seeds quite faster with a probability of 9.10^{-4} for an item to be rejected per minute for *Chelidonium majus* and 21.10^{-4} for *Viola odorata*. It clearly appears that *Lasius* ants kept items inside the nest during a longer period of time than *Myrmica* ants, and this effect was even stronger with *Viola odorata*.

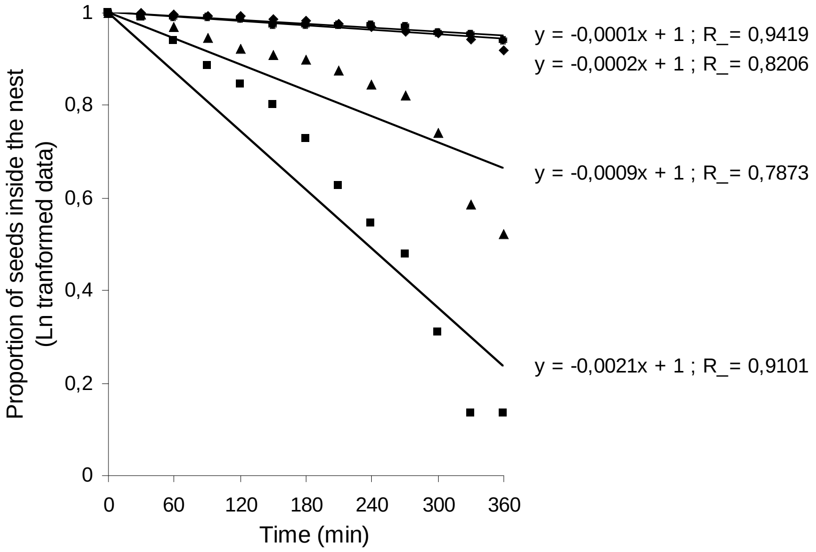

Figure 2. Dynamics of the percentage of items still present inside the nest (entire seeds + seeds without elaiosome) for *Lasius/Viola* (circles), *Lasius/Chelidonium* (diamonds), *Myrmica/Viola* (squares) and *Myrmica/Chelidonium* (triangles). Percentages are Ln transformed and best fitted with linear regression.

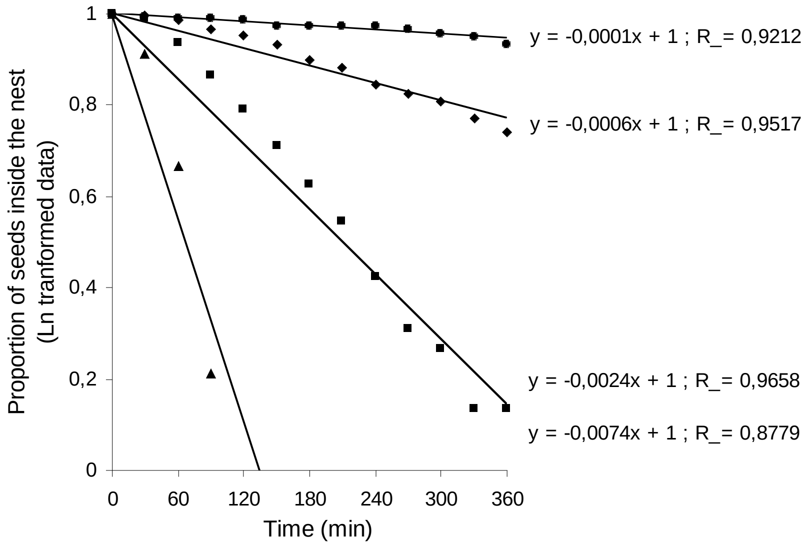

Figure 3. Dynamics of the percentage of entire seeds still present inside the nest for *Lasius/Viola* (circles), *Lasius/Chelidonium* (diamonds),

Myrmica/*Viola* (squares) and *Myrmica*/*Chelidonium* (triangles). Percentages are Ln transformed and best fitted with linear regression.

Once entered into the nest, seeds do not remain entire since inner-nest workers begins to detach their elaiosome. The dynamics of elaiosome removal differed depending on both partners (Fig. 1). As regard the ant effect, *Myrmica rubra* discarded elaiosomes quicker than *Lasius niger*. The results also indicated a plant effect: *C. majus* elaiosomes were discarded quicker by both ant species than *V. odorata* elaiosomes. When combined, dynamics of elaiosome removal show strong differences between ant-seed pairs. For example *Myrmica rubra* ants succeeded in removing all *C. majus* elaiosomes within only two hours, whereas it took about 6 hours to *Lasius niger* ants before removing only 3.3 % of the total number of *V. odorata* elaiosomes.

The number of entire seeds (E) inside the nest decreased because of two processes: the elaiosome removal (i.e. they were no more entire seeds) but also the rejection of entire seeds outside the nest. We then calculated the global probabilities of each event to happen: the probability to remove elaiosomes (k_1) and the probability to reject entire seeds out of the nest (k_{2e}). The decreasing number of entire seeds in the nest follows the equation (1):

$$E_{in} = e^{-(k_1+k_{2e})t} \qquad (1)$$

The curve of the number of entire seeds (E) inside the nest has an exponential shape and the slope of its regression line of ln-transformed data gives thus the term (k_1+k_2e), which represents the mean probability of entire seeds to decrease in the nest per time unit (Fig. 3). Besides, the number of entire seeds outside the nest increases over time and is given by the equation (2):

$$E_{out} = \frac{k_{2e}}{k_1 + k_{2e}}(1 - e^{-(k_1+k_{2e})t}) \qquad (2)$$

The coefficient $(\frac{k_{2e}}{k_1 + k_{2e}})$ is given by the slope of the regression line of the correlation between the number of entire seed inside the nest and outside the nest. This coefficient represents the relative contribution of rejection into the disappearance of entire seeds within the nest. With the two coefficients given by the equations (1) and (2), we deduce k_1 and k_{2e} (Table 1). The inverse function of these probabilities represents the mean time before the elaiosome is removed (T_1) and the mean time before an entire seed is rejected out of the nest (T_{2e}).

Table 1. Probabilities (seeds/min) of elaiosomes to be removed from entire seeds (k_1), of entire seeds to be rejected out of the nest (k_{2e}) and of seeds without elaiosome to be rejected (k_{2d}). Mean time (min) before seeds had their elaiosome removed (T_1), that entire seeds remain in the nest (T_{2e}), and that seeds without elaiosome remain in the nest (T_{2d}). "No data" means that the probabilities could not be calculated because no items were rejected.

	k_1	T_1	k_{2e}	T_{2e}	k_{2d}	T_{2d}
Lasius/Viola	8.3 E-6	12 E4	4.92 E-4	2034	no data	no data
Lasius/Chelidonium	2.7 E-3	371	6.03 E-4	1660	1.4 E-3	706
Myrmica/Viola	7.6 E-3	132	64.2 E-4	156	33 E-3	30
Myrmica/Chelidonium	45.3 E-3	22	no data	no data	5.1 E-3	196

The ratio between the probabilities to remove elaiosomes and to reject entire seeds (cf. Table 1) can be seen as a first estimate of the benefit of myrmecochory for ants. For instance, if $k_1 > k_{2e}$, elaiosomes are more likely to be removed than entire seeds to be rejected: ants are efficient in consuming the nutritive bodies of seeds, reducing the loss of entire diaspores and are thus expected to benefit from myrmecochory. On the contrary, if $k_1 < k_{2e}$, the rejection of entire seeds is more likely to occur before the elaiosome consumption and one should expect less nutritional benefit for the ant colony Here, only the ant/plant pair *Lasius/Viola* has a probability of remove the elaiosomes lower that the probability of rejecting entire seeds. In fact, nearly no elaiosome were detached within 6 hours (Fig. 1) and all items that were rejected were entire seeds (Fig. 4). For all the other ant-plant pairs, the probability of detaching elaiosome is higher than that of rejecting entire seeds. As rule, for a given seed species, *Myrmica* foragers are much more efficient than *Lasius* one in discarding the elaiosome. Moreover, for both ant species, we found out an important effect of the plant species on the elaiosome removal with *Chelidonium* seeds being more easily discarded than *Viola* ones. As a result, there is a variable composition of rejected items depending on the ants/plants involved. On the one hand, *Lasius niger*, that was a slow seed-rejecting and even slower elaiosome-discarding ant species, rejected only entire seeds of *Viola odorata* out of the nest (Fig. 4a). On the other hand *Myrmica rubra*, a fast seed-rejecting and elaiosome-discarding ant species, rejected *Chelidonium majus* seeds that were all deprived of their elaiosome (Fig. 4d). Between these two extreme cases, approximately half of the rejected seeds were entire ones and half were elaiosome-free for the pairs *Myrmica rubra/Viola odorata* and *Lasius niger/Chelidonium majus*, (Fig. 4b and 4c).

We showed here one of the mechanisms that generate differences between each ant/plant pair after the nest stay and that position the myrmecochory process in a continuum between antagonism and mutualism.

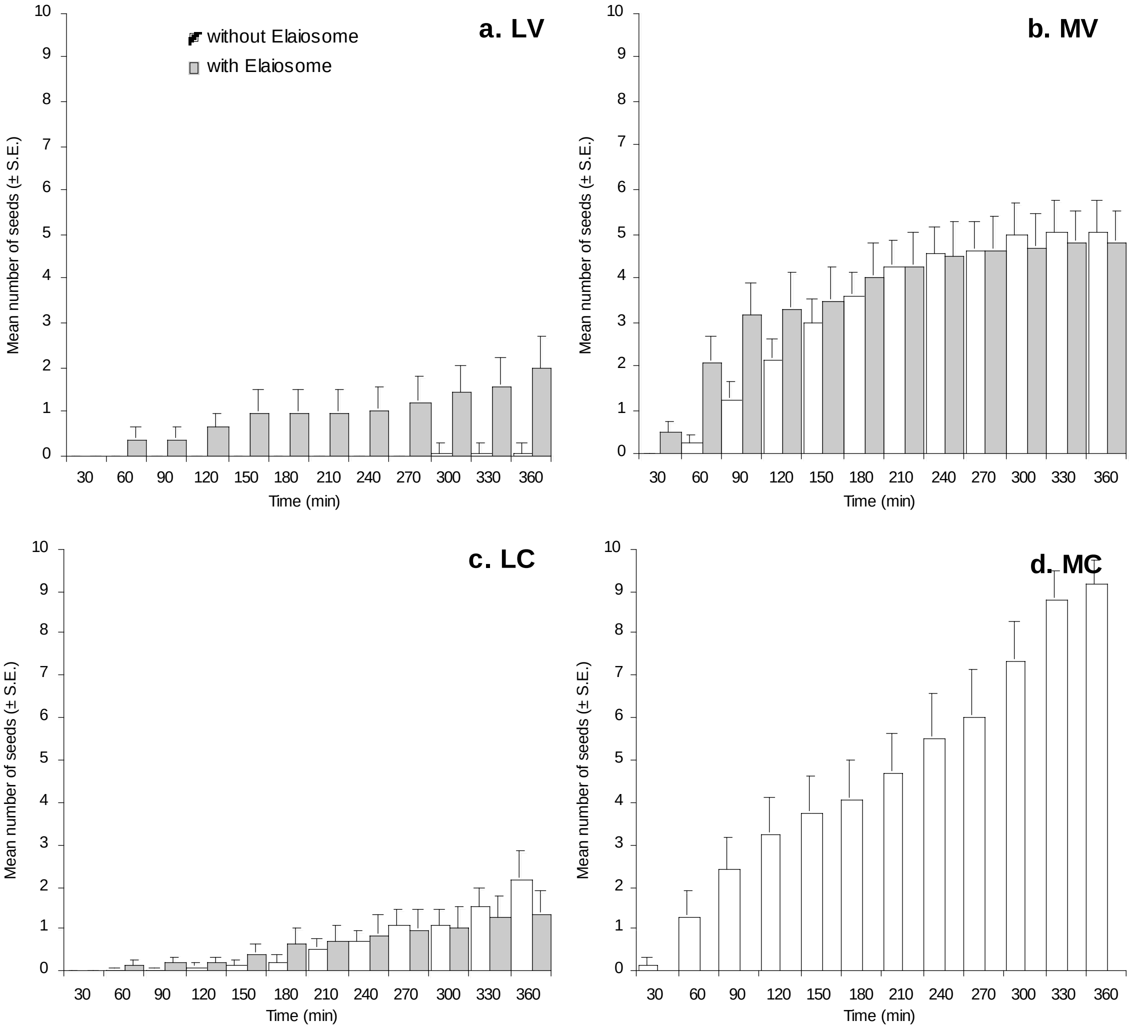

Figure 4. Cumulated number (mean ± S.E., n=18) of entire seeds (full) or seeds without elaiosome (hatched) that are rejected outside the ant nest.

As we saw, the removal of elaiosomes from seeds was only gradually achieved by the ants. Once discarded, seeds were not immediately rejected outside the nest and could be found concurrently with entire seeds by inner-nest workers. These workers behave in a quite different way depending on whether seeds still had or not an elaiosome. Indeed, a variable number of inner-workers (between none and four ants) could be seen manipulating and chewing seeds, but on average, there was a significantly lower number of ant workers in contact with seeds without elaiosome than with entire seeds of the same species (Table 2).

Table 2. Mean number of ants (± S.D.) in contact with entire seeds (E) and seeds without elaiosome (D) (all observations pooled per ant-seed pair); Mann-Whitney Test..

	E	n	D	n	p value	
Lasius/Viola	1.03 ± 0.92	1473	1 ± 0.82	4	Too few data	
Lasius/Chelidonium	0.61 ± 0.62	1227	0.31 ± 0.48	498	<0.0001	***
Myrmica/Viola	1.89 ± 1.17	736	0.33 ± 0.53	99	<0.0001	***
Myrmica/Chelidonium	1.51 ± 0.73	326	0.55 ± 0.61	970	<0.0001	***

This indicates that ants lost their interest in seed items as soon as the elaiosome was removed. But although the removal of elaiosomes leads to a loss of ants' interest for the seed, it is not consistently followed by a rejection of the seed out of the nest. Indeed, it was not rare to observe a seed without elaiosome inside the nest (Fig. 1, hatched). Conversely and quite unexpectedly, seeds could also be rejected outwards despite they still bore an elaiosome (Fig. 4). In order to assess the impact of elaiosome removal on the dynamics of rejection, we calculate the probability to reject seeds once their elaiosome was removed (k_{2d}). The number of seeds without elaiosome (D) increases with the number of elaiosome removed ($k_1.E$) and decreases with the rejection of seeds without elaiosomes ($k_{2d}.D$). The time evolution of the number of seeds without elaiosome inside the nest is thus given by the following equation (3):

$$\frac{dD}{dt} = k_1 E - k_{2d} D \qquad (3)$$

By integrating (3), we obtain the number of seeds without elaiosome inside the nest (4).

$$D = \frac{k_1\left(e^{-k_{2d}t} - e^{-(k_1+k_{2e})t}\right)}{k_1 + k_{2e} - k_{2d}} \qquad (4)$$

We then plotted our observed data and fitted them with the equation (4), thus obtaining the best value of k_{2d} (Table 1).

We see that the probability to reject a seed without its elaiosome is always higher than the probability to reject a seed with its elaiosome (Table 1). This shows the loss of the elaiosome enhances the rejection of the seed by the inner-nest workers.

Finally, during the whole experiment, we found no significant correlations between the percentage of entire seeds among items remaining inside the nest, and the percentage of entire seeds among rejected items during the following half hour (*Myrmica/Viola*, R^2=0.0215; *Myrmica/Chelidonium*, no entire seeds rejected; *Lasius/Viola*, R^2=-0.0066; *Lasius/Chelidonium*, R^2=-0.03). This means that what is rejected is not directly related on what remains inside the nest, and suggests that ants do not reject items on the basis of a global assessment of seed quality and seed quantity within the colony.

Spatial dynamics of seeds within the nest

Once seeds were brought by foragers inside the nest, they were deposited at one of three following locations: 1. Seeds were put near inner-workers that immediately contacted, antennated and manipulated them between their mandibles; 2. Seeds were deposited over the brood pile in contact with larvae and pupae; 3. Seeds were put in empty areas of the nest, they were not contacted and neglected by workers at least for a while. The proportions of seeds left at one of these three locations changed over time and depended on each ant-seed pair (see Fig. 5). A few instants after the seeds were brought inside the nest, the proportion of seeds left alone in empty places (0% for both plant species with *Myrmica rubra*; 31.2 % for *V. odorata* and 17.5% for *C. majus* with *Lasius niger*) was lower than the proportion of empty places in the nest, which represented nearly half of the nest surface (52.2 ± 8.1 %, n=36 for *Lasius niger*, and 42.2 ± 7.8 %, n=36 for *Myrmica rubra*). This means that the majority of retrieved seeds were actively directed at locations where they can be treated and/or eaten by either nestmate workers or brood.

However, we found out an ant-specific trend in the way seeds were brought in contact with brood. Once seeds had been brought inside the nest (t=0), *Lasius niger* workers let some seed items at empty places (*Viola odorata*: 2.9 ± 2.7 seeds, n=18; *Chelidonium majus*: 1.7 ± 0.5 seeds, n=18). Then, ants gradually brought seed items close to the brood so that the number of seeds in contact with larvae increased progressively (Fig. 5a and 5c). After 3 hours, the mean number of seeds in contact with larvae reached a maximum, growing from 0.3 to 2 for *Viola odorata* and from 1.4 to 6.3 for *Chelidonium majus* (n=18). By contrast, *Myrmica rubra* treated immediately all the seeds that were retrieved to the nest and the maximum number of seeds put in contact with larvae was reached since the beginning with *V. odorata* and after 30 min (a mean of 5.1 seeds) for *C. majus*. Since the very beginning, 0.7 ± 0.3 (n=18) *Viola odorata* seeds and 3.6 ± 3.3 (n=18) *Chelidonium majus* seeds (out of 10) were put in contact with brood. Over time, the number of seeds in contact with larvae decreased while their number concurrently increased at empty locations due to the loss of interest of workers for seeds of which elaiosome had been removed (see Table 1).

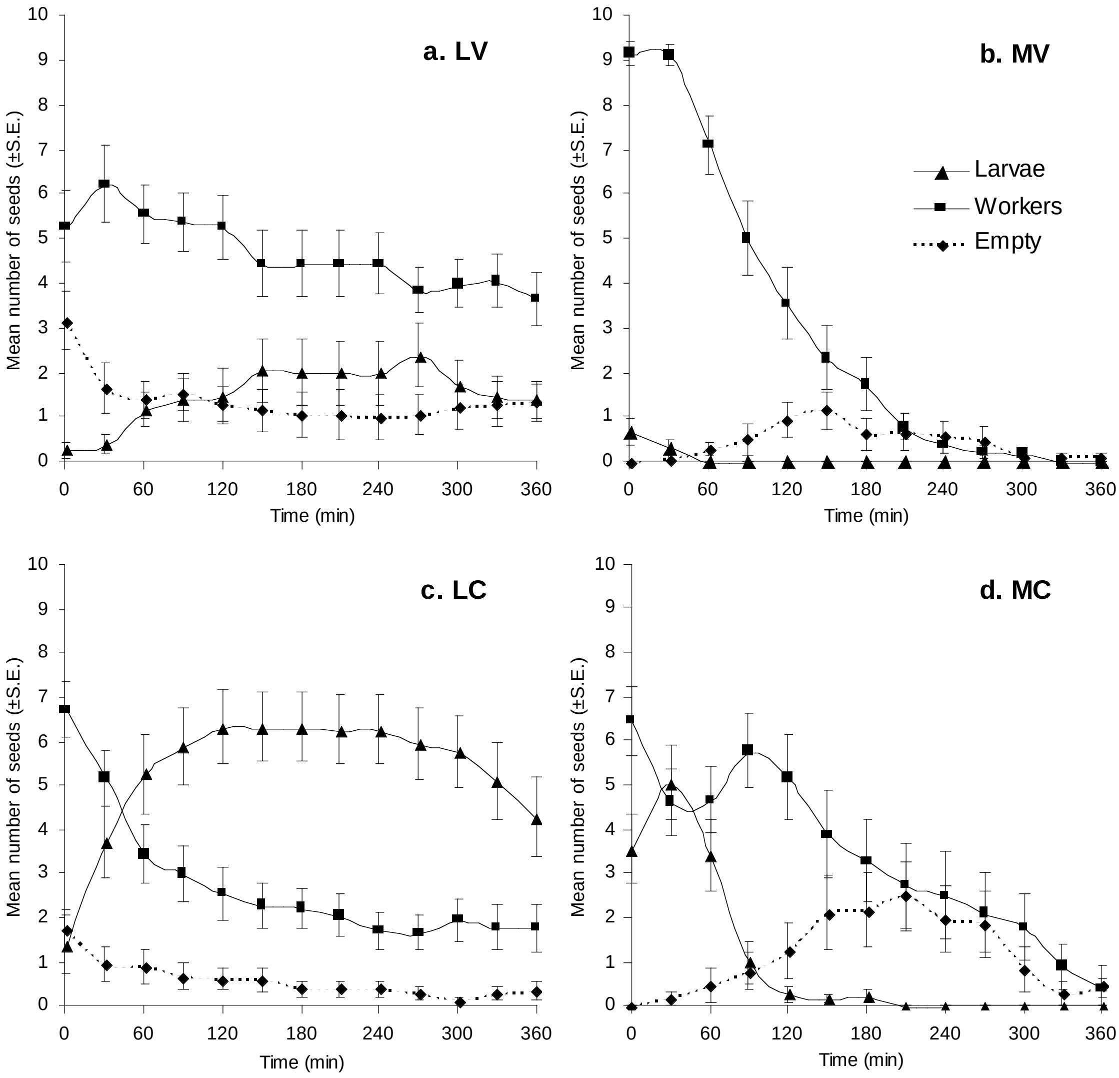

Figure 5. Mean number (± S.E., n=18) of seeds inside the nest in contact with larvae (triangle), workers (square) and in empty places (circle).

There was also a plant specific trend in the location of seeds. For each ant species, more *Chelidonium majus* seeds were put in contact with brood than *Viola odorata* ones. Thirty minutes after seed introduction into the nest, -when nearly all seeds still had their attractive elaiosome bodies, the percentage of *Chelidonium majus* seeds deposited by ants on brood (*Lasius niger*: 37.2% of seeds; *Myrmica rubra*: 51.4% of seeds) was higher than expected from a random distribution non-sensitive to brood presence (*Lasius niger*: 12% ; *Myrmica rubra*: 9%, which represents the percentage of nest surface occupied by the brood) (Wilcoxon signed ranks test, two-tailed P = 0.0003 for *Myrmica rubra* and P = 0.0304 for *Lasius niger*). Thus, workers perceived the brood piles as preferred sites for depositing *Chelidonium majus* seeds in comparison to the rest of the nest. Conversely, for *Viola odorata*, the percentages of seeds brought in contact with brood (*Lasius niger*: 4%; *Myrmica rubra*: 3.5%) were lower than expected from random (*Lasius niger*: 12%; *Myrmica rubra*: 9%) (Wilcoxon signed ranks test, two-tailed P = 0.0003 for *Myrmica rubra* and P = 0.0007 for *Lasius niger*). Indeed, *V. odorata* seeds were scattered all over the nest but mainly near the nest entrance so that they scarcely reached the brood that was usually located at the back of the nest.

When combined, the ant- and plant-specific trends resulted in extremely different patterns of seed processing within the nest. If we take into account the co-occurrence of seeds and larvae, we found out two extreme cases. On the one hand, in the *Myrmica rubra*/*Viola odorata* pair, seeds were immediately treated by workers and left in contact with larvae during a short period of time (less than one hour) in few quantities (mean of 0.08 ± 0.19 seeds in contact with brood during the 6h experiment; n=13). However, these few contacts with larvae do not prevent elaiosomes to be removed at high rates, probably because workers have a good ability to detach elaiosomes. Once the elaiosomes removed, seeds were left in empty places before being quickly rejected. On the other hand, in the *Lasius niger*/*Chelidonium majus* pair, seeds were progressively put in contact with brood for a long while so that seeds are accumulating over the brood pile and are rejected very slowly. Approximately half of the seeds (5.3 ± 1.4) were in contact with brood during the whole experiment. These quite different ways of seed processing ultimately lead to a similar proportion of elaiosomes discarded at the end of the experiment (*Myrmica rubra*/*Viola odorata*: 52%; *Lasius niger*/*Chelidonium majus*: 64% of the total number of seeds brought inside the nest).

Discussion

Elaiosome removal: a combined effect of ant and seed specificities

Elaiosome removal depends on ant species as well as on plant species. On the ant side, *Myrmica rubra* ants discarded elaiosomes faster than the black garden ant *Lasius niger*. On the plant side, ants discard *Chelidonium majus* elaiosomes in higher proportions than *Viola odorata* ones. Then, inside the nest, the ant-seed pair *Myrmica rubra* / *Chelidonium majus* appears to be the most "efficient" in the myrmecochory process: a rapid treatment of seeds and the highest rate of elaiosome removal. However, this does not necessarily mean that the benefits for ants and seeds are maximal. Further studies are needed to link the efficiency of seed treatment to increased benefits for partners in terms of seed fate, seedling survival or ant colony growth. The high efficiency of within-nest treatment of *Chelidonium majus* seeds by *Myrmica rubra* ants is coherent with previous "outside-the-nest" experiments that showed a faster retrieval of *Chelidonium majus* than for *Viola odorata* seeds by *Myrmica rubra* as well as a higher proportion of discarded elaiosome after seed transit within the nest [19]. The smaller size of *Chelidonium majus* elaiosomes (approx. 0.8 mm width) compared to *Viola odorata ones* (approx. 1.65 mm width), or probably their consistency, may explain their easier removal by ants. Indeed, the size of these attractive bodies better fits to the ant mandible opening width (approx. 1 mm), giving to the ants a mechanical facility to handle or chew elaiosomes.

Seed rejection: an ant-specific phenomenon

Surprisingly, the dynamics of seed rejection outside the nest depended mainly on the ant species. Seeds treated by *Myrmica rubra* were almost all rejected within six hours with a mean stay duration in the nest of 132 min (*V. odorata*) and 22 min (*C. majus*), rejecting them nearly ten times faster than *Lasius niger*. These retention times were comparable to tropical carnivorous ant species that "*bit elaiosomes during two to eight hours of intermittent attention*" [33]. Remarkably, the rejection dynamics were ant-specific and were similar for both plant species, whenever seed items were still bearing or not an elaiosome. As a consequence, the elaiosome removal and the seed rejection were two phenomena not as closely coupled as initially expected. Seeds could stay in the nest even if they had no elaiosome left, and conversely, they could be rejected even with an elaiosome still attached. Some few cases of entire seeds rejected have already been reported previously [19, 34, 35], and our results confirm that not all elaiosomes are automatically eaten by ants during the nest stage. It could be interesting to determine the role of worker age in this rejection process. Indeed, it was shown that young workers of *Aphaenogaster senilis* performed the nest cleaning activities and tended to transport the seeds of *Euphorbia characias* far from the nest while older workers tended to ignore seeds inside the nest and take them to the nest (Gomez and Espadaler 1998).

Future investigations could also focus on how the dynamics of seed rejection and dispersal by ants is related to the way ant colonies manage refuse items. One may wonder whether elaiosome have similar rejection dynamics as other items such as nest building materials, inedible parts of preys or nestmate corpses [32, 36]. Some carnivorous ant species (and particularly in warm and humid climates) may have developed hygienic behaviours in which they treat and reject preys quickly [37]: this "hygienic" behaviour may have facilitated the seed rejection phase of the myrmecochory process.

From an ultimate perspective, if we assume that the main benefit of myrmecochory for ants is a quantitative nutritional gain [38-40], our study showed that this gain can be highly variable, and depending on both partners. From the ant perspective, if the outcome is negative (i.e. the benefit of elaiosome consumption is lower than the cost of seed transport), we should be in a case of ants "parasitized" by plants for which ants may have developed mechanisms like learning or satiation to avoid such costs [5, 41]. Future studies should expect a high inter and intra-pair variability of outcomes, placing each case in a continuum that vary from antagonism to mutualism [42]. Moreover, one must also take in account that outcomes of interactions can also vary geographically and temporally in the field [43, 44], generating a dynamic geographic mosaic of coevolution [45, 46].

The brood

We showed here that some seeds brought in the nest were deposited directly in contact with brood. A reversed phenomenon has been observed with the ant *Aphaenogaster rudis* for which inner-nest workers brought directly larvae onto the seeds of *Carex pedunculata*. Larvae then chew elaiosomes before workers transported seeds towards refuse piles [11]. Furthermore, we showed that the period during which some seeds were put in contact with brood was ant-specific, shorter for *Myrmica rubra* than *Lasius niger*, which confirms that timing issues in the nest are mainly dependent on ant traits. Besides, the maximum number of seeds in contact with larvae varies with seed species and is higher for *Chelidonium majus*. This confirms that variations of interaction outcomes are dependent on combined effects of both partners [19]. An interesting perspective will be to understand why seeds are preferentially deposited close to larvae. The "brood mimicry hypothesis" [24, 47] stated that ants carry seeds to the brood chambers because they mimic the odour of larvae. Indeed, seed removals could be elicited by triolein compound [48, 49], which is known to be a major brood-tending pheromone in *Solenopsis invicta* [50]. This hypothesis has to be further investigated by comparing chemical compounds of *Viola* and *Chelidonium* seeds as well as of *Myrmica* and *Lasius* larvae, and by testing them in behavioural assays.

Our results showed that *Chelidonium majus* seeds were put preferentially in contact with brood and had their elaiosomes detached at higher rates than *Viola odorata*. This suggests that ant larvae feed directly on *Chelidonium* elaiosomes. However, it is likely that workers may also take the elaiosomes off the seeds in order to give them to the larvae, because *Myrmica rubra* ants put a very few *Viola odorata* seeds in contact with their larvae but still get 54% of elaiosomes removed. In both cases, larvae should be the final elaiosome sinks as shown for *Myrmica rubra* larvae that benefit from the nutritional gain of elaiosomes of *Corydalis cava* and *Scilla bifolia* [38, 39]. Larvae should be then a central factor controlling the elaiosome retrieval by foragers, and thus the whole myrmecochory process.

The time parameter

Timing plays a crucial role in seed dispersal by ants. First because ant-seed interactions strongly depend on seasonal and daily phenologies of both ant and plant species [6, 23]; secondly because the window of attractivity after seed release due to desiccation is variable (cf chapter 3); finally because the duration of the nest stay could play a role in seed germination by the degree of scarification, or by interfering with seed dormancy due to the elaiosome removal. It has been demonstrated that germination rates are higher when elaiosomes are removed after one or two days after the seed release than immediately after this release [33, 51]. The matching between this "delayed elaiosome removal" (plant trait) and the seed-rejection delay (ant trait) should influence seed fate. In fact, to germinate inside the nest can be unfavourable if the nest is too deep [52], but to be rejected outside the nest too early can expose to post-dispersal predation [53]. In this perspective, our experiments showed that the seed-rejection delay is ant-specific and do not depend on seed traits.

Furthermore, the nest stage is undoubtedly the longest period of contact between ants and seeds during the myrmecochory process. It is possible that the longer the stay, the higher the probability of scarification of seeds by workers. Such a correlation has never been measured, but we already know that seed scarification and elaiosome removal can have a positive [54] or negative [55] effects on germination rates. For example, seeds of *Chelidonium majus* that spent a few hours in a *Lasius niger* nest had lower germination rates (whatever they still have an elaiosome or not) than seeds that spent the same time without ants (Servigne, unpublished data).

We highlight here that outcomes of the nest stage depends strongly on both partners and that some issues like the role of larvae and the nest timing could be crucial parameters determining the partner choices in myrmecochory [56]. The nest stage is far from being clear and opens new research perspective in ant physiology and behaviour, as well as in ecology of seed dispersal.

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Résultats annexes

Influence de la scarification sur la germination des graines

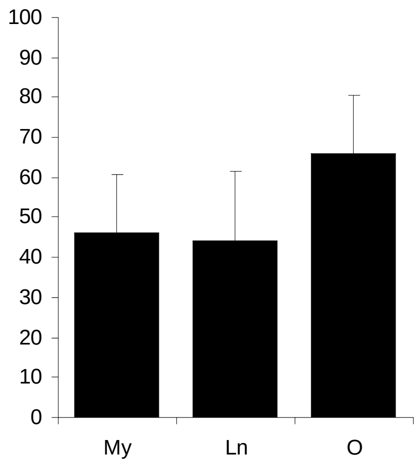
Si de nombreuses études montrent que le traitement des graines par les fourmis (scarification et arrachage de l'élaiosome) a un effet positif sur la germination des graines (Ridley 1930, Beattie 1978, Culver & Beattie 1980, Horvitz & Beattie 1980, Lobstein & Rockwood 1993, Gibson 1993, Horvitz & Schemske 1994, Gomez & Espadaler 1997, Martins et al. 2006), d'autres montrent qu'il n'a aucun effet (Slingsby & Bond 1985, Kaufmann et al. 1991, Lobstein & Rockwood 1993, Lisci 1996, Boyd 2001, Christian & Stanton 2004), voire un effet négatif (Bond & Slingsby 1984, Dalling & Wirth 1998, Zettler *et al. 2*001, Imbert 2006). Tout dépend donc des espèces en jeu. Des essais de germination ont été réalisés avec nos espèces pour mesurer l'impact des fourmis sur la germination des graines.

Matériels et méthodes

Pour chaque plante (*Viola odorata* et *Chelidonium majus*), trois groupes des graines ont été testées : un groupe ayant séjourné 24h dans une colonie de *Myrmica rubra*, un autre dans une colonie de *Lasius niger* et un autre était resté 24h à coté des colonies (mêmes conditions, 21 ± 1 °C). Chaque groupe de graines (avec l'élaiosome mâché ou arraché) a ensuite été planté dans six bacs de 25 graines (5 x 5). L'expérience a été répétée l'année suivante (2005 & 2006). La germination des graines de *Viola odorata* a échoué la première année. Ainsi, la deuxième année, les bacs contenant les graines de *Viola odorata* ont été placés 4 semaines à 8°C afin de lever la dormance des graines. Mais sans plus de résultats. Dans les bacs de *Chelidonium majus*, nous avons compté les plantules lorsque la plus petite (ou tardive) possédait une feuille (en plus des deux cotylédons).

Résultats et discussion

Les essais de germination montrent que les graines de *Chelidonium majus* germent mieux sans traitement par les fourmis (Kruskall Wallis test, $p = 0.0054$). Le traitement des graines par les deux espèces de fourmis n'est pas significativement différent (Kruskall Wallis test, post-hoc test, $p>0.05$). Les graines de *Chelidonium majus*, malgré leurs importants taux de prises, et leur importance numérique sur le terrain, germent donc moins bien après traitement par les fourmis.



Pourcentage de germination des graines de *Chelidonium majus* sans traitement (0), après traitement par *Myrmica rubra* (Mr) et *Lasius niger* (Ln) (moyenne ± ET, n=12).

Chapitre 3

Seed desiccation limits removal by ants

Abstract

1. Time matching between partners is a key issue in the evolution of plant-animal mutualisms. In seed dispersal by ants, timing of seed release has been studied but little is known about the availability window of seeds once they are released. Many diaspores of myrmecochorous plant species in mesic habitats have elaiosomes typically soft, desiccation-sensitive and which attractivity toward ants decreases quickly.

2. The purpose of this study was to measure the availability window of two mesic-habitat seed species (*Chelidonium majus* and *Viola odorata*) by linking their desiccation and rehydration rates with the carrying behaviour of the ant *Myrmica rubra*.

3. We find out in laboratory experiments that seeds of both species loose one third of their weight during the first 24h after the seed release. This loss is concurrent with a 92% decrease of *Viola odorata* seed removal rates, but not concurrent with the slower decrease of *Chelidonium majus* seed removal rates, which keep one third of their attractivity after one month of desiccation.

4. Seeds recover almost entirely their initial weight after being soaked in water during 18h. This rehydration restores the whole attractive potential of *Chelidonium majus* seeds but only a part of *Viola odorata* seeds.

5. We show here that the window of seed attractivity towards ants depends strongly on seed desiccation and is very variable among plant species. Seed desiccation is an influential temporal parameter in seed dispersal.

Keywords: *Chelidonium majus*, elaiosome, myrmecochory, rehydration, seed dispersal, *Viola odorata*.

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Introduction

Many animals pollinate flowers or disperse seeds in exchange of food rewards like nectar or fleshy fruits (Herrera, 2002; Pellmyr, 2002). Pollination and seed dispersal mutualisms involve large networks of species which composition and abundance vary considerably through space and time (Bascompte & Jordano, 2007; Elzinga *et al.*, 2007; Manzaneda *et al.*, 2007). Then, the evolution of these plant-animal mutualisms depends strongly on the spatial and temporal co-occurrence of partners. In seed dispersal by ants (myrmecochory), the timing of seed release is a plant trait that can match with the activity peaks of seed-disperser ants either at the seasonal scale or at particular moments of the day (Turnbull & Culver, 1983; Oberrath & Bohning-Gaese, 2002; Boulay *et al.*, 2007). But if temporal variations of seed release have been studied, little is known about the attractivity window of a seed once it has been released.

Myrmecochory is a worldwide mechanism that involves more than 3000 plant species from more than 80 families (Beattie & Hughes, 2002). Ants are then the most important invertebrate taxa that disperse seeds. Usually, seeds are carried by ants to their nest, where the lipid-rich appendage called elaiosome is discarded. Seeds can then germinate inside or outside the nest, most often in refuse piles. Elaiosomes, which are considered as an adaptation for ant dispersal, have diverse morphological origins showing a conspicuous example of independent convergence (Mayer *et al.*, 2005; Edwards *et al.*, 2006). It is admitted that elaiosomes of the xeric habitats (like Mediterranean climate regions of Australia and South Africa) are firm, long-lived and can remain attractive even after several years (Berg, 1975; Beattie & Hughes, 2002). In comparison, elaiosomes of temperate plant species of mesic habitats (mainly in Northern Europe and North America) are typically softer and short-lived (Sernander, 1906; Berg, 1958). Contrarily to the seed, the elaiosome is devoid of a hard coat testa and is unprotected from desiccation in dry weather (Werker & Fahn, 1975). Thus, as Berg (1966) said, “evanescence” is the typical feature of most elaiosomes: “After a short period of time in dry air they normally shrink, collapse, and loose most, although not all, of their attractiveness to ants”. Mayer and colleagues (2005) classified the temperate elaiosomes in three groups following their consistency: 1- *Soft with* *large cells* that contain a lot of water, desiccate quickly and lose their original shape; 2- *Soft with small to medium cells* that contain lower water, rarely collapse and do not lose their original shape when desiccating; 3- *Hard* with a prominent cuticula protecting a small cells layer that do not collapse.

In nearly 130 papers that deal with myrmecochory in mesic habitats, the link between elaiosome aging and rates of removal by ants has been reported only in the five following references. In a North American open habitat, elaiosomes of *Corydalis aurea* seeds remain very attractive even after three days outside (Hanzawa *et al.*, 1985). On the contrary, in European mesic habitats, Guitián and colleagues (2003) noted that ants clearly preferred fresh *Erythronium dens-canis* seeds and retrieved them as long as their surface remained smooth. Likewise, ants removed faster fresh seeds of *Hepatica nobilis* (Mark & Olesen, 1996) and *Carex pillulifera* (Kjellsson, 1985) than two and three day-old seeds respectively. In the same way, in a North American deciduous forest, one-day-old seeds of *Jeffersonia diphylla* were removed faster by ants than three-day-old seeds (Smith *et al.*, 1986).

Such short-term loss of ants' interest for aging seeds suggests that the elaiosome attractiveness might have been altered by desiccation in the open air. Hence, the purpose of this study was to measure the attractivity window of seeds by quantifying the influence of desiccation and rehydration of mesic-habitat elaiosomes on ant carrying-behaviour. We hypothesized that desiccation (*i.e.* seed weight loss) is related to the ant-attracting capacity of seeds (*i.e.* their removal rates). Besides, we know that elaiosomes play a major role in water control by absorbing and temporarily retaining water before passing it to the rest of the seed (Bianchini & Pacini, 1996). We then hypothesized that rehydration (for example by rain) could re-enhance the ant-attracting capacity of seed elaiosomes, and thus their functional life as a dispersal factor. To test these hypotheses, we choose one ant species (*Myrmica rubra)* to which was given two seed species (*Chelidonium majus* and *Viola odorata*) with soft and large elaiosomes (see above Group 1).

Materials and methods

Ant species, collecting and rearing

The red ant *Myrmica rubra* L. is 4 to 6 mm in length, forms polygynous colonies common in European temperate open habitats. Its diet is composed principally by dead or alive insects or small animals, but it has also been reported as consuming sugars (aphid honeydew, plant nectar) or elaiosomes (Cammaerts, 1977; Bülow-Olsen, 1984; Le Roux *et al.*, 2002; Servigne & Detrain, 2008). We collected four colonies of *Myrmica rubra* from earth banks in La Gorgue (France). In the laboratory, colonies were reared in plaster nests (Janet type, 20 x 20 x 0.4 cm) and placed in arenas (50 x 38 x 10 cm) with Fluon-coated borders to prevent ants' escape. Nests were regularly moistened and kept at 21 ± 2 °C, 35 ± 5 % relative humidity and a constant photoperiod of 12h per day. We covered the nest chamber with a red glass plate through which the ants could be observed. All colonies contained approximately 500 to 600 workers and 5 to 10 gynes. We supplied ants *ad libitum* with water and sucrose solution (1M), and twice a week with cockroaches (*Periplaneta americana*) and mealworms (*Tenebrio molitor*).

Plant species

Chelidonium majus L. is a common perennial plant present in open and ruderal habitats like debris, hedges, open woodlands or old walls (Lambinon *et al.*, 1992). It grows in European temperate climate and produces seeds from May until approximately the end of November. Seed dispersal follows two steps (diplochory): seeds are first scattered around the parent plant (autochory) and are then removed by ants from the soil surface (myrmecochory). Seeds are dark brown and measure approximately 2 mm length. Elaiosomes are white and fleshy, measure approximately 1.5 mm length and are formed by the tissue of the raphe (Sernander, 1906; Bresinsky, 1963).

Viola odorata L. is a common perennial plant of understory or edge of temperate forests and sometimes open and ruderal habitats (Lambinon *et al.*, 1992). It is strictly myrmecochorous (without previous autochory) but capable of vegetative reproduction. Seeds are yellow-brown and measure approximately 3 mm length. Elaiosomes are white-coloured, large, soft, cone-like basal, approximately 2 mm length and formed mainly by tissue of the raphe (Sernander, 1906; Bresinsky, 1963).

Elaiosomes of both species are classified in the first group ("soft with large cells") (Mayer *et al.*, 2005). Seeds were collected less than 20 min before each experiment (fresh seeds).

Seed weighting

We collected 20 seeds of *Viola odorata* and 100 seeds *of Chelidonium majus* from different capsules and left them uncovered at room temperature (21 ± 2 °C, 35 ± 5 % HR) in the laboratory. We weighted both species immediately after their collection and after being left in the open air at room temperature for 1, 6, 12, 18, 24, 48, 96 hours, and one month (n=4).

Besides, we made three groups of seeds by desiccating them during 24h, 96h or one month. We rehydrated these groups during 18 hours by placing seeds (20 seeds of *Viola odorata* and 100 seeds *of Chelidonium majus*) in a folded piece of filter paper (5.5 cm diameter) and into a Petri dish with water (3.5 cm diameter, 1 ml water). We then weighted fresh seeds and these three rehydrated group seeds (n=4 for each group). In order to measure the water gain in the elaiosome and in the seed itself, we measured separately the elaiosome, the seed, and both together (*i.e.* the entire seed).

Experimental procedure

Groups of 15 seeds having undergone different desiccation treatments were given to the four ant colonies (fresh seeds, seeds after 1h, 6h, 12h, 24h, 48h, 96h or one month of desiccation). We counted the number of seeds removed after 20 minutes. We choose small groups of seeds (i.e. 15) in order to avoid satiation effects (Heithaus *et al.*, 2005). Instead, we tried to increase the number of replications. Colonies were starved three days before starting a set of experiments. A set was composed of four days following a precise order of testing depending on the desiccation and rehydration timing. During the first set, we gave *Viola odorata* seeds to colonies A and B, and *Chelidonium majus* seeds to colonies C and D. One week later, a second set of 4-days testing was done by inverting the seed species given to ant colonies (*V. odorata* to colonies C, D and *C. majus* to colonies A, B). After one month of desiccation, we rehydrated seed groups of each of the two species and compared their removal rate to that of one-month-desiccated seeds. The whole procedure (two 4-days sets and "one-month-desiccated" test) was done in July and replicated in August and September. We thus obtained 12 replications (4 colonies x 3 replications) per seed treatment and seed species.

Before each experimental set, the colony was connected by a cardboard bridge (40 cm x 1 cm) to a foraging arena (20 x 15 x 4 cm) with Fluon-coated borders to which ants had always access. The seed source was a small 3 x 3 cm square in the middle of the foraging arena. In the morning, one hour before starting the experiment, we deposited 5 fresh seeds at the seed source. By doing this, a minimum number of scouts was already present in the foraging arena when the experiment started (6.9 ± 2.1 scouts, n=120) what reduced the latency time needed for seed discovery (less than 10 sec.). The number of scouts present in the arena did not significantly differ at the beginning of each experimental day of the 4-days testing (*Chelidonium majus*: p=0.7710; *Viola odorata*: p=0.9942; Friedman test, n = 12).

Removal rates were pooled since there was no colony effect (Friedman test; *Viola odorata*: p=0.0924; *Chelidonium majus*: p=0.0796) and no replication effect (*i.e.* July *vs.* August *vs.* September) (Friedman test; *Viola odorata*: p=0.2691; *Chelidonium majus*: p=0.2206).

Results

Desiccation

First we noticed that, on average, fresh diaspores of *Viola odorata* were about five times heavier than *Chelidonium majus* ones. Desiccation dynamics were similar for both species: diaspore weights steeply decreased until they stabilized after 24 hours (*V. odorata*) or 48 hours (*C. majus*) (Figure 1). After 96 h of desiccation, a diaspore of *Viola odorata* had lost 43.1 % of its initial weight (78 % weight loss of the elaiosome and 27 % weight loss of the seed itself), whereas a diaspore of *Chelidonium majus* had lost 32.6 % of its weight (70 %

weight loss of the elaiosome and 21.5 % weight loss of the seed itself). The striking weight loss of elaiosomes shows that these bodies are more sensitive to desiccation than the seed core. While seeds did not change in shape or size but just got a little paler, elaiosomes of both species shrank drastically, wrinkled and became yellow pale instead of white (Figure 3).

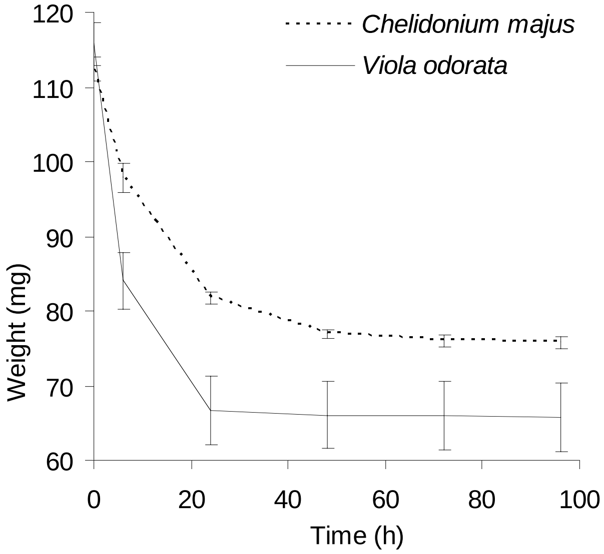


Figure 1. Mean diaspore (seed + elaiosome) weight (± S.D., n=4) during desiccation at room temperature of 100 seeds of *Chelidonium majus* (doted line) and 20 seeds of *Viola odorata* (full line).

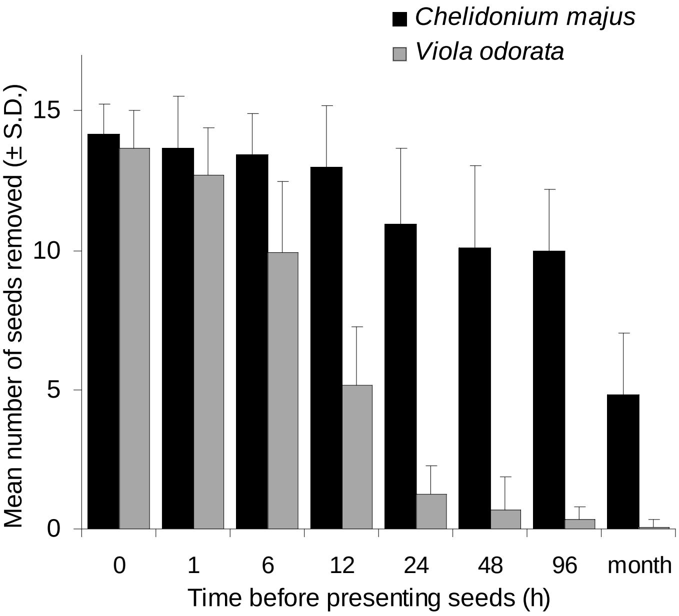


Figure 2. Mean number (± S.D., n=12) of seeds removed after 20 minutes in the foraging arena among the group of 15 seeds.

As both seeds got desiccated, seed removal rates decreased following an exponential decrease during the first 24h (*V. odorata*: $R^2 = 0.98$; *Chelidonium majus*: $R^2 = 0.91$). (Figure 2) (*Viola odorata,* p<0.0001; *Chelidonium majus* p<0.0001; Friedman Test, n=12). However, the effects of desiccation on seed removal differed depending on plant species: it was markedly faster for *Viola odorata*, of which the removal showed a 92% decrease within 24 h (only 1.25 ± 1 seeds removed during 20 min, n=12). We infer from the exponential decay that *Viola odorata* looses half of its attractivity in seven hours. Surprisingly, despite their weight loss over time, *Chelidonium majus* seeds were still highly removed after 96 hours (10 ± 2.2 seeds in 20 min, n=12), and even after one month (4.8 ± 2.2 seeds in 20 min, n=12). The impact of desiccation duration on *C. majus* seed removal rates was then very different from that on seed weight. Moreover, even when the seed weight was stabilized (at 24 hours), its attractiveness continued to decrease slowly. After 48 hours, seeds of *Chelidonium majus* were 15 times more removed than those of *Viola odorata.*

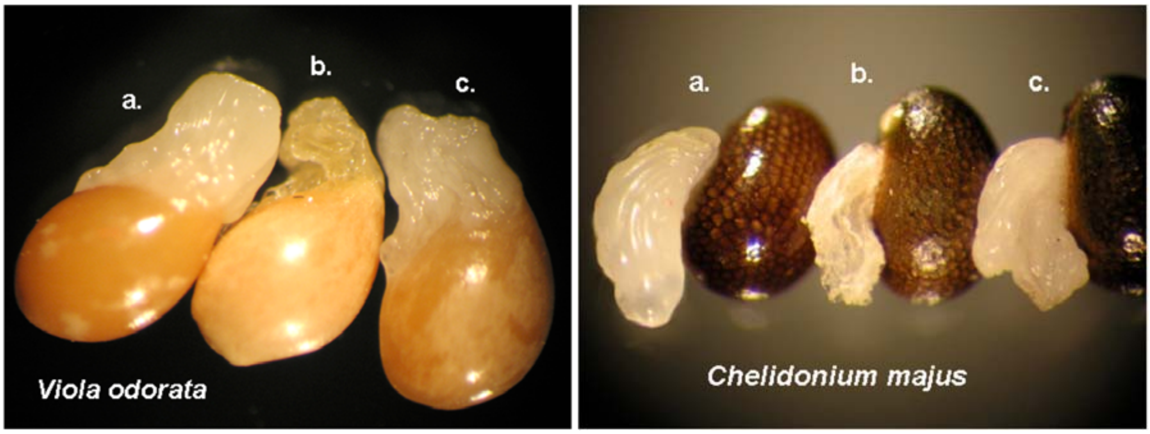
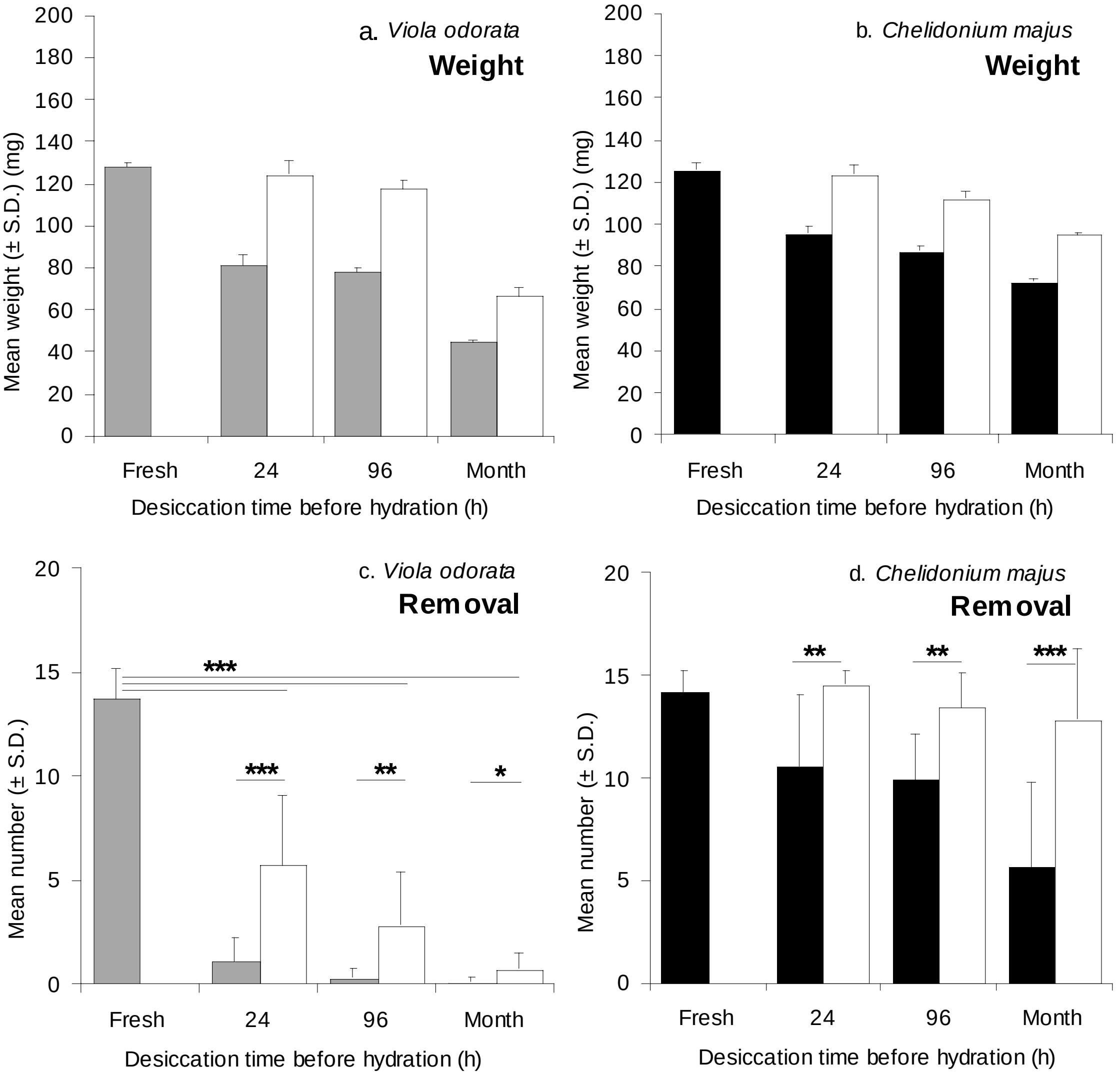


Figure 3. Fresh (a), desiccated (b) and rehydrated (c) seeds of *Viola odorata* and *Chelidonium majus.*

Rehydration

After hydration, elaiosomes looked back again as fresh ones (*i.e.* white coloured and smoother) (Figure 3). Seeds almost recovered their fresh weight after being in contact with water for 18h (Figures 4ab). After a 96h desiccation, entire seeds of *Viola odorata* recovered 92.4 % of their initial weight (93.8 % for *C. majus*). The seed itself recovered 97.6 % of the fresh weight and the elaiosome 74.5 % (96 % and 82.5 % respectively for *C. majus*).

Following rehydration, seeds recovered part of their attractive potential towards ants (Figure 4cd). For *Viola odorata*, the rehydrated seeds were removed 5.3 times more than the 24h-desiccated seeds (8.5 times more after 96 hours, and 9 times more after one month) (Figure 4c) (Mann-Whitney test, n=12; 24 *vs.* 24-water: p=0.0005; 96 *vs.* 96-water: p=0.0058; one-month *vs.* one-Month-water: p=0.0324). Nevertheless, the "fresh" removal rate was never reached back after rehydration (Mann-Whitney test, n=12; Fresh *vs.* 24-water: p<0.0001; Fresh *vs.* 96-water: p<0.0001; Fresh *vs.* one-month-water: p<0.0001). The removal rates of rehydrated seeds differ depending on the duration of desiccation undergone by the diaspore (Friedman test, n=12, p=0.0018). Indeed, the longer-lasting the desiccation duration, the lower the removal rate of rehydrated seeds. In other words, desiccation reduced chances to re-activate seed potential for dispersal by ants. Ultimately, after one month of desiccation, rehydrated seeds were almost not removed (0.75 ± 0.7 seeds in 20 min).



Figures 4. Mean weight (± S.D., n=4) of 100 seeds of *Viola odorata* (a) and 20 seeds of *Chelidonium majus* (b); mean number (± S.D., n=12) of desiccated (colored) vs. rehydrated (white) seeds removed after 20 minutes for *Viola odorata* (c) and *Chelidonium majus* (d).; Mann-Whitney Test results: p<0.05 (*); p<0.01 (**); p<0.001 (***). All statistical tests described in text are not included in the figures.

For *Chelidonium majus*, rehydration also significantly re-enhance the attractivity of desiccated seeds (Figure 4d) (Mann-Whitney test, n=12; 24 *vs.* 24-water: p=0.0062; 96 *vs.* 96-water: p=0.0018; one-month *vs.* one-Month-water: p=0.0009). Quite surprisingly, rehydrated seeds recovered attractivity close to that of fresh ones, even after having undergone one month of desiccation. Thus there were no significant differences between fresh and rehydrated seed removal rates (Mann-Whitney test, n=12; Fresh *vs.* 24-water: p=0.4975; Fresh *vs.* 96-water: p=0.3962; Fresh *vs.* one-month-water: p=0.3217) and removal rates were similar for all categories of rehydrated seeds (Friedman test, n=12, p=0.1486).

Discussion

During the first day of desiccation, both seed species lost about one third of their initial weight before stabilizing. Desiccation dynamics were similar for both species even in the percentage of weight loss undergone by the elaiosome or by the seed itself. Concurrently, there was a decline over time in the removal rate of seed species which was rapid for *Viola odorata* diaspores and quite slower for *Chelidonium majus* ones. The steep decline of *Viola odorata* removal rates (which lost half of the removal rate in seven hours) is comparable to that of other temperate species with soft elaiosomes that are no more removed within two days like *Hepatica nobilis* (Mark & Olesen, 1996) or three days like *Carex pillulifera* (Kjellsson, 1985) and *Jeffersonia diphylla* (Smith *et al.*, 1986). Surprisingly, seeds of *Chelidonium majus* remain attractive during a much longer period of time in spite of their rapid weight loss. Nearly one third of seeds were still taken after one month of desiccation. This is the first case reported of a long-life elaiosome in a temperate plant species. Nevertheless, seed attractivity is still shorter-lasting from that of plant species that live in xeric habitats and which elaiosomes can remain attractive for years (Berg, 1975).

We suggest that each seed species could have an intrinsic desiccating rate that defines their time-window of attractivity to dispersing ants. This is certainly one of the factors that explain the high specific-differences of seeds removed during 24h by a single ant species in a temperate deciduous forest (Delatte & Chabrerie, 2008). Besides, the environmental conditions like sun exposure or air humidity are major factors that change the desiccation rates of plant diaspores. For example, it has been shown that the seed desiccation speed is greater in an open meadow than in a woodland habitat (Guitián *et al.*, 2003). It is thus possible that this seed species-specific window of attractivity could be an adaptation to the microclimatic conditions of the plant habitat. Moreover, in temperate mesic-habitats, one can assume that most myrmecochorous species could be positioned in a continuum from short-availability window, like *Viola odorata*, to a long-availability window, like *Chelidonium majus*.

Chemical compounds and water content

We found that both seed species loose approximately the same weight proportions during desiccation. In the case of *Viola odorata*, it is likely that the compounds that evaporate during the first hours after the seed release could play a major role in the ant-attracting capacity. It has been demonstrated that many chemical compounds trigger seed attractivity, such as fatty acids, diglycerides and triglycerides (Marshall *et al.*, 1979; Skidmore & Heithaus, 1988; Brew *et al.*, 1989; Boulay *et al.*, 2006), amino-acids (Brew *et al.*, 1989), starch and proteins (Szemes, 1943). But due to their high molecular weight, it is unlikely that these molecules could be volatile. We do not exclude the possibility that the decline of the ant-attracting capacity could be due to a physical constraint: desiccated elaiosomes could be harder to handle or to chew for the *Myrmica rubra* workers. In the case of *Chelidonium majus*, there is a far less marked effect of desiccation on seed removal rates. This suggests firstly that chemical compounds triggering carrying behaviour are less volatile and could remain on the seed surface for weeks, and secondly that water does not play an important role.

Besides, rehydrated seeds recovered an ant-attractivity potential, although to a different extent for each species. This phenomenon is still not clear. On the one hand, rehydration could help the chemical compounds that remained on desiccated seeds to have a second chance to be carried along by water during evaporation and then to be perceived by the ants. On the other hand, hydrated elaiosomes could be softer, easier to chew and cut with mandibles what makes seeds more attractive to ant workers. In our case, the restoration of attractiveness was partial for *Viola odorata* and total for *Chelidonium majus*. In any case, water content is undoubtedly important for both species as it affects seed removal rates, while surface chemical compounds are also key factors that seem to vanish slower in *Chelidonium majus* seeds.

Elaiosomes are known to play a role in water control of seeds (dehydration, rehydration and germination). In the case of *Ricinus communis*, it can absorb water from soil, retain it temporarily and transfer it later to the rest of the seed during germination process (Bianchini & Pacini, 1996). The elaiosome should be seen as an appendage that may change in size, consistency and hydration level, and influence germination rates. We demonstrate in this study that this function of water control also affects ant removal rates.

Timing matters

The duration while seeds remain attractive to the ants may have strong consequences on the plant fitness. The two plant species studied in this paper show two opposite strategies of seed dispersal. On one hand, *Chelidonium majus* seeds keep their attractiveness for weeks despite desiccation, restored it totally after being soaked (for example by rain) and are produced within a large seasonal window from May to November. As a result, there are thousands of long-term attractive seeds released on the ground by autochory (Servigne and

Detrain, unpublished data). On the other hand, the attractiveness of *Viola odorata* seeds is highly sensitive to desiccation and only slightly restored by rehydration (*i.e.* short window of availability). Moreover, *Viola odorata* seed production is time-limited from March to June and corresponds to only a dozens seeds kept clustered in the opened capsule. A time-limited seed availability can lead to a matching phenology of plant fructification period with diurnal (Boulay *et al.*, 2007) or seasonal (Oberrath & Bohning-Gaese, 2002) peaks of removalist-ant activity. The seed-release synchronizations could have the indirect effect of saturating ant's diets and then creating a competition for dispersal among plants (Mark & Olesen, 1996).

This time issue should also depend strongly on local removal pressures exerted by patrolling scouts. Indeed, the desiccation parameter can become negligible if seeds are placed in a high removal pressure site (i.e. if the number of patrolling scouts interested in elaiosomes is high) where seeds will be taken within a few minutes. For example, this can be the case if seeds fall down near an ant nest entrance. Nevertheless, high local removal pressures are not the rule. It may happen that attractive seeds of *Chelidonium majus* remain untouched even after 6 hours in the field (Oberrath & Bohning-Gaese, 2002; Peters *et al.*, 2003); and we noticed in several plots in preliminary field observations that many seeds of *Viola odorata* remained dry and untouched in their opened capsule (most probably undiscovered) at the end of the season. So in sites of low removal pressure where seeds remain hours or days on the soil before being taken by ants, the desiccation parameter should play a key role. In this case, the desiccation-sensitive seeds of *Viola odorata* may be more dependent on the local density of scouts than *Chelidonium majus* seeds. We assume that seeds which attractivity remains high when desiccated should be more likely to be dispersed in habitats with low ant populations. This is undoubtedly a central issue in the evolution of myrmecochory in xeric habitats, where removalist density can be low and resistance to desiccation a physical key factor for seed survival.

Besides, seed predation may be another key factor that alters the importance of duration of seed attractivity in the evolution of myrmecochory. Seed predators like granivorous ants, rodents, birds or beetles (Berg, 1966; Heithaus, 1981; Ohara & Higashi, 1987) are common and may be still interested in desiccated seeds that have been progressively neglected by seed-disperser ants. Interestingly, field observations in Mediterranean xeric habitats showed that desiccated myrmecochorous seeds lost their attractivity for removalist species like *Formica* and *Camponotus* but not for granivorous species like *Aphaenogaster* or *Messor* (Boulay, pers. com.). Then, a longer lasting attractivity of a seed (e.g. seed that resists to desiccation or has heavy attractive compounds) could maintain a removalist/predator ratio high and long enough to be still dispersed. However, one should keep in mind that seed-predation pressures are highly variable at large scales (e.g. granivorous ants are generally a dominant ant guild in xeric habitats; Wehner, 1987) and at regional or populational scales (Fedriani *et al.*, 2004; Manzaneda *et al.*, 2005; Boulay *et al.*, 2007; Manzaneda *et al.*, 2007). Thus desiccation may be indirectly another geographical-dependent parameter of this complex ant-seed mutualism. This opens avenues for future field research.

The present study has highlighted the role of desiccation on the timing of seed dispersal by ants. It should be completed by further studies investigating how temporal changes in seed surface compounds may act upon seed removal by ants as well as upon seed management inside the nest. Comparative studies should be carried out in different habitats to see whether time windows of seed attractiveness may have been selected as a function of plant microclimatic conditions, seed predation pressures or removalist-ant density. In the future, the understanding of the myrmecochory process will certainly gain from an integrative approach taking into account the phenology of plant species, their seed production patterns, the foraging behaviour of dispersing ants as well as the environmental constraints exerted on both partners.

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Chapitre 4

What features make ants major seed dispersers?

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Introduction

Insects have developed a wide array of interactions with plants (Barbosa & Letourneau, 1988; Gullan & Cranston, 2005; Rico-Gray & Oliveira, 2007). Many of these interactions are antagonistic since they are unfavourable to plants, like predation, grazing or parasitism. By contrast, insects may develop mutualistic interactions with plants such as pollination or seed dispersal that benefit to both partners (Thompson, 1994). In these two forms of mutualisms between free-living species (i.e non-symbiotic species) (Thompson, 1994; Thompson, 2005), insects provide the movement of reproductive structures across space that permit plants to complete their reproductive cycle (Herrera, 2002). Pollination involves many insect taxa, both solitary and social species and mainly flying insects like flies, wasps, bees or butterflies (Pellmyr, 2002; Van der Pijl, Dodson & Calaway, 1969). Although ants are constant plant visitors and avid nectar collectors, they rarely pollinate flowers. Many reasons have been proposed, but the most probable is that ant antibiotic secretions from the metapleural gland kill pollen grains (Beattie et al., 1985; Beattie et al., 1984; Hull & Beattie, 1988).

By contrast, ants are undoubtedly the major invertebrate taxa that disperse seeds (Beattie, 1985; Rico-Gray & Oliveira, 2007), whereas a very few cases of seed dispersal by insects have been reported. A few cases of seed dispersal by social insects other than ants have been described, like melitochory (bees) (Bacelar-Lima et al., 2006; Wallace, Howell & Lee, 2007; Wallace & Trueman, 1995), vespichory (wasps) (Bale et al., 2003; Jules, 1996; Zettler, Spira & Allen, 2001a) and termitochory, for which grass seeds in the food chambers can germinate only if a termitophagous mammal breaks the termite nest (Jolivet, 1996). Generally, seeds dispersed by animals can be transported through gut digestion (endozoochory), by unintentional adhesion to the vector body (epizoochory), or by intentional transport as food items (exozoochory). Contrarily to mammals, endozoochory is very rare for insects and has been reported once by Darwin in Nepal where seeds found in the excreta of migratory locusts were still viable (Darwin, 1859). Adhesion on insect bodies is reported in Diptera, Homoptera and Hemiptera but only with mosses and fungus spores (Jolivet, 1996). The third last method of seed dispersal –i.e. exozoochory, is used widely successfully by ants, which collect diaspores fallen under the plant or directly over twigs, bring them back to the nest, eat the nutrient-rich appendage called elaiosome and reject still viable diaspores within or outside the nest (Sernander, 1906).

Myrmecochory has generally been considered as a mutualism, but in fact, seed dispersal by ants can be placed in a continuum between mutualism and antagonism (Rico-Gray & Oliveira, 2007; Thompson, 1982). Dispersal of elaiosome-bearing seeds is usually carried out by non-granivorous ants, considered as “high quality dispersers” or “removalists” (Giladi, 2006), which are interested only in the nutritive reward provided by the elaiosome. However, seed dispersal by ants is a little bit more complex and elaiosome-bearing seeds can also be taken by harvester ants that eat the elaiosome, but also the seed itself (Aronne & Wilcock, 1994; O'Dowd & Hay, 1980). Seed dispersal can also occur for seeds not bearing an elaiosome, that are taken by harvester ants (or "poor quality dispersers" or "granivorous"). Although those harvester ants kill the majority of seeds by eating them and destroying the embryo, foragers always loose accidentally a few seeds on their way back to the nest (Detrain & Tasse, 2000), or let seeds undamaged on the nest refuse piles (Aronne & Wilcock, 1994; Wolff & Debussche, 1999). These lost seeds are dispersed *de facto* and have the opportunity to germinate far from their parents (i.e. dyszoochory). Although harvester ants can be considered as seed dispersers (Aronne & Wilcock, 1994; Retana, Picó & Rodrigo, 2004), in this review, we will refer only to myrmecochory *sensu stricto*, which is the dispersal of elaiosome-bearing seeds by non-harvester ants.

Myrmecochory occurs worldwide in a variety of ecosystems like arid habitats in Australia (Berg, 1975), South African Fynbos (Bond, Yeaton & Stock, 1991), Mediterranean regions (Baiges, Espadaler & Blanché, 1991), South American tropical forests (Horvitz & Beattie, 1980) or temperate deciduous forests of Europe and North America (Beattie & Culver, 1981; Sernander, 1906). It has appeared several times in very different plant taxa, showing a good example of convergent evolution (Beattie, 1985; Dunn et al., 2007). More than 3000 plant species from more than 80 families around the world are dispersed by ants (Beattie & Hughes, 2002). In each habitat, guilds of myrmecochorous plants are involved in multiple interactions with local guilds of ants (Beattie, Culver & Pudlo, 1979; Sernander, 1906). However, not all ant species have the same dispersal potential (Hughes & Westoby, 1992b; Ness & Morin, 2008), and one or a few ant species can be the main dispersal vector of the plants' guild (Gove, Majer & Dunn, 2007). Since the seminal work of Sernander in 1906 -in which he invented the words *myrmecochory* and *elaiosome*, about 300 research papers have been published on this topic. It has been a fruitful biological model for the study of seed dispersal (Bossard, 1990), because field studies have been facilitated by the limited areas foraged by ant workers, the rather short dispersal distances that can be walked by ants in comparison with birds, as well as by the persistence of ant nests and of their foraging trails at a same location.

In general, the reviews published about myrmecochory took the plant perspective by listing the hypotheses of selective advantages to plants (dispersal distance, sibling-competition avoidance, predator or fire avoidance, and directed dispersal to nutrient-rich microsites) (Beattie & Hughes, 2002; Beattie, 1983; Beattie, 1985; Bennett & Krebs, 1987; Bronstein, Alarcon & Geber, 2006; Giladi, 2006; Gorb & Gorb, 2003; Mayer, Ölzant & Fischer, 2005; Rico-Gray & Oliveira, 2007). Almost all of them also described the geographical areas where myrmecochory occurs, the number of families and genus of plants involved, as well as the general characteristics of elaiosomes. From the ant side, two reviews list the ant genera involved in myrmecochory and found that seed disperser ants can be omnivorous and exhibit a wide variety of nesting and foraging habits (Beattie, 1983; Gomez & Espadaler, 1997a). A recent review tackled the role of ant satiation and ant hierarchies in the myrmecochory process (Giladi, 2006). As it is often the case in research dealing with animal-plant mutualisms, the plant partner is always more studied than the animal (Bronstein, 1994; Cushman & Beattie, 1991) and much can still be gained by a more explicit focus on the animals (Waser, 1998). In the latest main review about myrmecochory (Rico-Gray & Oliveira, 2007, p 62), it is suggested that *"future research should stress the study of the "quality" component of ants as dispersers"*, introducing the idea that *"the presence of particular colony organizations and morphological and behavioural traits is clearly required for an ant species to be considered an effective disperser"*. The search for such ant features is the main objective of the present paper. We will review how ant traits - in particular social ones-, do contribute to seed dispersal, trying to answer why ants are the only social insects that disperse seeds so widely.

The ubiquity and diversity of ant species

Ants are not only found almost everywhere, but their diversity and abundance make them ecologically dominant taxa in many ecosystems, and permit them to access a wide range of niches (Beattie & Hughes, 2002; Hölldobler & Wilson, 1990). Their social behaviour is undoubtedly what causes their tremendous success (Wilson, 1987). Seed-disperser ants have a worldwide distribution and involve more than 47 genera and 200 species (Beattie, 1983; Gomez & Espadaler, 1997a). To rely on a diversity of dispersal agents has been an advantage for plants (Wheelwright & Orians, 1982). Because of this ubiquity and ecological importance, a seed that stays on the plant or falls down on the ground will have a high probability to be first found by an ant. But as we have seen previously, ant-seed interactions can be antagonistic as well as mutualistic. The ubiquity of ants should not always be an advantage: depending on the local proportion of harvester/disperser species among the ant community, different local outcomes that vary from granivory to seed dispersal may emerge. However, one should keep in mind that the picture is not so simple because granivorous ants can also disperse seeds unintentionally. It also occurs that they bring myrmecochorous seeds to their nest but finally could not break their thick coat (O'Dowd & Hay, 1980). In an evolutionary perspective, it has been suggested that seed dispersal by harvesting ants preceded myrmecochory (i.e. seed dispersal by removalist ants) during the evolution of ant-seed interactions (Rissing, 1986). Thus, even if seed interaction with ants may vary from predation to dispersal at ecological or evolutionary scales, there is always at least a small chance for the ant to disperse a seed.

The ant size

Seed selection

Seeds larger than 100 mg tend to be dispersed by vertebrates and those smaller than 0.01 mg tend to be unassisted (Hughes et al., 1994a). Between 0.1 and 100 mg, all dispersal modes are observed, including myrmecochory. It has been well demonstrated that granivorous ants respond to a specific range of seed sizes, inside of which they remove the largest seeds they can. Above their seed-size scale, ants can not remove seeds that are too large, and below, ants do not detect the seeds as a potential food (Davidson, 1978; Detrain & Pasteels, 2000; Heredia & Detrain, 2005; Kaspari, 1996; Retana & Cerda, 1994; Willott, Compton & Incoll, 2000). Moreover, whereas small species mainly carry small seeds, larger ants may collect a broader range of seed sizes (Kaspari, 1996). For seed-disperser ants and myrmecochorous seeds, the size-matching issue is still controversial because the reward is displaced from the seed itself to the elaiosome (i.e. ants eat only the elaiosome). Then, the high energy expenditure related to the transport of large seed items does not necessarily provide any supplementary food (Hughes & Westoby, 1992b; Schoener, 1971). Ideally, from the ant disperser perspective, the smallest seeds with the largest elaiosome are the most valuable food items. Indeed, some studies confirm that removal rates increase with the ratio between elaiosome and diaspore sizes and weights (Bas, Oliveras & Gomez, 2007; Gunther & Lanza, 1989; Hughes & Westoby, 1992b; Servigne & Detrain, 2008). But others showed that removal rates were dependent only on seed size (Gorb & Gorb, 2000; Gorb & Gorb, 1995b) or on elaiosome size (Mark & Olesen, 1996; Oostermeijer, 1989). We suggest that seed-dispersing ants may obey to more complex decision criteria than granivorous ants, combining two factors: a size-matching that opens a window of seed sizes that can be potentially transported, and within this window, ants may choose diaspores bearing the largest elaiosome or showing the highest elaiosome/seed ratio.

Seed transport

In addition to its influence on seed choice –i.e. size matching, the ant body size also determines the capacity of foragers to transport seeds and their dispersal distance. Several studies have reported that small ants may consume the elaiosome *in situ*, contrarily to large ants that tend to bring seeds back to their nest (Auld, 1986; Passos & Oliveira, 2002; Pudlo, Beattie & Culver, 1980). Obviously, the consumption of the elaiosome *in situ* without being transported is not beneficial to the plant. It has also been reported that ants drop accidentally seeds during foraging trips, and that the likelihood that it occurs decreases with large ant sizes (Gorb & Gorb, 1999a). Besides, it has been shown that the dispersal distance of myrmecochorous seeds can increase with ant body size (Auld & Denham, 1999; Gomez & Espadaler, 1998c; Ness et al., 2004; Pfeiffer, Nais & Linsenmair, 2004; Pudlo et al., 1980 *but see* Zelikova & Breed, 2008). A meta-analysis including 23 ant genera, 24 myrmecochorous species and 24 sites in 6 continents concluded that the relationship is remarkably strong to predict average dispersal distances from ant body size, and to be generalizable across ant species and communities (Ness et al., 2004). This relationship also exists with non-myrmecochorous fleshy diaspores in a Brazilian tropical forest, where large Ponerines (*Pachycondyla*, *Odontomachus*) tend to transport diaspores up to 10 m or more, whereas smaller ants (*Pheidole*, *Crematogaster*) usually recruit many workers and consume the fleshy bodies at the source or transport diaspores below 2 m (Pizo & Oliveira, 1999; Pizo, Passos & Oliveira, 2005). By this relationship, the ant body size directly influences plant benefits, as we know that increasing dispersal distance help in colonising new habitats or to reduce the competition between mother plants and their offspring (Handel, 1976; Handel, 1978b).

From the ant community perspective, intra and inter-specific differences of ant sizes should strongly contribute to shaping their impact as dispersers on plant populations. For example, whereas large and small seeds were found in nests of the large ant *Formica polyctena*, only small seeds (of the same plant species, *Ballota nigra* and *Chelidonium majus*) were found in nests of the smaller ant *Lasius fuliginosus* (Gorb & Gorb, 2003). Ant size appears then to be a key factor explaining why variations in the ant community composition provoke strong seed dispersal variations (Garrido et al., 2002; Manzaneda, Rey & Boulay, 2007; Ness et al., 2004).

The ant diet

Although elaiosomes can be an essential part of ant diet during a period of time (Kjellsson, 1985; Oostermeijer, 1989) or can be nutritionally sufficient for foundress's survival (Marussich, 2006), to our knowledge there is no ant species of which diet is exclusively made of elaiosomes. The wide spectrum of nutrients contained in elaiosomes opens a large range of potential consumers among ants, but also among predators like rodents, beetles or birds (Gomez & Espadaler, 1997b; Heithaus, 1981; Ohara & Higashi, 1987; Smith, Rosenheim & Swartz, 1986). One of the main advantages of elaiosomes is certainly the presence of compounds like linoleic acid (Brew, O'Dowd & Rae, 1989) or sterols (Gammans, Bullock & Schönrogge, 2005) that are essential for the metabolism of insects and synthesize *de novo* by only a few insects (Dadd, 1973). Elaiosomes are above all a food source for growing larvae (Fischer et al., 2005), but studies that measured the effects of elaiosome consumption on ant colonies have unclear and contradictory results (Bono & Heithaus, 2002; Fokuhl, Heinze & Poschlod, 2007; Gammans et al., 2005; Marussich, 2006; Morales & Heithaus, 1998). For example a punctual elaiosome supply to naïve *Aphaenogaster rudis* colonies did not increase the colony size (number of workers), the queen mass nor the number of alates, but changes the sex ratio (Bono & Heithaus, 2002; Morales & Heithaus, 1998). But these results could not be obtained with *Myrmica* species (Gammans et al., 2005).

Omnivorous diet

Most seed-disperser ant species have large diet spectrum (Beattie & Hughes, 2002; Boulay et al., 2005). For example, among the major seed dispersers around the world, we find the genera *Lasius*, *Myrmica* and *Formica* in European temperate habitats (Beattie, 1983; Gorb & Gorb, 2003), *Rhytidoponera* in Australia (Dunn, Gove & Majer, 2006), *Camponotus* in arid environments in Mexico (Mendoza & Castaño-Meneses, 2007), *Aphaenogaster* and *Camponotus* in Mediterranean region (Boulay et al., 2007a) or *Pheidole* and *Ectatomma* in tropical dry forest of Costa Rica (Horvitz & Beattie, 1980; Zelikova & Breed, 2008). Those omnivorous species, which represent nearly half ant species of the world (Tobin, 1993), can forage on a large array of food containing carbohydrates, lipids or proteins such as honeydew, extrafloral nectaries, Müllerian bodies, dead or alive animals, honey-dew or seeds. But in fact, the "omnivorous" category is a catch-all term. We know that some species have preferences for honey-dew and are mainly aphid tender (e.g. *Lasius*), others have carnivorous preferences (e.g. *Ectatomma*, *Myrmica*), or can be at least partially granivorous (e.g. *Pheidole*, *Rhytidoponera*). Experimental results tend to suggest that the diversified diet of omnivorous ants influence seed choices and dynamics of removal. For example, omnivorous aphid tenders *Lasius fuliginosus* and *Lasius niger* remove seeds at lower rate than the more carnivorous ants *Formica polyctena* and *Myrmica rubra* respectively (Gorb & Gorb, 1999b; Servigne & Detrain, 2008). But more data are needed to determine in which way and proportion the diet preference is influential. And the influence of ant diet on seed fate of a local myrmecochory network is certainly much more complex than a simple carnivorous preference. For example, it is interesting to note that attraction of nectar-feeding ants can be reinforced by the presence of extra-floral nectaries on plants, what can encourage frequent visitations of ants to the plant and thus increase the probability for a seed to be removed later by a non-granivorous ant (Cuautle, Rico-Gray & Diaz-Castelazo, 2005; Fowler, 1983).

Carnivorous diet

In the neotropical forest, one can find large specialized carnivorous hunter *Odontomachus* and *Pachycondyla* (Ponerinae) removing seeds of *Calathea ovandensis* "frenetically" to the nest, discarding the elaiosome, giving it to their larvae and rejecting the still viable diaspore (Horvitz, 1981; Horvitz & Beattie, 1980). Many other examples of carnivorous ants dispersing seeds have been described (Hughes, Westoby & Jurado, 1994b; Orivel & Dejean, 1999; Pizo & Oliveira, 1998). It is well known that carnivorous Ponerines are attracted by the lipid fraction of elaiosomes (Hughes et al., 1994b; Pizo et al., 2005), and probably also by proteins, but their role as attractant and nutritional requirement for the colony has still to be assessed (Rico-Gray & Oliveira, 2007). Interestingly, it has also been shown that some bird-dispersed fleshy diaspores that contain fatty acids similar to the composition of elaiosomes could also be taken and dispersed by ants in a secondary stage (Passos & Oliveira, 2002; Pizo & Oliveira, 2001) (note that this case of opportunistic seed dispersal is a peculiar case of myrmecochory *without* elaiosome). In fact, the ability for a plant species to attract carnivorous ants can be highly beneficial since those carnivorous species tend to be large, forage individually, bring back seeds to the nest, and overall are not interested in the seed itself but in the elaiosome. If several other conditions are gathered, like high density of ant colonies, long-lived nests, and prey-poor habitat, carnivorous ants may become very good agents of seed dispersal (Pizo et al., 2005). The main advantages of attracting carnivorous ants is that they are not interested in the seed itself, combined with the fact that they usually reject elaiosome-free diaspores and do not store seeds in deep nest granaries for future consumption -like harvester ants usually do (Brown, Reichman & Davidson, 1979; Délye, 1971). As suggested, "*in habitats where seed predation by granivorous ants is a major source of seed mortality, the adaptive value of elaiosomes is to shift the odds of a seed being removed by a granivore, towards being removed by a carnivorous or omnivorous ant species*" (Hughes et al., 1994b).

The insect-prey convergence hypothesis

From a foraging standpoint, elaiosomes can be considered as a "dead insect analogue" (Carroll & Janzen, 1973). Hughes and colleagues (1994b) have tested this hypothesis by comparing the chemical profile of elaiosome to that of insect preys. They found that the levels of saturated and monounsaturated fatty acids between 16 and 18 carbon chain (i.e. palmitic, palmitoleic, stearic, oleic) were very similar across 12 elaiosomes species and 7 insect orders. They showed that these fatty acids are actually prevailing in the removal of items as shown for two major seed-dispersers in Australia (*Aphaenogaster longiceps* and *Rhytidoponera metallica*). By contrast, two nectar-feeder ant species (*Camponotus* and *Polyrhachis*), that were not sensitive to the fatty acid 1,2 diolein, did not remove elaiosome-bearing seeds in the field (Hughes et al., 1994b). This suggests that the insect-prey convergence of elaiosomes, which relies on the insectivorous part of the ant diet to attract them, is an important adaptive advantage of elaiosomes (Boulay et al., 2007a; Boulay et al., 2005; Gorb & Gorb, 1999b; Horvitz & Beattie, 1980; Hughes et al., 1994b; Pizo & Oliveira, 1998).

Besides, the chemical composition of elaiosomes appears to be different from the seed itself (Fischer et al., 2008; Hughes et al., 1994b; Soukup & Holman, 1987). Elaiosomes contain more digestible compounds (i.e. low molecular weight) than the seeds; for example 7.5 times more amino acids than proteins, or more monosaccharides than polysaccharides (Fischer et al., 2008). Moreover, it has been shown that the diglycerid fraction of elaiosomes of *Ulex* sp. is attractive only towards removalist ants (mutualists) and not towards granivorous ones (antagonists) (Gammans et al., 2005). Thus chemical composition of elaiosomes has converged with insect preys of ants and has differed from the seed itself, attracting other mutualistic ants than strictly granivorous ants. This suggests that ants have been a major selective force in the evolution of elaiosomes (Boulay, Coll-Toledano & Cerdá, 2006; Fischer et al., 2008; Hughes et al., 1994b).

The hygienic hypothesis

In the nest, diaspores which of the elaiosome has been removed do not interest ants anymore. Therefore, the fate of seeds after elaiosome consumption depends on what ant species usually do with refuse items. It has been shown in laboratory experiments that elaiosome removal and speed of seed rejection (i.e. the treatment of seeds inside the nest) depended strongly on the ant species (Servigne & Detrain, Chapter 2 of this thesis). Moreover, *Myrmica rubra* managed all items quicker then *Lasius niger*, whereas the former has a more carnivorous diet, and the latter is preferentially aphid-tender. Rejection of refuse items out of the nest may be part of "hygienic" measures that maintain the nest clean. One may assume that the investments of ant colonies in waste management are variable among species and should be stronger for carnivorous species, because preys are fast-perishable food that should be quickly discarded at a distance of the nest. These hygienic behaviours exist also for the transport of dead-bodies away from the nest (necrophoric behaviours) and may be elicited by the presence of oleic acid on the dead bodies (Wilson, Durlach & Roth, 1958) although this may depend on the social context of the colony (i.e. the number of ants engaging in the different tasks) (Gordon, 1983), and interestingly, we know that elaiosomes contain high proportions of oleic acids (Boulay et al., 2006; Hocking & Kortt, 1987). Moreover, the worker age is also known to influence this kind of behaviour: young workers perform the nest cleaning activities and are likely to transport the seeds far from the nest, whereas older workers tend to retrieve seeds to the nest and to ignore them once inside the nest (Gomez & Espadaler, 1998a).

To bring refuses outside the nest or far away from the nest in a centrifugal movement is then potentially influential for seed dispersal systems. For instance, *Formica cunicularia* workers deposit waste deeply inside the nest in special chambers, whereas *Formica cinerea* reject waste around the nest entrance, and *Formica execta* transport waste up to the border of their foraging territory (Dlussky 1967, *cited by* Gorb & Gorb, 2003). In flat grounds, *Solenopsis invicta* does not make refuse piles but scatters debris widely around the nest in random patterns (Howard & Tschinkel, 1976); *Myrmica ruginodis* relocated 16-20% of *Carex pilullifera* seeds outside the nest (Kjellsson, 1985), and *Myrmica rubra* and *Lasius niger* carried elaiosome-free diaspores out of the nest up to five meters (a nearly 15 min trip for one ant rejecting one seed) (Servigne, *pers. obs.*). Although there is still much work to do for disentangling the ins and outs of the rejection phenomenon, we make here the hypothesis that differences in seed rejection could result -at least partially- from a diet-dependent hygienic behaviour. By way of consequence, this could be another factor why attraction of carnivorous ants should have been one of the main selective advantages of elaiosome-bearing seeds.

The foraging strategies

As social and non-flying insects, ants show some traits of foraging that may be important for seed dispersal. Firstly, they forage on the ground, searching food in a two-dimensional world; secondly, they live in a nest and are central place foragers; thirdly, they can elicit recruitment to collect food. We discuss in this section how these three traits can be crucial for seed dispersal.

Finding seeds: a "diffuse" foraging

Two fundamental differences between pollination and seed dispersal concern the final location of the moving reproductive structures (pollen and seeds), and the control of animal vector movements (Herrera, 2002; Wheelwright & Orians, 1982). Seed dispersal systems have spatially unpredictable targets (germination sites) with no incentives that control the movements of the dispersing agent. By contrast, animals that pollinate flowers are directed towards distinctive and predictable targets (the stigma of conspecific flowers), and guided by colour incentives and nutritional rewards (pollen, nectar) (Herrera, 2002; Wheelwright & Orians, 1982). In the case of seed dispersal, fleshy fruits that attract vertebrates often bear incentive colours and are often clustered (Herrera, 2002). By contrast, myrmecochorous seeds are mostly small with no particular colour, bear a whitish elaiosome and are often scattered on the ground after a ballistic phase (Beattie, 1985; Nakanishi, 1994; Narbona, Arista & Ortiz, 2005; Ohkawara & Higashi, 1994). Ballistic dispersal is typically found in myrmecochorous plants (Beattie & Lyons, 1975; Passos & Ferreira, 1996; Stamp & Lucas, 1990; Van der Pijl, 1982). For instance, nearly all ballistic dispersal species in a tropical dry forest of South America are ant-dispersed (Griz & Machado, 2001). In many cases however, seeds can be found very clustered, when ants can get them directly on the reproductive structure of the plant or fallen at the base of the parent-plants. In the temperate plant *Viola odorata*, a dozen of seeds are clustered within a non-exploding capsule opened barely. The peduncle carrying the capsule is weak and prostrated under the leaves towards the litter level, invisible to flying animals. This has been considered as an adaptation for ant dispersal (Beattie & Culver, 1981). In the same way, seeds can also be found clustered in vertebrate dejections on the soil, branches or leaves, when myrmecochory follows a primary vertebrate phase (Pizo et al., 2005). In any cases, clustered or scattered, available myrmecochorous seeds generally do not have far-distance incentives, are invisible from far, and do not attract ants at a distance (Kjellsson, 1985; Servigne & Detrain, 2008; Sheridan, Iversen & Itagaki, 1996, *but see* Youngsteadt et al., 2008). Then, they are more likely to be found by opportunistic scavengers that forage by scanning the soil surface for dead insects, bird droppings, animal waste, fruit fragments or scraps left by larger predators. Typically, this concerns animals like ants, beetles or rodents, which have been largely reported as myrmecochorous seed collectors (e.g. Heithaus, 1981; Higashi & Ito, 1991).

Transporting seeds: central place foraging and recruitment

Preys or seeds are collected around the nest and brought back in order to be stored, eaten or shared between nestmates. The food sinks of the colony (brood, young workers, queen, mates) are then centralized. Likewise, in the case of myrmecochory, foragers rarely consume elaiosomes at the source but transport the seeds back to the nest covering in some cases hundreds of meters between the foraging ground and their nest. Some ant species can nevertheless consume elaiosomes in situ (cf. section "the ant size"), but this has been attributed to their smaller body size in comparison to the diaspore. But it is the *ex situ* consumption that allows seed dispersal by ants, and this carrying trait excludes many insect species from the list of the potential seed dispersers. For instance, solitary species that tend to consume preys at the source are predators instead of dispersers, as it is the case with beetles (Ohkawara & Higashi, 1994).

Besides, recruitment is one of the main foraging traits of ants, but its status in myrmecochory is still not clear. In fact, there is no correlation between the abilities of one ant species to recruit nestmates and to disperse seeds. For example in the same site, two species (*Ectatomma ruidum* and *Pheidole fallax*) were responsible of 92% of all observed seed removals. The first species is a solitary forager whereas the second species recruit heavily to resources, including myrmecochorous seeds (Zelikova & Breed, 2008). To our knowledge, the local increase of foragers at myrmecochorous seed sources has been rarely reported (*but see* Bond et al., 1991; Nesom, 1981). Moreover one cannot say whether this local increase is due to an active recruitment of nestmates or from repeated visits by ant workers having found seeds independently. Most of the studies measuring seed removals did not report recruitments (e.g. Culver & Beattie, 1978; Delatte & Chabrerie, 2008; Mark & Olesen, 1996). In European temperate forests, for example, the rate of ant arrival at the seed source was stable or slowly increasing, but without showing an exponential increase nor trail marking behaviours (*Myrmica rubra*, *Formica polyctena, Lasius fuliginosus*) (Gorb & Gorb, 2003; Gorb & Gorb, 1999b). In laboratory conditions, starved colonies of *Myrmica rubra* and *Lasius niger* (that are considered as efficient mass-recruiters) never recruited nor lay any trail to a dozen of aggregated seeds (Servigne & Detrain, 2008). One may assume that the recruitment decision rule should be similar for seeds than for preys of similar size. Provided these items are small enough to be individually retrieved, they do not elicit recruitment (Detrain & Deneubourg, 1997; Detrain, Deneubourg & Pasteels, 1999).

The behavioural flexibility: sensory learning

A great variety of insect species rely extensively on learning for major activities like feeding, defence, aggression or sexual and social interactions (Dukas, 2008). Ants are known to display landmark learning abilities, for example in wood ants (Graham & Collett, 2002; Judd & Collett, 1998; Nicholson et al., 1999) that are important seed dispersers (Gorb & Gorb, 2003). They also have motor learning abilities that permit experienced foragers to manipulate seeds during less time than unexperienced one (Gorb & Gorb, 2003). Although these two types of learning are certainly crucial for the colony, they appear not to be central for seed fate. On the contrary, sensory learning appears to be much more important.

Sensory learning is the acquisition of sensory information (visual, auditory or olfactory) and permits the ants to learn the quality of food sources and to behave acccordingly. Among this learning type, habituation (or sensitization) are examples of non-associative learning in which there is a progressive diminution (or amplification) of behavioural responses where the same stimulus is repeatedly encountered. Several evidences of habituation are reported for seed dispersal by ants. In deciduous forest of North America, seed removal was greater in areas where the myrmecochorous plant *Jeffersonia diphylla* was absent compared to areas where it was abundant (Smith et al., 1986). Ants preferred seeds of *Sanguinaria canadensis* over *Asarum canadense* when they were placed in *Asarum* populations (Heithaus, 1986); seeds of *Asarum canadense* were preferred in sites with dense population of *Jeffersonia diphylla*, and conversely (Smith et al., 1989b). Likewise, seed removals of *Hepatica acutiloba* and *Trillium nivale* were slower in dense populations of these seed species (Smith, Forman & Boyd, 1989a). This indicates first that ants were able to discriminate the elaiosome compounds of different plant species, second that ants preferred newly introduced seeds, and third that ants can progressively loose their interest for a seed species. Finally, seed removals (but not insect larvae) declined to nearly zero over the scale of hours or days (Heithaus, Heithaus & Liu, 2005). These last authors called this phenomenon "satiation" and suggested that it was due to the limited space in the nest or to individual decisions.

But in fact we still do not know whether this loss of interest results from an individual sensory learning (habituation), a physical limitation of the volume ingested in the stomach (individual satiation), or from a negative feedback from the larvae, considered usually as protein sinks (collective satiation). In fact, the phenomenon of habituation should not be a general rule. For example, in six populations of *Asarum canadense* of different density, removal rates were found to be similar (Heithaus, 1986). Furthermore, sensitization also has been reported: removal of myrmecochorous seed was faster when they were presented within the population of the same plant (Pemberton, 1988; Ruhren & Dudash, 1996).

Although the learning phenomenon is still not clear, it appears to be a fundamental parameter in the mechanisms of partner choice between ants and seeds. The consequence of habituation or satiation (i.e. the cessation of foraging) in a given habitat could have tremendous ecological consequences for a seed population, acting as a negative feedback in a local myrmecochory network. It could be one of these negative feedbacks (like predation and competition) that mathematical models have shown to be essential for the stability of seed dispersal mutualisms (Heithaus, Culver & Beattie, 1980). Besides a direct beneficial effect has been proposed and states that in habitats with small colonies and small overlapping territories, "*the satiation of one colony may promote the distribution of seeds into several ant nests, thereby reducing competition between siblings*" (Giladi, 2006). Learning is undoubtedly one of the great avenues of future research in myrmecochory.

The phenology and rhythms of the colony

Time matching between partners is a key issue in the interactions of plant-animal mutualisms. Myrmecochory outcomes will be successful only if the schedule of one partner matches with that of the other, i.e. when the windows of seed availability are open for ants in a particular moment of the season (Oberrath & Bohning-Gaese, 2002; Thompson & Willson, 1978) or of the day (Boulay et al., 2007b).

At seasonal time scales, timing of seed release often coincides with the activity peaks of seed-disperser ants, mostly in spring in temperate regions (Guitián & Garrido, 2006; Oberrath & Bohning-Gaese, 2002; Ohkawara, Ohara & Higashi, 1997; Thompson, 1981; Thompson & Willson, 1978; Turnbull & Culver, 1983). From the ant perspective, the colony has an annual life cycle that can change the seed-disperser activity levels. In temperate regions, the period of high seed-removal rates is spring when alternative preys for ants may be scarce, and preceded the summer period of alternative food for ants (Hulme, 1992). It was shown for some species that during the summer period, seed removal by ants steeply decreased until being negligible (Culver & Beattie, 1978; Guitián & Garrido, 2006; Servigne & Detrain unpublished data). Foragers were clearly less interested in seeds, probably because ants like *Myrmica*, began to forage for other food sources like lepidopteran larvae that appeared on the new herbaceous plants (Ohkawara et al., 1997). As we know that larvae are the main recipient of elaiosome intakes (Fischer et al., 2005; Fokuhl et al., 2007) and can stimulate prey and seed collection by workers when no alternative food is available (Boulay et al., 2005), another important cause of this steep summer decrease is probably the absence of proteins sinks like sexual pupae or brood. We observed this steep decrease immediately after the mating flight, in July for the ant species *Lasius niger* (obs. pers.). Larvae and alternative food should be two central factors controlling the seasonal phenology of elaiosome retrieval by foragers, and thus the whole myrmecochory process.

At daily scales, foraging activity can depend strongly on temperature (Boyd, 1996). Some myrmecochorous plants of dry habitats release their seeds mostly in the morning, which often corresponds to the period of maximum foraging activity of many effective ant dispersers such as *Euphorbia characias* (Espadaler & Gomez, 1996), *Euphorbia* sp., (Narbona et al., 2005) or *Helleborus foetidus* (Boulay et al., 2007b). In other habitats, ants removed the majority of freshly-released seeds during the day, and by this way, seeds avoid strong nocturnal predation by rodents (Gibson, 1993a; Kjellsson, 1985; Ness & Bressmer, 2005; Turnbull & Culver, 1983).

The nest

Nest structure and depth

After elaiosome consumption, ants can reject diaspores outside the nest (secondary transport) or abandon them inside the nest. To be buried can be advantageous for a seed: it permits to survive a fire (Berg, 1981; Majer, 1982) or predation pressures (Drake, 1981; Heithaus, 1981), and gives the best conditions for germination (Beattie & Culver, 1982; Gibson, 1993a). However, if seeds are buried too deep, seedlings may never reach the soil surface, because each seed species has an optimal germination depth that depends on its mass (Bond, Honig & Maze, 1999). Then, the nest depth and the location of chambers where seeds are abandoned (indirectly the structure of the nest) should be crucial for seed fate. Although the basic structure of a nest is generally descending tunnels that interconnect flat horizontal chambers, the depth and form of soil-nests can vary considerably among species from a few centimeters (1-2 cm) mainly in tropical and temperate habitats to desert-dwelling ants that dig the deepest nests up to several meters (Tschinkel, 2003; Williams & Lofgren, 1988). Considering a case of seed-disperser ant species, *Myrmica ruginodis* is a slightly nomadic species that build shallow nests with large nest chambers and thin walls using available vegetation (Brian, 1977). It could be considered as a good seed disperser as it has been reported transporting myrmecochorous seeds of 32 plant species (e.g. Delatte & Chabrerie, 2008; Sernander, 1906). Its close-related species *Myrmica scabrinodis* is comparatively sedentary and build stronger nests with smaller cells and thick mud-plastered and water-retaining walls that permit to live in hot and dry weather. These kinds of nests may not be favourable places for abandoned seeds. Interestingly, this last ant species can be considered as a poor removalist (only two seed species removal reported) (Baiges et al., 1991). One may then wonder whether there could be a general relationship between the quality of dispersal and the nest structure, like form, depth or strength (durability). In this perspective, the species *Lasius flavus*, *Lasius alienus* and *Tetramorium caespitum* are skilled nest builders that can dig wide and deep (> 1m) networks of chambers (Brian, 1977). But despite these ants are common, *Lasius flavus* is reported as non seed-disperser (Dostal, 2005; Woodell & King, 1991), and *Lasius alienus* and *Tetramorium caespitum* as poor dispersers: they have been seen transporting seeds of only one and five plant species respectively (Baiges et al., 1991; Dostal, 2005; Oostermeijer, 1989; Sernander, 1906), and in some cases, their seed-disperser status is controversial. For exemple, *Tetramorium caespitum* do not respond to seeds of the myrmecochorous plants *Ulex europaeus* and *U. minor* (Gammans et al., 2005). We suggest here that the nest structure trait (depth, form and durability) could be related to the ability of ant species to disperse myrmecochorous seeds. The positive or negative effect of nest structure on seed survival may have been one of the factors that shaped the evolution partner choice in ant-seed interactions. More precisely, this hypothesis predicts that ants with shallow nests should be more efficient in seed dispersal than ants with deep and long-lived nests. This could be tested in future investigation. But as we have shown, the nest structure is mainly related to the nomadic or sedentary living trait. So to complete the explanation of this hypothesis, we detail in the next section the links between nest relocation and seed dispersal.

Nest relocation

To be left inside the nest can have other negative consequences for seeds. They can be submitted to continuing disturbance by ants, for example by nibbling the new shoots, by maintaining water-retaining walls of nest chambers that prevent the seeds to find good conditions for germination, or by gathering seeds in clusters thus increasing seedling competition (Beattie, 1985; Hughes, 1991). Then, the advantages of remaining inside the nest may be offset not only by the nest depth effect (see previous section) but also by all these negative behavioural effects. Nest relocation can however permit seeds to avoid some of these effects and might have a role to play in seed fate and structure of plant communities (Culver & Beattie, 1978). Nest relocation was thought to be a rare phenomenon as a consequence of strong disturbances, but it appears that it is very frequent in several taxonomic groups of ants and many habitats (Hughes, 1991; Smallwood, 1982; Smallwood & Culver, 1979). It may be a flexible response to changing environmental abiotic factors (shading, moisture of soil, deterioration of nests, etc.) as well as biotic factors (accumulation of fungi in the nest, competition for food, parasites or predators pressure, etc.) (Smallwood, 1982; Smallwood & Culver, 1979). For example the ant species *Myrmica rubra* (a well-known seed-disperser) relocates frequently its nests in relation to microclimatic conditions like cold, drought and shade (Brian, 1952). One of the best-known North-American seed-disperser ant species, *Aphaenogaster rudis*, is also nomadic and has shallow and temporal nests under leaves or next to decaying logs (Ruhren & Dudash, 1996). Future research may include this parameter in the search for the conditions and selective pressures that permit the evolution and maintenance of myrmecochory.

However, the complexity of the myrmecochory system catches us up here, because if nest relocation presents many advantages for seeds (e.g. a reduced competition between seedlings), it may not offer the best nutrient-enriched soils, which are generally associated with long-lived nests (Higashi et al., 1989) and are considered one of the main selective forces that could explain the evolution of myrmecochory (Bond & Slingsby, 1983; Culver & Beattie, 1980; Davidson & Morton, 1981a; Davidson & Morton, 1981b; Westoby, Rice & Howell, 1990).

Nest distribution and community structure

The movement of seeds and their fate depends on the ant community structure and composition (Gorb & Gorb, 2003; Le Corff & Horvitz, 1995). But in fact, only a few studies address this community-level issue. At the spatial scale, the distance to nest entrance is one of the main factors that influence the distance of dispersal (Gomez &

Espadaler, 1998c). Species with larger territories (e.g. *Formica polyctena* and *Lasius fuliginosus*) contributed in a larger way to diaspore removal than species with small and numerous foraging territories (e.g. *Myrmica rubra* and *Leptothorax nylanderii*) (Gorb & Gorb, 2003). But large territories may bring a risk of seed concentration if final seed location is in the nest, near the nest entrance or at precise dump piles at the territory borders (Gorb & Gorb, 2003). The spatial structure of ant community is a complex trait that depends on many other factors, and there are a few chances that a single rule could be found to explain the seed fates. But one can however focus on the correlation between the ant community diversity and the occurrence of myrmecochory, to determine how competition between territories influences the fate of the myrmecochorous guild.

At the behavioural scale, *Rhytidoponera* sp. are subordinate species that are numerous when other species are not. In fact, it is the most important seed remover in Australia. In habitats where the ant community is more diverse, one can find more small and aggressive ant species that eat the elaiosome *in situ* (Gove et al., 2007). In Northern America, the seed-disperser ant species *Aphaenogaster rudis* is a subordinate species that tend to discover food and recruit much more quickly than other ant species in order to avoid competition for food resources (Fellers, 1987). In the same way, workers of the sub-dominant ant *Myrmica rubra* that forage in a *Formica polyctena* territory removed seeds quickly, and are disturbed when the dominant ants are present (Gorb & Gorb, 2003). Little is known about the influence of ant hierarchies and community structure on seed dispersal quality, but one can already put forward that it may play a crucial role on the dynamics of seed removal and further on the plant community structure. Future studies may verify whether these few examples are exceptions or the rule, and confirm whether behavioural subordinance is a favourable ant trait that seeds can exploit.

Conclusions

Ants are the only broad seed dispersers among insects. Although there is no clear morphological specialization of ants for seed dispersal and elaiosome consumption, we found particular colony organizations, morphological and behavioural traits that could explain why ants are indeed effective dispersers. First, traits that make ants *broad* seed dispersers are "generalist" traits: their ecological ubiquity and taxonomical diversity, their opportunistic and omnivorous diet, and their "diffuse" foraging pattern. All these traits increase conspicuously the probabilities of encounter between foragers and myrmecochorous seeds. Second, among insect taxa, ants are unique as gathering the following traits: the ground foraging that permit them to find small seeds on the soil and to relocate them in precise microsites; their ability to carry items efficiently and to transport seed far from the source (i.e. central place foragers); and the nest-living that opens the possibilities for seeds to be buried.

Besides, several ant traits that are not typical of ants, have influential consequences on ant-seed interactions: their body size can influence dispersal distances and the size matching between partners may exert selective pressures on seed size populations; the phenology of ant activity may also exert selective pressures on the phenology of the plant guild, shifting the flowering phase towards early spring in temperate regions; the ant diet, for which the carnivorous preferences have the advantage of shifting the consumption from the seed itself to the elaiosome, and by this way shifting the ant-seed interaction from antagonism (granivory) to mutualism (dispersal); the learning which can modify drastically removal rates at the scale of populations; and the ability of nest relocation from which seed fate and seedling survival depends on.

Seed dispersal by ants differs from bird-dispersal by their small size of diaspores, their shorter dispersal distances, their earlier phenology in temperate regions, their non-visual attracting cues, and by the transport of diaspore between the mandible and not into the gut. Despite that, these two mutualistic networks of interactions show similarities in the presence of generalist traits of dispersers that generate multi-specific interactions. This non-specificity is likely to be one of the conditions that stabilize the seed dispersal mutualistic networks (Bascompte & Jordano, 2007).

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Chapitre 5

Myrmecochory in European mesic habitats: which plant species are involved?

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Abstract

We drew up the first list of myrmecochorous plants of temperate Europe. We found 260 species, among them 124 are myrmecochorous (i.e. observed in the field) and 136 are potentially myrmecochorous (i.e. bearing an elaiosome but never observed in the field). We show that these plant species are mainly herbaceous. They tend to flower earlier then other plant species of the flora. This confirms that ants may have exerted selective pressures on the phenology of myrmecochorous plants towards an earlier flowering. Moreover, the proportion of seed species comprised between 1 and 3 mm width and between 0.6 and 10 mg is higher for myrmecochorous plants than for the total flora, showing the size and weight range that ants may have selected through time.

Introduction

Seed dispersal by ants occurs in various habitats around the world, from tropical forests of South America to dry habitats of Australia and South Africa, through deciduous forests of the northern hemisphere. It is generally admitted that myrmecochory involves more than 200 ant species of almost 50 genera (Gomez & Espadaler 1997) interacting with more that 3000 plant species of 80 families, with both monocotyledons and dicotyledons (Beattie 1985, Beattie & Hughes 2002, Dunn et al. 2007).

Australia and South Africa are the two hotspots of myrmecochory. In Australia, the phenomenon has been well studied (51 papers) and it is assessed that it occurs in about 1500 species of 87 genera and 24 families (Berg 1975, Westoby et al. 1991). It occurs mainly in dry sclerophyll forests, woodlands and heath, where 30 to 50% of the flora and more than 50 % of the woody shrub species could be touched by this syndrome (Westoby et al 1981, Willson et al 1990, Beattie & Hughes 2002). In South African Fynbos (23 papers), it could involves about 1300 species of 78 genera and 29 families are concerned (Slingsby & Bond 1981, Bond & Slingsby 1983). Both assessments of the number of species were based on field observations of ant behaviour, on seed morphology, literature data, and global extrapolations for the large genera such as *Acacia* (500 species in Australia), and many species still need to be tested in the field. In these habitats, elaiosomes are firm and persistent and can remain attractive for several months or even years (Berg, 1975, Beattie & Hughes, 2002). The advantages of ant-dispersal for plants are mainly the protection of seeds from granivorous ant and rodent predation, as well as their escape from fire by being placed inside the nest at a depth appropriate for germination (Drake 1981, Mayer 1982, Bond & Breytenbach 1985, Parr et al. 2007).

In Northern mesic habitats (Northern Europe, Northern America and Japan), the list of myrmecochorous plant species has never been established, but it is generally reported that about 300 myrmecochorous species could be found, with a majority of herbaceous species of the understorey of temperate deciduous forests (Sernander 1906, Beattie & Culver 1981, Beattie & Hughes 2002). We know for example that 26 to 35% of the herbaceous layer of the temperate forests of Northern America can be ant-dispersed (Beattie & Culver 1981). Although the study of myrmecochory started one century ago in Northern Europe (Sernander 1906) and despite a comparable number of studies as in Australia (54 papers), we expect to find in northern Europe only a few dozens of plant species involved in this type of dispersal. The adaptations of myrmecochorous plants living in the mesic habitats of Northern hemisphere differ from Southern habitats. Elaiosomes are usually soft, short-lived, and become desiccated and unattractive after a few days. Advantages for plants from ant-dispersal are mainly the protection of seeds against predation (rodents, birds or beetles), their placement in nutrient-rich microsites (directed dispersal) and/or the reduction in competition with siblings (Handel 1976, 1978, Culver & Beattie 1978, Culver

& Beattie 1980, Beattie & Culver 1983, Turnbull & Culver 1983, Gibson 1993, Ohkawara et al. 1997, Gorb et al. 2000). Furthermore, one of the main features of myrmecochory in Northern temperate regions is the seasonal separation between early flowering (spring) ant-dispersed plants and late-flowering (late summer and fall) other plants such as bird-dispersed ones (Thompson 1981, Oberrath & Böhning-Gaese 2002, Rico-Gray & Oliveira 2007).

We aim in this paper to draw up the first list of the myrmecochorous species of Northern Europe, and to find features or trends that characterise myrmecochorous plant species among the flora of European temperate climate.

Material and methods

Species lists

The list of the plant species was established compiling two sources of data: the plant species of which the seeds bear a nutritional appendage (*i.e.* an elaiosome), and the plant species of which seeds have been observed transported by ants. First, the species that bear an elaiosome were taken out from the German flora database *Biolflor* (3660 species) (Klotz et al. 2002). In a second time, we added all plant species that have been observed as transported by ants in some articles published about myrmecochory in this geographical area (Casazza et al 2008, Culver & Beattie 1980, Delatte & Chabrerie 2007, Delatte & Chabrerie 2008, Dostal 2005, Fischer et al 2005, Fokuhl et al 2007, Gammans et al 2005, Gammans et al 2006, Gibson 1993b, Gorb & Gorb 1995, Gorb & Gorb 1996, Gorb & Gorb 1999a, Gorb & Gorb 1999b, Gorb & Gorb 2000a, Gorb & Gorb 2003, Gorb et al 1997b, Gorb et al. 2000a, Guitian & Garrido 2006, Handel 1978, Heinken 2004, Kjellsson 1985^{a}, Kjellsson 1985b, Kjellsson 1985c, Lisci 1996, Mark & Olesen 1996, Marshall 1979, Mayer & Svoma 1998, Oberrath et al 2002, Oostermeijer 1989, Pacini 1990, Peters et al 2003, Prinzing et al 2007, Prinzing et al 2008, Servigne & Detrain 2008, Weiss 1909, Winkler & Heinken 2007), and including the Sernander's monograph (1906). We removed the species for which the presence of the elaiosome was "controversial" in *Biolflor* and that were never observed in the field with ants. Some species were reported myrmecochorous in the field but were not reported as bearing an elaiosome in the database *Biolflor*. For those species, we checked for the presence of the elaiosome in the *Comparative Plant Ecology* database (Grime et al. 2005) as well as in additional papers that studied the structure and properties of seeds (Bresinsky 1963, Pemberton 1988, 1990, Li vigni & Melatti 1999, Fischer et al 2008). Finally, the list should contain myrmecochores (with elaiosome and observation) and potential myrmecochores (with elaiosome but without observation). We analyse data on both classes pooled.

Data

For the analysis of plant traits, we used only the data from *Biolflor*. We choose to analyse several plant traits such as life form, vegetative reproduction, ecological strategy, breeding system, begin and end of flowering, flowering time, floral reward, and seed weight and width. We then calculated the proportions of the presence/absence of this trait among our list of myrmecochorous species. Then, we compared these proportions with those of the entire list of angiosperm species for which the flora *Biolflor* had data (we did not take into account the 95 species of mosses and gymnosperms compiled in *Biolflor*). We performed Chi-square tests to compare the proportions of myrmecochorous species with the proportions of the total angiosperm flora.

Results and discussion

Species, genera, families

Among the 3565 angiosperm species of the flora *Biolflor*, we found 260 species (7.2 %) that can be considered myrmecochorous (Table 1). Among these, 106 (41.2 %) bear a nutritive appendage (elaiosome) on their seeds and have been observed being transported by ants. Besides, 18 species (7 %) have been observed as being dispersed by ants, but we did not find any confirmation about the presence of a nutritive appendage on seeds in all the databases. Finally, 136 (52.9 %) plant species are known to bear a nutritive appendage but we did not find any data in the literature confirming that they are transported by ants, so they can be considered as potentially myrmecochorous.

Twenty-nine (out of 152) families were concerned with seed dispersal by ants. The eight families that contain the highest number of myrmecochorous species (Violaceae, Euphorbiaceae, Boraginaceae, Asteraceae, Juncaceae, Lamiaceae, Ranunculaceae, Poaceae) represent 57 % of the myrmecochorous species. The two families Euphorbiacae (22 sp.) and Fabaceae (9 sp.) are known to have the highest diversity of ecological interactions with ants (McKey 1989). It is worth to notice that five among the largest flora families (with more than 50 species) are not involved in myrmecochory (Brassicaceae, Apiaceae, Orchidaceae, Onagraceae and Chenopodiaceae). Conversely, two families have all their species touched by the myrmecochorous syndrome (Polygalaceae and Resedaceae), and three families are represented by only one species, which is myrmecochorous (Apocynaceae, Buxaceae and Trilliaceae).

Eighty-four genera were concerned with seed dispersal by ants. The following genera show the highest number of myrmecochorous species and represent more than half of all myrmecochorous species (*Viola, Euphorbia, Luzula, Carex, Centaurea, Potentilla, Polygala, Primula, Pulmonaria, Melica, Lamium, Melampyrum, Ranunculus*). Nearly half of the genera involved (44) have only one myrmecochorous species.

The presence of both myrmecochorous and non-myrmecochorous species in one genus suggests a considerable plasticity in mode of dispersal adapted to ecological circumstances. Moreover, the number of genus and families involved confirms that myrmecochory has evolved independently many times (Beattie 1983, 1985, Dunn et al 2007), showing a conspicuous example of convergence.

Morphology

Life form refers to the vertical position of vegetative buds, classified following the Raunkiær system (Fig 1). Myrmecochores have a relatively low proportion of phanerophytes (5 %) in comparison with the flora (17 %) (Fig. 2), and interestingly no macrophanerophytes (i.e. trees). The proportion of geophytes and hemicryptophytes are higher for myrmecochorous plants. Both classes pooled indicates that the majority of myrmecochorous plants (77 %) have vegetative buds on or in the soil. Finally, there is no hydrophyte among myrmecochores, which can be easily explainable. These European myrmecochorous traits differ from the South African Fynbos, in which the ant-dispersal syndrome is frequent in tall treelike shrubs, shrubs and perennial herbs, but rare in geophytes and absent from annuals (Bond & Slingsby 1983, 1984, Bond et al. 1991).

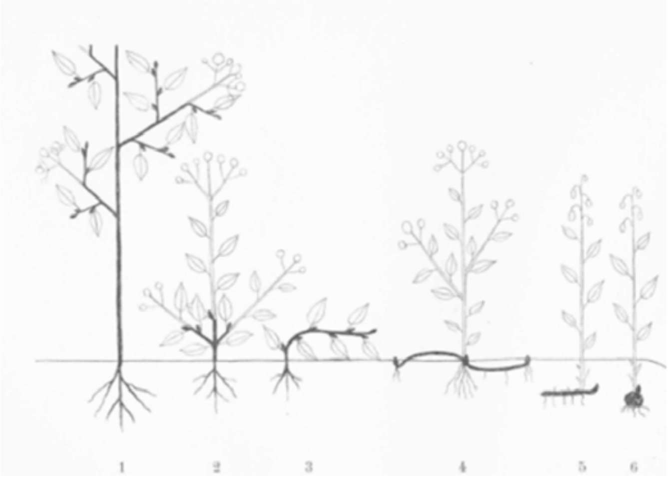

Fig 1. Raunkiær system of life forms. **Phanerophyte** (1): any woody plant that carries its dormant buds openly on branches above the ground. **Chamaephytes** (2-3): any low perennial plant whose buds overwinter just above soil level. **Hemicryptophyte** (4): any plant whose buds are situated on herbaceous shoots on the soil surface, protected by foliage or dead leaves. **Geophytes** (cryptophytes) (5-6): resting buds are subterranean, often on storing organs protected within the soil. **Therophyte** (not represented): summer annuals, which can only reproduce by means of generative diaspores. **Hydrophyte** (not represented): resting buds are situated under water on the bed or in the mud.

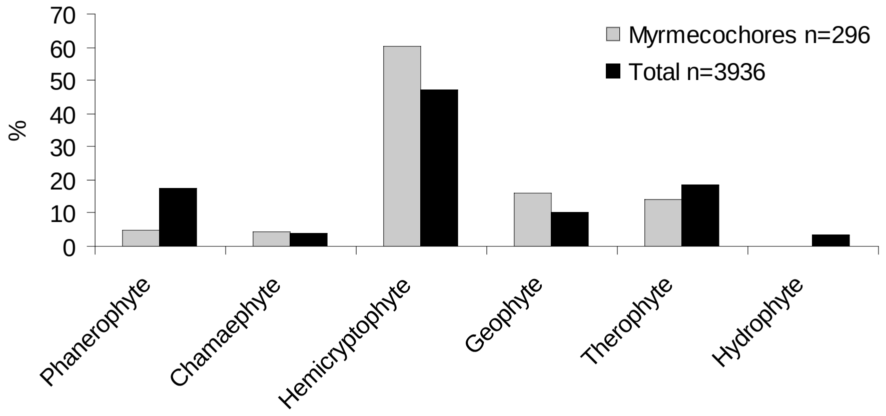

Figure 2. Proportions of life forms among myrmecochores (grey) and the total flora (black).

Floral and reproductive biology

As regards the reproductive strategy, there is no significant difference between the proportions of species with vegetative reproduction and others (Chi-square Test, p=0.1824). Nearly 60 % of myrmecochorous species can reproduce by both vegetative and generative mean, which is approximately the same as in the angiosperm flora (57.3 %).

Interestingly, the beginning and ending of the flowering time tend to be earlier for myrmecochorous species than for other species (Fig. 3 and 4) (p = 0.002, Wilcoxon Test). The proportions of flowering duration, however, are quite the same (Chi-square Test, p=0.9954), and are mainly comprised between 2 and 3 months (73 % of species). It is to note that the end of the flowering season does not mean that the fructification has begun. In fact, in some species, the fructification can be concurrent to the flowering. This evolution toward an earlier seasonal distribution of flowering and seed production is a typical trait of the community organisation in the temperate forests that has been already reported (Thompson 1981, Oberrath & Böhning-Gaese 2002, Guitián & Garrido 2006). In early spring, alternative preys for ants may be scarce and thus seed removal rates can be high. In summer, when food sources get diversified, one can observe a decrease in seed removal rates (Culver & Beattie, 1978, Ohkawara et al., 1997, Guitián & Garrido, 2006).

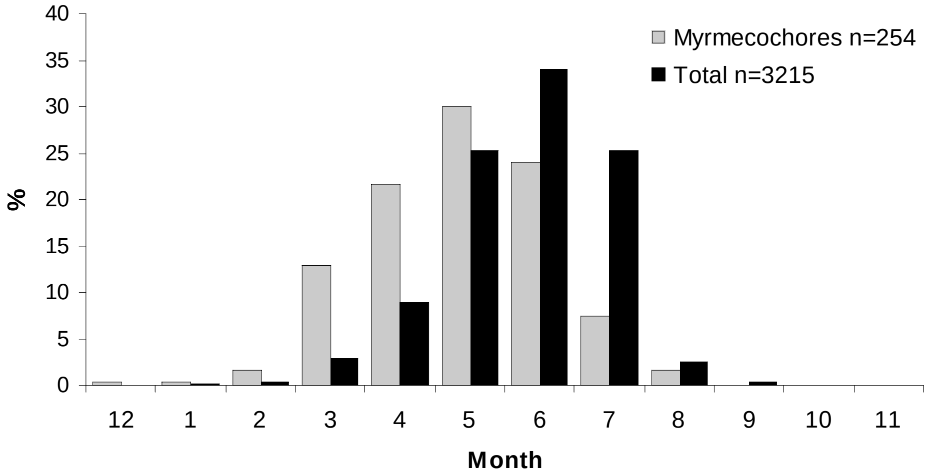

Figure 3. Proportions of species beginning their flowering phase.

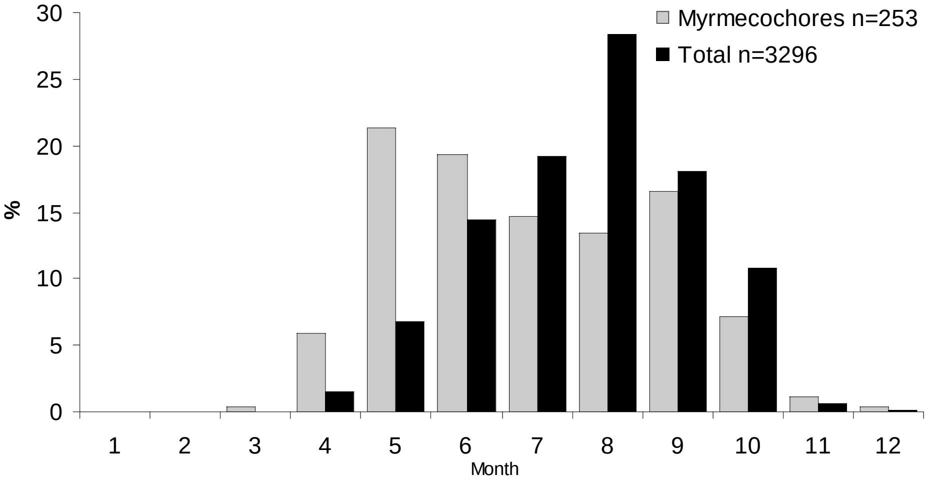

Figure 4. Proportions of species ending their flowering phase

On the flowers, 166 species of 208 myrmecochores (79.81 %) have nectar as floral reward. This proportion is not significantly different than the total flora (76.8 %, n=2289) (Chi-square test, p= 0.7654). We know that in the tropics, the attraction of nectar-feeding ants can be reinforced by the presence of extra-floral nectaries on plants, which can encourage frequent visitations of ants to the plant and thus increase the probability for a seed to be removed later by a non-granivorous ant (Fowler 1983, Cuautle et al. 2005). This needs to be confirmed in the field, but apparently there is no such trend in European species.

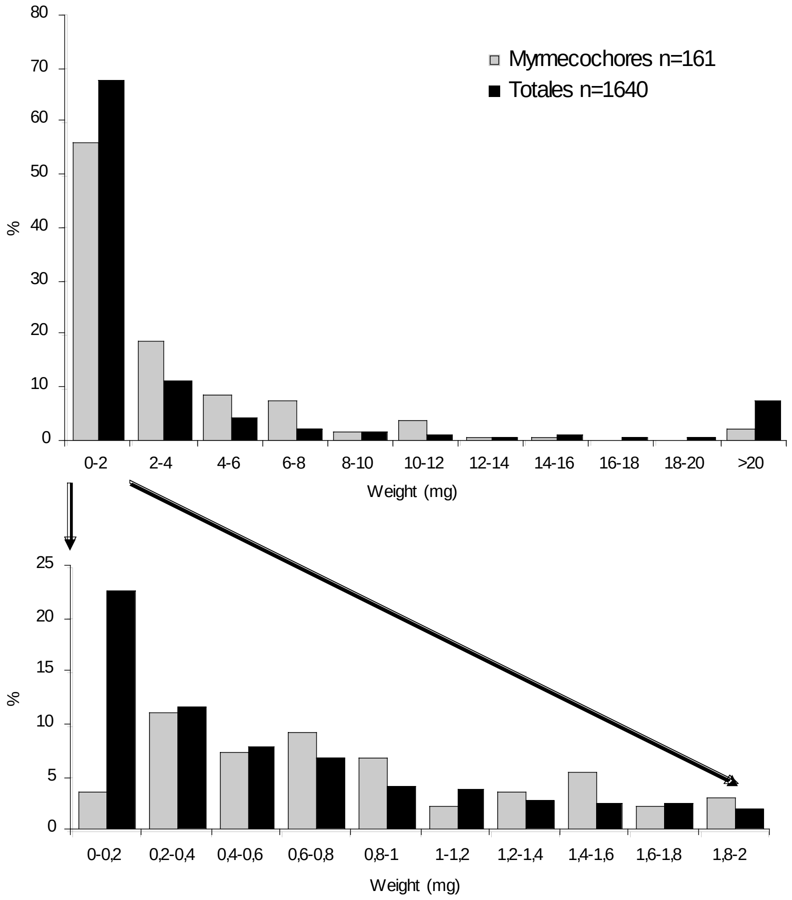

Figure 5. Proportions of seed weights

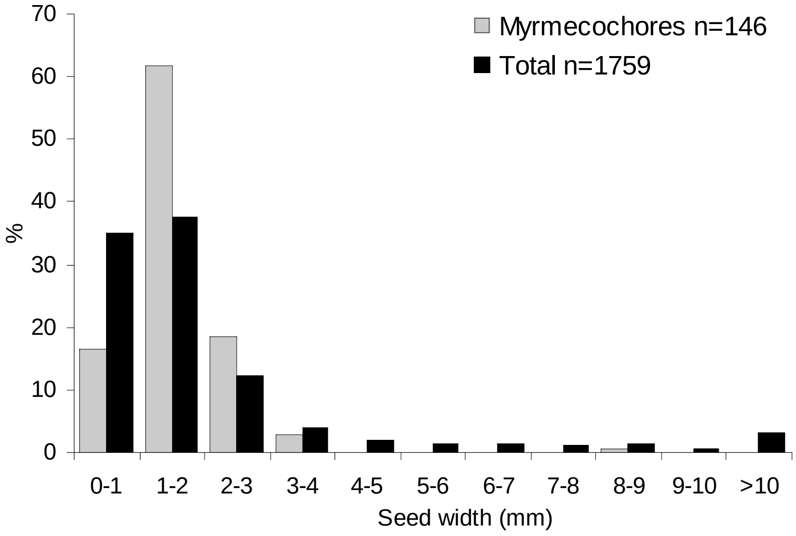

Figure 6. Proportions of seed widths

Seeds

As regard seed weight, most of the myrmecochorous seeds (56 %) have a weight lower than 2 mg and a few species with seed heavier than 10 mg (7%) (Fig. 5). When focusing on the weight class below 0.2 mg, myrmecochores are more relatively abundant beyond 0.6 mg (34 % vs. 25 % between 0.6 mg and 2 mg). The remarkable exception is for the class of seed weight lower than 0.2 mg (4 % vs. 23 %) indicating that sporochory is rare among myrmecochorous plants. In the same way, ants are likely to prefer small seeds (0-3 mm width), with a strong preference for seed width comprised between 1 and 3 mm (80 % of myrmecochorous seeds versus 50 % for all angiosperms) (Fig. 6). This width is likely to correspond with the average opening width of the ant mandibles. It has been well demonstrated that granivorous ants respond to a specific range of seed sizes, inside of which they remove the largest seeds they can. Above their seed-size scale, ants can not remove seeds that are too large, and below, ants do not detect the seeds as a potential food (Davidson, 1978; Retana and Cerdá, 1994; Kaspari, 1996; Detrain and Pasteels 2000; Willott et al., 2000; Heredia and Detrain, 2005). For myrmecochory (not granivory), we may expect a size-matching that permit ants to handle the elaiosome or the seed itself. These results suggest that ants have exerted selective pressures on seed weight and size.

Conclusions

The number of myrmecochorous plants of temperate Europe is quite higher than expected and remains far lower than the assessement in Australia and South Africa. Although there is still much work to confirm the status of many species in the field, the number of species involved in myrmecochory in temperate Europe should not vary significantly in the future. We show at large scale that the myrmecochorous species tend to flower earlier than other plant species of the flora. This suggests that ants may have exerted selective pressures on the phenology of myrmecochorous plants, shifting it towards an earlier flowering. Moreover, we have a good assessement of the size-matching between ants and seeds: ants prefer seed species between 1 and 3 mm width and between 0.6 and 10 mg. Seed size and weight are also traits that have been submitted to direct selective pressures by ants. These results should be compared with features of other floras of the world in order to determine to what extend these selective traits are typical from temperate Europe.

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Table 1. List of myrmecochorous species of temperate Europe that have been reported bearing an elaiosome and/or which seeds have been observed taken by ants. The elaiosome could be always present (1), sometimes (2) or controversial (3) (Biolflor classification), or if absent of Biolflor, could be present in the other database (Grime et al. 2005) (G) or in (Pemberton 1990 and Krückl 2001) (B).

Family	*Genus*	*species*	Presence of an elaiosome	Observed with ants
Lamiaceae	*Acinos*	*alpinus*	1	
Lamiaceae	*Acinos*	*arvensis*	1	
Ranunculaceae	*Adonis*	*vernalis*	1	X
Poaceae	*Agrostis*	*capillaris*	1	X
Lamiaceae	*Ajuga*	*chamaepitys*	1	X
Lamiaceae	*Ajuga*	*genevensis*	1	
Lamiaceae	*Ajuga*	*pyramidalis*	1	X
Lamiaceae	*Ajuga*	*reptans*	1	X
Liliaceae	*Allium*	*ursinum*	1	X
Boraginaceae	*Anchusa*	*arvensis*	1	X
Boraginaceae	*Anchusa*	*azurea*	1	
Boraginaceae	*Anchusa*	*officinalis*	1	X
Ranunculaceae	*Anemone*	*nemorosa*	1	X
Ranunculaceae	*Anemone*	*ranunculoides*	1	
Rosaceae	*Aremonia*	*agrimonoides*	1	X
Aristolochiaceae	*Asarum*	*europaeum*	1	X
Boraginaceae	*Borago*	*officinalis*	1	X
Buxaceae	*Buxus*	*sempervirens*	1	
Asteraceae	*Calendula*	*arvensis*	1	
Asteraceae	*Carduus*	*acanthoides*	B	
Asteraceae	*Carduus*	*nutans*	1	X
Cyperaceae	*Carex*	*caryophyllea*	1	
Cyperaceae	*Carex*	*digitata*	1	X
Cyperaceae	*Carex*	*ericetorum*	1	X
Cyperaceae	*Carex*	*halleriana*	1	X
Cyperaceae	*Carex*	*melanostachya*	1	
Cyperaceae	*Carex*	*montana*	1	X
Cyperaceae	*Carex*	*ornithopoda*	1	X
Cyperaceae	*Carex*	*pallescens*	1	X
Cyperaceae	*Carex*	*pillulifera*	G	X
Cyperaceae	*Carex*	*praecox*	1	X
Cyperaceae	*Carex*	*flacca*	1	X
Cyperaceae	*Carex*	*humilis*	1	
Asteraceae	*Centaurea*	*calcitrapa*	1	
Asteraceae	*Centaurea*	*cyanus*	1	X
Asteraceae	*Centaurea*	*diffusa*	1	
Asteraceae	*Centaurea*	*jacea*	1	X
Asteraceae	*Centaurea*	*montana*	1	

Asteraceae	*Centaurea*	*nigra*	1	
Asteraceae	*Centaurea*	*nigrescens*	1	
Asteraceae	*Centaurea*	*phrygia*	1	
Asteraceae	*Centaurea*	*pseudophrygia*	1	
Asteraceae	*Centaurea*	*scabiosa*	1	X
Asteraceae	*Centaurea*	*stoebe*	1	
Asteraceae	*Centaurea*	*triumfettii*	1	
Fumariaceae	*Ceratocapnos*	*claviculata*	G	
Fabaceae	*Chamaecytisus*	*ratisbonensis*	1	
Fabaceae	*Chamaecytisus*	*supinus*	1	
Papaveraceae	*Chelidonium*	*majus*	1	X
Liliaceae	*Chionodoxa*	*luciliae*	no	X
Asteraceae	*Cirsium*	*acaule*	no	X
Asteraceae	*Cirsium*	*arvense*	B	
Asteraceae	*Cirsium*	*vulgare*	1	
Portulacaceae	*Claytonia*	*perfoliata*	1	
Portulacaceae	*Claytonia*	*sibirica*	1	
Ranunculaceae	*Clematis*	*vitalba*	1	
Asteraceae	*Cnicus*	*benedictus*	1	
Liliaceae	*Colchicum*	*autumnale*	1	X
Fumariaceae	*Corydalis*	*cava*	1	X
Fumariaceae	*Corydalis*	*intermedia*	1	X
Fumariaceae	*Corydalis*	*pumila*	1	X
Fumariaceae	*Corydalis*	*solida*	1	X
Iridaceae	*Crocus*	*albiflorus*	1	
Primulaceae	*Cyclamen*	*purpurascens*	1	
Fabaceae	*Cytisus*	*scoparius*	1	X
Fabaceae	*Cytisus*	*striatus*	1	X
Poaceae	*Danthonia*	*decumbens*	1	X
Poaceae	*Deschampsia*	*flexuosa*	1	X
Celastraceae	*Euonymus*	*europaeae*	G	
Euphorbiaceae	*Euphorbia*	*amygdaloides*	1	X
Euphorbiaceae	*Euphorbia*	*cyparissias*	1	X
Euphorbiaceae	*Euphorbia*	*dulcis*	1	X
Euphorbiaceae	*Euphorbia*	*epithymoides*	1	
Euphorbiaceae	*Euphorbia*	*esula*	1	
Euphorbiaceae	*Euphorbia*	*exigua*	1	
Euphorbiaceae	*Euphorbia*	*falcata*	1	
Euphorbiaceae	*Euphorbia*	*helioscopia*	1	X
Euphorbiaceae	*Euphorbia*	*lathyrus*	1	X
Euphorbiaceae	*Euphorbia*	*lucida*	1	
Euphorbiaceae	*Euphorbia*	*palustris*	1	
Euphorbiaceae	*Euphorbia*	*peplus*	1	X
Euphorbiaceae	*Euphorbia*	*platyphyllos*	1	
Euphorbiaceae	*Euphorbia*	*salicifolia*	1	
Euphorbiaceae	*Euphorbia*	*segetalis*	1	X
Euphorbiaceae	*Euphorbia*	*seguieriana*	1	

Euphorbiaceae	*Euphorbia*	*stricta*	1	
Euphorbiaceae	*Euphorbia*	*verrucosa*	1	
Euphorbiaceae	*Euphorbia*	*villosa*	1	
Euphorbiaceae	*Euphorbia*	*virgata*	1	
Rosaceae	*Fragaria*	*vesca*	no	X
Fumariaceae	*Fumaria*	*capreolata*	3	X
Fumariaceae	*Fumaria*	*officinalis*	3	X
Fumariaceae	*Fumaria*	*parviflora*	B	
Liliaceae	*Gagea*	*lutea*	1	X
Liliaceae	*Gagea*	*minima*	1	X
Liliaceae	*Gagea*	*pratensis*	1	
Liliaceae	*Gagea*	*villosa*	1	
Amaryllidaceae	*Galanthus*	*nivalis*	1	X
Lamiaceae	*Galeobdolon*	*argentatum*	3	X
Lamiaceae	*Galeobdolon*	*luteum*	1	X
Lamiaceae	*Galeobdolon*	*montanum*	B	
Fabaceae	*Genista*	*anglica*	G	
Fabaceae	*Genista*	*tinctoria*	G	
Lamiaceae	*Glechoma*	*hederacea*	no	X
Ranunculaceae	*Helleborus*	*foetidus*	1	X
Ranunculaceae	*Helleborus*	*niger*	1	
Ranunculaceae	*Helleborus*	*viridis*	1	X
Ranunculaceae	*Hepatica*	*nobilis*	1	X
Poaceae	*Holcus*	*lanatus*	2	X
Poaceae	*Holcus*	*mollis*	2	X
Liliaceae	*Hyacinthoides*	*non-scripta*	no	X
Dipsacaceae	*Knautia*	*arvensis*	1	X
Dipsacaceae	*Knautia*	*dipsacifolia*	1	X
Dipsacaceae	*Knautia*	*drymeia*	1	
Dipsacaceae	*Knautia*	*kitaibelii*	1	
Lamiaceae	*Lamium*	*album*	G	X
Lamiaceae	*Lamium*	*amplexicaule*	G	X
Lamiaceae	*Lamium*	*hybridum*	G	
Lamiaceae	*Lamium*	*maculatum*	G	X
Lamiaceae	*Lamium*	*purpureum*	G	X
Scrophulariaceae	*Lathraea*	*squamaria*	1	X
Amaryllidaceae	*Leucojum*	*vernum*	1	
Juncaceae	*Luzula*	*alpina*	1	
Juncaceae	*Luzula*	*alpinopilosa*	1	
Juncaceae	*Luzula*	*campestris*	1	X
Juncaceae	*Luzula*	*congesta*	1	
Juncaceae	*Luzula*	*desvauxii*	1	
Juncaceae	*Luzula*	*divulgata*	1	
Juncaceae	*Luzula*	*forsteri*	1	X
Juncaceae	*Luzula*	*glabrata*	1	
Juncaceae	*Luzula*	*luzulina*	1	
Juncaceae	*Luzula*	*luzuloides*	1	

Juncaceae	*Luzula*	*multiflora*	1	X
Juncaceae	*Luzula*	*nivea*	1	
Juncaceae	*Luzula*	*pallidula*	1	
Juncaceae	*Luzula*	*pilosa*	1	X
Juncaceae	*Luzula*	*spicata*	1	
Juncaceae	*Luzula*	*sudetica*	1	
Juncaceae	*Luzula*	*sylvatica*	1	
Papaveraceae	*Macleaya*	*cordata*	1	
Scrophulariaceae	*Melampyrum*	*arvense*	1	X
Scrophulariaceae	*Melampyrum*	*cristatum*	1	
Scrophulariaceae	*Melampyrum*	*nemorosum*	1	X
Scrophulariaceae	*Melampyrum*	*pratense*	1	X
Scrophulariaceae	*Melampyrum*	*sylvaticum*	1	X
Poaceae	*Melica*	*altissima*	1	
Poaceae	*Melica*	*ciliata*	1	
Poaceae	*Melica*	*nutans*	1	X
Poaceae	*Melica*	*picta*	1	
Poaceae	*Melica*	*transsilvanica*	1	
Poaceae	*Melica*	*uniflora*	1	X
Euphorbiaceae	*Mercurialis*	*annua*	1	X
Euphorbiaceae	*Mercurialis*	*perennis*	1	X
Caryophyllaceae	*Moehringia*	*ciliata*	1	
Caryophyllaceae	*Moehringia*	*muscosa*	1	X
Caryophyllaceae	*Moehringia*	*trinervia*	1	X
Poaceae	*Molinia*	*arundinacea*	1	X
Boraginaceae	*Myosotis*	*scorpioides*	no	X
Boraginaceae	*Myosotis*	*sparsiflora*	1	X
Amaryllidaceae	*Narcissus*	*pseudonarcissus*	1	
Boraginaceae	*Nonea*	*pulla*	1	
Boraginaceae	*Nonea*	*rosea*	1	X
Boraginaceae	*Omphalodes*	*scorpioides*	2	X
Liliaceae	*Ornithogalum*	*nutans*	no	X
Trilliaceae	*Paris*	*quadrifolia*	no	X
Scrophulariaceae	*Pedicularis*	*sylvatica*	1	
Poaceae	*Poa*	*trivialis*	1	X
Polygalaceae	*Polygala*	*alpestris*	1	
Polygalaceae	*Polygala*	*amara*	1	
Polygalaceae	*Polygala*	*amarella*	1	
Polygalaceae	*Polygala*	*calcarea*	1	
Polygalaceae	*Polygala*	*chamaebuxus*	1	
Polygalaceae	*Polygala*	*comosa*	1	
Polygalaceae	*Polygala*	*serpyllifolia*	1	
Polygalaceae	*Polygala*	*vulgaris*	1	X
Portulacaceae	*Portulaca*	*oleracea*	1	
Rosaceae	*Potentilla*	*alba*	1	X
Rosaceae	*Potentilla*	*anserina*	2	
Rosaceae	*Potentilla*	*collina*	1	

Rosaceae	*Potentilla*	*crantzii*	1	
Rosaceae	*Potentilla*	*erecta*	1	
Rosaceae	*Potentilla*	*recta*	B	
Rosaceae	*Potentilla*	*sterilis*	1	
Rosaceae	*Potentilla*	*tabernaemontani*	1	
Rosaceae	*Potentilla*	*thuringiaca*	1	
Primulaceae	*Primula*	*auricula*	1	
Primulaceae	*Primula*	*clusiana*	1	
Primulaceae	*Primula*	*elatior*	1	X
Primulaceae	*Primula*	*farinosa*	1	
Primulaceae	*Primula*	*hirsuta*	1	
Primulaceae	*Primula*	*minima*	1	
Primulaceae	*Primula*	*veris*	1	
Primulaceae	*Primula*	*vulgaris*	1	X
Fumariaceae	*Pseudofumaria*	*alba*	1	
Fumariaceae	*Pseudofumaria*	*lutea*	1	
Boraginaceae	*Pulmonaria*	*angustifolia*	1	
Boraginaceae	*Pulmonaria*	*collina*	1	
Boraginaceae	*Pulmonaria*	*mollis*	1	X
Boraginaceae	*Pulmonaria*	*montana*	1	
Boraginaceae	*Pulmonaria*	*obscura*	1	X
Boraginaceae	*Pulmonaria*	*officinalis*	1	X
Boraginaceae	*Pulmonaria*	*rubra*	1	
Ranunculaceae	*Pulsatilla*	*micrantha*	1	
Ranunculaceae	*Ranunculus*	*cassubicus*	2	X
Ranunculaceae	*Ranunculus*	*ficaria*	1	X
Ranunculaceae	*Ranunculus*	*flammula*	1	
Ranunculaceae	*Ranunculus*	*lanuginosus*	1	
Ranunculaceae	*Ranunculus*	*walo-kochii*	1	
Resedaceae	*Reseda*	*alba*	no	X
Resedaceae	*Reseda*	*lutea*	1	X
Resedaceae	*Reseda*	*luteola*	3	X
Resedaceae	*Reseda*	*odorata*	3	X
Liliaceae	*Scilla*	*amoena*	1	X
Liliaceae	*Scilla*	*bifolia*	1	X
Liliaceae	*Scilla*	*siberica*	1	X
Poaceae	*Setaria*	*verticillata*	1	
Asteraceae	*Silybum*	*marianum*	B	
Lamiaceae	*Stachys*	*sylvatica*	no	X
Caryophyllaceae	*Stellaria*	*holostea*	no	X
Boraginaceae	*Symphytum*	*asperum*	1	
Boraginaceae	*Symphytum*	*bulbosum*	1	X
Boraginaceae	*Symphytum*	*officinale*	1	X
Boraginaceae	*Symphytum*	*tuberosum*	1	
Santalaceae	*Thesium*	*alpinum*	1	X
Santalaceae	*Thesium*	*ebracteatum*	1	
Santalaceae	*Thesium*	*linophyllon*	1	

Santalaceae	*Thesium*	*pyrenaicum*	1	
Fabaceae	*Ulex*	*europaeus*	1	X
Fabaceae	*Ulex*	*gallii*	G	
Fabaceae	*Ulex*	*minor*	G	X
Scrophulariaceae	*Veronica*	*agrestis*	1	X
Scrophulariaceae	*Veronica*	*chamaedrys*	no	X
Scrophulariaceae	*Veronica*	*hederifolia*	1	X
Scrophulariaceae	*Veronica*	*persica*	1	X
Apocynaceae	*Vinca*	*minor*	1	
Violaceae	*Viola*	*alba*	1	
Violaceae	*Viola*	*ambigua*	1	
Violaceae	*Viola*	*arvensis*	1	X
Violaceae	*Viola*	*biflora*	1	
Violaceae	*Viola*	*calaminaria*	1	
Violaceae	*Viola*	*canina*	1	X
Violaceae	*Viola*	*collina*	1	
Violaceae	*Viola*	*elatior*	no	X
Violaceae	*Viola*	*epipsila*	1	
Violaceae	*Viola*	*guestphalica*	1	
Violaceae	*Viola*	*hirta*	1	X
Violaceae	*Viola*	*kitaibeliana*	1	
Violaceae	*Viola*	*mirabilis*	1	X
Violaceae	*Viola*	*montana*	1	
Violaceae	*Viola*	*odorata*	1	X
Violaceae	*Viola*	*palustris*	G	
Violaceae	*Viola*	*pumila*	1	
Violaceae	*Viola*	*pyrenaica*	1	
Violaceae	*Viola*	*reichenbachiana*	1	X
Violaceae	*Viola*	*riviniana*	1	X
Violaceae	*Viola*	*rupestris*	1	
Violaceae	*Viola*	*schultzii*	1	
Violaceae	*Viola*	*stagnina*	1	
Violaceae	*Viola*	*suavis*	1	X
Violaceae	*Viola*	*tricolor*	1	

Conclusions et perspectives

Le système d'interactions mutualistes que constitue la myrmécochorie contient plusieurs niveaux de complexité, rendant la compréhension globale du système difficile. A un premier niveau, chaque habitat possède des caractéristiques propres qui influencent le système via des pressions de sélection différentes (feux, prédation, compétition, ressources disponibles, etc.). A un deuxième niveau, au sein d'un habitat, les interactions sont non seulement multi-spécifiques, mais elles montrent également des intensités différentes pour chaque couple plante-fourmi. Il en résulte que chaque interaction se place dans un continuum entre antagonisme et mutualisme. A cela, il faut ajouter un troisième niveau, la variabilité géographique (locale et régionale) et temporelle (jour et saison) dont nous commençons à peine à entrevoir l'étendue (e.g. Garrido et al. 2002, Boulay et al. 2006, 2007).

Le présent travail s'est focalisé sur une quatrième dimension de cette complexité, à savoir le caractère *séquentiel* du processus de myrmécochorie. Cette dimension a rarement été prise en considération (sauf Gorb & Gorb 2003). Le plus souvent, les études se sont concentrées seulement sur une étape du processus : la chute des graines au sol, le comportement de prise à la source, la relocalisation des graines, la germination, etc. Ici, nous avons retracé, en gardant les mêmes espèces et conditions expérimentales, les étapes du processus impliquant les fourmis[8] : prise des graines à la source, transport vers le nid, traitement dans le nid, rejet hors du nid. Chaque étape de la séquence a montré une variabilité propre à chaque couple fourmis-graines. Nous avons tenté de mettre en évidence les mécanismes à l'origine de ces variabilités interspécifiques.

À la première étape, c'est-à-dire lors du contact entre fourmis et graines, nos résultats montrent que la présence d'un élaiosome ne modifie pas la probabilité qu'une fourmi a de contacter une graine. Il n'y a donc pas d'attraction à distance. Les fourmis suivent toujours la même séquence de comportements : antennation, manipulation et prise des graines, et peuvent abandonner le contact à chaque étape. Une fois la graine contactée avec les antennes, les fourmis réagissent différemment selon leur espèce et celle de la graine rencontrée **(Chapitre 1)**. Ainsi, les fourmis *Myrmica rubra* ont été plus rapides et efficaces dans la prise de graines à la source que les fourmis *Lasius niger*, en particulier avec les graines de Chélidoine. Le nombre d'antennations à la source, variable selon les paires fourmis-graines, peut être considéré comme un indicateur de l'« hésitation » des fourmis à prendre les graines **(Chapitre 1)**.

L'observation de la deuxième étape, c'est-à-dire le traitement des graines à l'intérieur du nid, a été possible grâce à une approche de laboratoire. A l'instar de la première étape de collecte des graines, les fourmis *Myrmica rubra* ont montré une plus grande rapidité dans les dynamiques d'arrachage de l'élaiosome et de rejet des items hors du nid que les fourmis *Lasius niger* **(Chapitre 2)**. Les dynamiques d'arrachage de l'élaiosome ont aussi été influencées par l'espèce de graine (plus important pour les graines de *Chelidonium majus*). De manière inattendue, les dynamiques de rejet des items hors du nid ont été peu influencées par l'espèce de graine. Ceci montre une grande spécificité des fourmis dans les dynamiques de traitement des items dans le nid. De plus, pour une graine, le fait de ne plus avoir d'élaiosome diminue le nombre moyen d'ouvrières qui la contactent et la manipulent simultanément. La perte de l'élaiosome diminue donc l'attractivité de la graine pour les fourmis à l'intérieur du nid. Même si nous avons montré que l'absence d'élaiosome accroit la probabilité qu'une graine a d'être rejetée, le rejet par une ouvrière d'une graine qui vient de perdre son élaiosome n'est cependant pas automatique. Ainsi, nous observons des cas de graines entières rejetées hors du nid, et inversément, des graines dépourvues d'élaiosomes restant dans le nid. Nous avons par ailleurs mis en évidence qu'une proportion variable de graines rapportées au nid (moins de la moitié) étaient déposées

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[8] L'ensemble du processus de dispersion comprend deux étapes où les fourmis n'interviennent pas : une première phase de mise à disposition des graines par la plante, et une étape de germination et de survie des plantules qui a lieu après l'abandon des graines par les fourmis.

directement au contact des larves, les autres étant traitées ailleurs par les ouvrières ou laissées temporairement à l'abandon dans le nid **(Chapitre 2).**

Coté plantes, plusieurs facteurs peuvent entrer en jeu pour expliquer ces différences de comportement lors de la récolte des graines à la source et du traitement des graines dans le nid. Ces facteurs sont la taille des graines, leur consistance, leur forme, ou leur composition chimique. Même si nous n'avons pas isolé chaque facteur indépendamment pour en mesurer leurs influences respectives, nos résultats peuvent cependant appuyer les hypothèses suivantes : le rapport de poids élaiosome/graine influencerait le choix des fourmis (Gunther & Lanza, 1989, Hughes & Westoby, 1992b, Bas el al., 2007), ainsi que le fait que l'élaiosome serve de « poignée » facilitant la prise des graines (O'Dowd & Hay 1980, Horvitz 1981, Gomez et al. 2005) **(Chapitre 1)**. La composition chimique des deux espèces de graines semble être différente **(Annexe)**, et il est probable que certains composés chimiques présents à la surface des graines soient responsables d'un comportement de transport, comme cela a été montré pour l'acide oléique (Wilson 1958, Boulay et al. 2006) ou le 1,2 dioleine (Marshall et al. 1979, Brew et al. 1989). Mais leur rôle sur le comportement des fourmis ne pourra être déterminé que par des expériences ultérieures.

Coté fourmis, l'hypothèse la plus probable qui expliquerait un taux de prise plus rapide par les *Myrmica rubra*, est la convergence d'odeurs entre élaiosome et proies (Hughes et al. 1994). Ainsi, une espèce carnivore (ou à tendance carnivore comme les *Myrmica*) serait plus efficace dans le comportement de prise de graines à élaiosomes « mimant » chimiquement une proie. Ce scénario a également permis de générer une nouvelle hypothèse de travail prometteuse : la rapidité de traitement des graines dans le nid et de rejet hors du nid serait une conséquence d'un comportement hygiénique plus développé chez les fourmis carnivores, habituées à gérer des proies rapidement périssables **(Chapitre 4)**. Dès lors, la myrmécochorie pourrait s'avérer être un bon modèle d'étude de la gestion des déchets par les fourmis dans le nid. De plus, il est également possible que les contacts fréquents et prolongés entre les graines de *Chelidonium majus* et le couvain des deux espèces de fourmis **(Chapitre 2)** soient dus à une convergence entre les odeurs des graines et celles du couvain. Les composés responsables d'un tel comportement pourraient être des trioléines, qui sont non seulement connues pour être des phéromones du comportement de soin au couvain *Solenopsis invicta* (Bigley & Vinson 1975), mais ont été observées comme ayant une influence sur le comportemnt de récolte des graines (Boulay et al. 2006).

Par ailleurs, nous avons isolé expérimentalement le paramètre de dessiccation des graines afin mesurer son influence sur le taux de prises **(Chapitre 3)**. Nous avons montré que la dessiccation réduisait les taux de prises des fourmis, et que la réhydratation des mêmes graines leur permettait de retrouver une attractivité et donc une valeur fonctionnelle prolongée. L'effet de la dessiccation sur le comportement des fourmis est cependant très différent d'une espèce de graine à l'autre : les graines de *Viola odorata* perdent presque toute attractivité après 4 jours de dessiccation et leur réhydratation ne rétabli que faiblement leur attractivité ; alors qu'à l'inverse, les graines de *Chelidonium majus* gardent un tiers de leur attractivité après un mois de dessiccation et leur réhydratation restaure presque entièrement leur attractivité. Le facteur « dessiccation » des graines et des élaiosomes pourrait prendre une place centrale dans la compréhension du système de la myrmécochorie, et en particulier dans l'élaboration d'un scénario évolutif de l'apparition des élaiosomes. En effet, il a été montré d'une part que les élaiosomes avaient une fonction de régulation des flux d'eau entre la graine et l'extérieur (Bianchini & Pacini, 1996) et d'autre part que la myrmécochorie était très présente dans les milieux secs et semi-arides du globe. Il est dès lors probable que les élaiosomes soient apparus au cours de l'évolution suite à des pressions de sélection abiotiques (chaleur et sécheresse) (Forest et al 2007) et aient ensuite évolués sous la pression des fourmis vers une augmentation de la taille des élaiosomes (Edwards et al. 2006) et vers un changement de leur composition chimique indépendamment de la graine elle-même (Hughes et al. 1994, Fischer et al. 2008). Ce phénomène de dessiccation constitue l'une des perspectives de recherche les plus intéressantes actuellement pour la myrmécochorie (Rob Dunn, comm. pers.).

L'étude comparative que constitue la deuxième partie de ce travail nous a permis de prendre du recul et d'analyser plus globalement le système de la myrmécochorie aussi bien du point de vue des fourmis que des plantes. Ainsi, nous avons dressé un aperçu des principales caractéristiques des fourmis dispersatrices de graines myrmécochores **(Chapitre 4)**. Certaines caractéristiques « généralistes » des fourmis rendent les rencontres entre fourmis et graines très probables : leur ubiquité et diversité taxonomique, leur régime alimentaire omnivore, et leur fourragement « diffus » et opportuniste. Les fourmis possèdent des traits qui les rendent uniques par rapport aux autres insectes : le fourragement au sol, la capacité à transporter de la nourriture, ainsi que la nidification. Enfin, certains traits des fourmis ont une influence considérable sur la dispersion des graines : leur taille, les préférences de régime alimentaire, la phénologie, la capacité d'apprentissage et la fréquence de déménagement des nids. Alors que dans les études sur les mutualismes plantes-animaux, les animaux sont toujours moins étudiés que les plantes (Cushman & Beattie 1991, Bronstein 1994), cette synthèse constitue une étape importante dans l'étude détaillée et intégrée du partenaire « animal » de la myrmécochorie.

Côté plante, nous avons entamé une réflexion globale sur la myrmécochorie Européenne. Pour la première fois, une liste des plantes myrmécochores et potentiellement myrmécochores des régions d'Europe tempérée a été constituée (260 spp.) et nous avons pu dessiner des tendances quant à leurs caractéristiques. Nous montrons que ces dernières sont majoritairement herbacées, et ont tendance à fleurir plus précocement que les autres espèces, et que la proportion de graines myrmécochores comprises entre 1 et 3 mm et entre 0,6 et 10 mg est plus importante que dans le reste de la flore **(Chapitre 5)**. Ces résultats sont interéssants en soi pour la flore de nos régions, mais gagneraient à être comparés aux caractéristiques des flores myrmécochores des autres régions du monde (Australie, Afrique du Sud, forêt amazonnienne, etc.). Hors, notre étude a bénéficié de la mise à disposition d'une exceptionnelle base de données botanique (Biolflor) qui n'a actuellement pas d'équivalent dans le monde. Gageons que la compilation prochaine de données botaniques dans les autres régions du monde permettra d'entamer une fructueuse étude comparative globale de la myrmécochorie.

Vers une compréhension de la complexité des réseaux d'interactions mutualistes

L'analyse de la « séquence myrmécochorique » a montré que chaque étape du processus générait de la variabilité. Or, il est fort probable que la variabilité soit cumulée voire décuplée au fil des étapes. Des variations à la source **(Chapitre 1)** se retrouvent dans le nid, qui lui-même génère d'autres différences **(Chapitre 2)**, menant au final à des taux de consommation d'élaiosome différents, ainsi que par extension, des distances de dispersion ou des devenirs de graines variables. A l'issue de ce travail, il n'est cependant pas possible de se prononcer sur la valeur adaptative globale de chaque partenaire (fitness) car il manque des mesures expérimentales de la survie des plantules (et de leur capacité à se reproduire), ainsi que des mesures de la croissance des colonies de fourmis (et de leur capacité à se reproduire). Nous pouvons juste supposer que les importantes différences de traitement des graines (et de consommation de l'élaiosome) peuvent être reportées, à l'issue de l'ensemble des interactions d'une saison entière, sur les valeurs adaptatives de chaque partenaire. En effet, nos expériences de laboratoire doivent être confrontés à l'avenir avec des données de terrain et replacés dans un contexte écologique.

Pendant longtemps, la plupart des recherches sur les mutualismes s'est concentrée sur les interactions spécifiques entre deux partenaires, négligeant la majorité des mutualismes, c'est-à-dire les interactions multi-spécifiques complexes comme la pollinisation ou la dispersion des graines (Stanton 2003, Strauss & Irwin 2004). Dans les années 70, quelques modèles de coévolution entre graines et agents de dispersion ont été élaborés sur base des modèles connus de coévolution spécialisées (Snow 1971, McKey 1975, Howe 1977, Howe & Eastbrook 1977). Or, les études de terrain qui ont suivi ont contredit les prédictions, observant une imposante majorité de liens « diffus » entre partenaires, c'est-à-dire non-obligatoires et non-spécialisés (Wheelright & Orians 1982, Howe 1984, Herrera 1985). Depuis, pour désigner un réseau d'interactions mutualistes (e.g. les réseaux de pollinisation ou de dispersion de graines par les oiseaux, ou par les fourmis), on emploie généralement le terme de « mutualisme diffus » (e. g. Beattie 1985). Or, le terme « diffus » reste vague et ne permet pas de décrire ni de comprendre la complexité des phénomènes en jeu (Thompson 1994). Ainsi, depuis quelques années, plusieurs équipes ont ouvert la « boite noire » des mutualismes diffus grâce à la construction de cadres théoriques tels que la *Mosaïque Géographique de la Coévolution* (Thompson 1994, 2005), et la théorie des réseaux appliqués aux interactions mutualistes (Bascompte & Jordano 2007).

Dans le système myrmécochorie, d'une part nous avons montré que les interactions graines-fourmis pouvaient être très variables à toutes les étapes du processus et *a fortiori* en conditions naturelles. D'autre part, de nombreux résultats contradictoires ont été accumulés depuis des dizaines d'années sur les effets positifs (ou négatifs) de la myrmécochorie sur les plantes (distances de dispersion, dispersion dirigée, protection contre les prédateurs, etc.) et sur les fourmis (augmentation du nombre d'ouvrières ou de sexués, biais du sex-ratio, taux de survie des reines, etc.). De telles variations de résultats dans les interactions graines-fourmis, combinées à une variabilité géographique et temporelle constitue le « matériel brut » de la *Mosaïque Géographique de la Coévolution* (Thompson 1994, 2005). Ce cadre conceptuel intégrant les approches de l'écologie évolutive, la génétique des populations, la phylogéographie, la systématique, la biochimie, la physiologie et la biologie moléculaire, tente d'élaborer une théorie générale de la coévolution. Elle postule que chaque interaction se situe dans un continuum entre mutualisme (par exemple la dispersion) et antagonisme (par exemple la granivorie) et englobe dans la définition de coévolution aussi bien les interactions dites « diffuses » que les spécifiques et obligatoires. Enfin, elle génère des scénarios de coévolution et hypothèses précises et testables. Par exemple, on suppose l'existence de localités (hotspots) où les interactions mutualistes seraient soumises à une sélection réciproque (coévolution). Ces hotspots seraient intriquées dans une matrice géographique sans sélection coévolutive particulière (coldpots). La théorie prédit que les interactions inter-spécifiques forment une mosaïque géographique d'intensités d'interaction différentes et variables dans le temps. La compréhension du fonctionnement de cette mosaïque pourrait ensuite servir à prédire les formes et les trajectoires coévolutives tant à l'échelle temporelle que spatiale (Thompson 1994, 2005). Il serait intéressant à l'avenir d'utiliser ce cadre théorique pour tenter de dresser un schéma général de la myrmécochorie. Bien sûr, les connaissances actuelles sur la myrmécochorie sont encore insuffisantes, mais d'ores et déjà, une série d'études dans la péninsule ibérique ont ouvert le chantier en mesurant les variations géographiques et temporelles de la dispersion des graines par les fourmis suivant les hypothèses de ce cadre théorique (Garrido et al. 2002, Fedriani et al. 2004, Boulay et al. 2006, 2007, Alcantara et al. 2007, Manzaneda et al. 2007, Rey & Manzaneda 2007). Notre étude, combinant deux espèces de plantes avec deux espèces de fourmis, s'inscrit dans cette logique d'analyse de la variabilité interspécifique des interactions mutualistes.

Toujours dans l'optique d'analyser la diversité des interactions au sein d'un mutualisme diffus, l'utilisation de la théorie des réseaux pourrait s'avérer très utile (Bascompte & Jordano 2007). Il a été montré que les réseaux d'interactions mutualistes de pollinisation et de dispersion des graines par les oiseaux avaient des caractéristiques structurelles semblables. Les réseaux mutualistes sont construits sur des liens faibles (non-obligatoires) et asymétriques, dont la structure est dite « nested », c'est-à-dire que la majorité des espèces (par exemple d'oiseaux) a des interactions avec peu d'espèces de partenaires (par exemple les fruits charnus) et que peu d'espèces généralistes ont développé des interactions avec un grand nombre d'espèces. Ceci diffère fondamentalement des réseaux d'interactions prédateur/proie qui sont dits « compartementalisés », c'est-à-dire comprenant des petits groupes d'espèces spécialisées n'interagissant que très peu entre eux (Bascompte et al. 2003, Bascompte & Jordano 2007). Il semble que la prépondérance des interactions à faibles dépendances des partenaires explique la persistance et la stabilité de ces réseaux mutualistes, en répartissant les perturbations du système à l'ensemble de la communauté (McCann et al. 1998, Bascompte et al. 2005, Bascompte & Jordano 2007). Nous sommes encore loin d'avoir assez de données sur les liens entre plantes et fourmis du réseau de myrmécochorie européen, mais notre étude des espèces de plantes **(Chapitre 5)** ainsi que la compilation des espèces de fourmis impliquées dans la myrmécochorie (cf Introduction, p 14) en constituent une première étape indispensable. La myrmécochorie représente certainement un modèle de mutualisme idéal pour étudier le fonctionnement des mutualismes « diffus ».

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Annexe

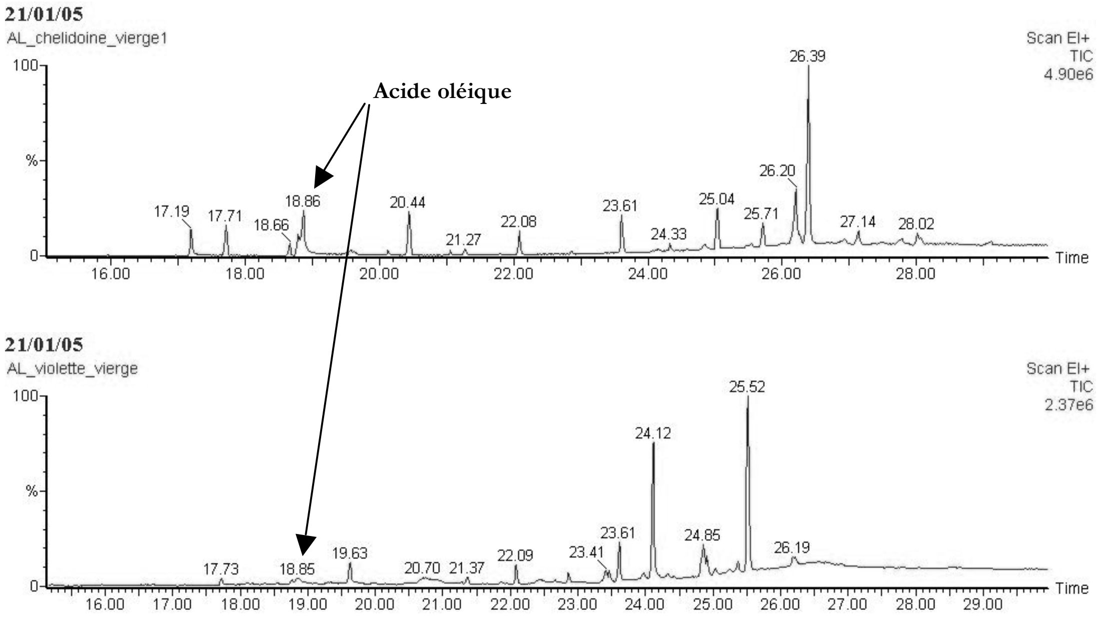
Analyses chimiques préliminaires de la surface des graines de chélidoine et de violette

L'une des hypothèses permettant d'expliquer les différences de taux de prises entre les graines de *C. majus* et *V. odorata* est la composition chimique des graines. En effet, certains composés sont connus pour influencer le comportement des fourmis. C'est le cas de l'acide oléique, par exemple, qui peut déclencher un comportement nécrophorique, c'est-à-dire de transport des cadavres hors du nid (Wilson 1958) ou vers le nid selon l'état de la colonie (Gordon 1983). En 2004, nous avons réalisé des analyses préliminaires de la composition chimique à la surface des graines de chélidoine et de violette.

Les analyses ont été faites à l'Université de Tours (avec le Pr Alain Lenoir). Nous avons analysé par chromatographie en phase gazeuse (GC), des échantillons de graines fraiches de violette (n = 5) et de chélidoine (n = 10). Chaque échantillon a été immergé 15 min dans 200µl de pentane. De cette solution, 2 µl ont été prélevés et injectés dans un chromatographe (VGM250Q) avec détecteur à ionization de flame et une colonne de silice DB-5.

Les profils de chromatographie des deux espèces de graines sont différents (cf. figure ci-dessous). Il est dès lors probable que les fourmis puissent percevoir ces différences au contact antennaire. Une analyse préliminaire de spectrographie de masse a permis de détecter la présence d'acide oléique chez les deux espèces (à plus forte proportion chez *Chelidonium majus*). Or, il a été montré que l'acide oléique pouvait jouer un rôle majeur dans le déclenchement du comportement de transport de graines d'*Helleborus foetidus* par les fourmis, et qu'une variation de sa composition pouvait affecter les probabilités de prises de graines (Boulay et al 2006).

En plus de terminer l'identification des composés, il serait intéressant d'utiliser d'autres solvants (par exemple pour extraire et analyser seulement les lipides), et de tester les substances détectées sur le comportement d'espèces de fourmis différant par leur régime alimentaire, plus ou moins carnivores.



Profils des graines de chélidoine (haut) et de violette (bas) en chromatographie en phase gazeuse.

Résumé

Ce travail porte sur la dispersion des graines par les fourmis et se divise en deux parties : l'une expérimentale (Chapitres 1, 2 & 3) et l'autre synthétique (Chapitres 4 & 5). L'approche expérimentale a consisté en une exploration en conditions de laboratoire des comportements des fourmis à deux étapes du processus de myrmécochorie: à la source de graines et dans le nid. Des graines des plantes myrmécochores *Viola odorata* et *Chelidonium majus* ont été présentées aux fourmis *Lasius niger* et *Myrmica rubra*. Chaque étape de la séquence myrmécochorique a généré une variabilité des comportements propre à chacun des quatre couples fourmis-graines.

L'élaiosome n'attire pas les fourmis à distance. Les fourmis suivent toujours la même séquence de comportements : antennation, manipulation et prise des graines. Le nombre d'antennations et de manipulations avant la prise de graines peut être considéré comme un indicateur de l'« hésitation » des fourmis à prendre les graines. L'espèce à tendance carnivore *Myrmica rubra* a été plus rapide et efficace dans la prise de graines que l'espèce éleveuse de pucerons *Lasius niger*. Parallèlement, les fourmis ont moins antenné, moins manipulé et plus pris de graines de *Chelidonium majus*, ce qui montre un intérêt particulier pour cette espèce. Un jour après l'expérience, toutes les graines des deux espèces se trouvaient dans les déchets à l'extérieur du nid, avec au moins la moitié des élaiosomes consommés **(Chapitre 1)**.

Lors du passage des graines à l'intérieur du nid, les fourmis *Myrmica rubra* ont également montré une capacité à traiter les graines rapidement, en montrant une dynamique d'arrachage de l'élaiosome et de rejet des graines hors du nid plus rapide. Le taux

d'arrachage de l'élaiosome a été influencé par l'espèce de graine, plus important pour les graines de *Chelidonium majus*. Nous avons montré qu'une proportion variable de graines rapportées au nid (moins de la moitié) étaient déposées directement au contact des larves, les autres étant traitées ailleurs par les ouvrières ou laissées temporairement à l'abandon dans le nid. Par ailleurs, les dynamiques de rejet des items hors du nid ont curieusement été peu influencées par l'espèce de graine. Pour une graine, le fait de ne plus avoir d'élaiosome diminue le nombre moyen d'ouvrières qui la contacte simultanément. Parallèlement, même si la réponse n'est pas de type « tout ou rien », l'absence d'élaiosome accroit aussi la probabilité qu'une graine a d'être rejetée. **(Chapitre 2).**

Nous avons isolé expérimentalement le paramètre de dessiccation des graines afin mesurer son influence sur le taux de prises. La dessiccation progressive des graines réduit les taux de prises par les fourmis *Myrmica rubra*. La réhydratation des mêmes graines leur permet de retrouver une attractivité et donc une valeur fonctionnelle prolongée. Les graines de *Viola odorata* perdent presque toute attractivité après 4 jours de dessiccation et leur réhydratation ne rétabli que faiblement leur attractivité. A l'inverse, les graines de *Chelidonium majus* gardent un tiers de leur attractivité après un mois de dessiccation et leur réhydratation restaure presque entièrement leur attractivité **(Chapitre 3)**.

La synthèse bibliographique a permis de dresser un aperçu des principales caractéristiques des fourmis dispersatrices de graines myrmécochores. Certains traits « généralistes » rendent les rencontres entre fourmis et graines très probables : leur ubiquité et diversité taxonomique, leur régime alimentaire omnivore, et leur fourragement « diffus » et opportuniste. Les fourmis possèdent des traits qui les rendent uniques par rapport aux autres insectes : le fourragement au sol, la capacité à transporter de la nourriture, ainsi que la nidification. Certains traits des fourmis ont une influence considérable sur la dispersion des graines : leur taille, les préférences de régime alimentaire, la phénologie, la capacité d'apprentissage et la fréquence de déménagement des nids. Nous développons également l'hypothèse que la rapidité et l'efficacité du traitement des graines par les fourmis seraient une conséquence d'un comportement hygiénique des fourmis à tendance carnivore, habituées à gérer des proies périssables **(Chapitre 4)**.

Nous avons dressé pour la première fois une liste des espèces de plantes myrmécochores et potentiellement myrmécochores des régions d'Europe tempérée (260 spp.). Nous montrons que ces dernières sont majoritairement herbacées, et ont tendance à fleurir plus précocement que les autres espèces. La proportion de graines myrmécochores comprises entre 1 et 3 mm et entre 0,6 et 10 mg est plus importante que dans le reste de la flore **(Chapitre 5).**

Mots-clés: myrmécochorie, élaiosome, dispersion des graines, dessiccation des graines, nid, comportement hygiénique, fourmis, *Lasius niger, Myrmica rubra, Viola odorata, Chelidonium majus*.

Abstract

This study concerns seed dispersal by ants and is divided in two parts: one experimental (chapters 1, 2 & 3), and one synthetic (chapters 4 & 5).

Experimental work consisted in a series of laboratory experiments, in which ant behaviour was studied at two stages of the dispersal process: at the seed source and inside the nest. Seeds of the myrmecochorous plants *Viola odorata* and *Chelidonium majus* were presented to two ant species: *Lasius niger* and *Myrmica rubra*. Each stage of the myrmecochory sequence generated a variability of behaviours for each of the four ant-seed pairs.

The elaiosomedo not attract seed at a distance. Ants followed always the same behavioural sequence: antennations, manipulations, and removal. The number of antennations and manipulations before removal can be considered as a measure of ants' "hesitation" to remove seeds. The carnivorous species *Myrmica rubra* was faster and more efficient at taking seeds than the aphid-tending *Lasius niger*. At the same time, ants antennated and manipulated less *Chelidonium majus* seeds, which shows a particular interest for this seed species. One day after the experiment, all seeds of both species were located outside the nest in the refuse piles. At least half of their elaiosomes had been consumed (**chapter 1**).

Inside the nest, *Myrmica rubra* also showed a great ability to treat seeds quickly, i.e. quicker dynamics of elaiosome removal and seed rejection outside the nest. Elaiosome removal rates were influenced by seed species (higher for *Chelidonium majus*). We showed that a variable proportion of seeds (less than half) was directly deposited in contact with larvae. The rest of the seeds were handled elsewhere by workers, or left temporarily unattended in the nest. Dynamics of seed rejection outside the nest were curiously little influenced by the seed species. For a seed, to loose the elaiosome decreased the number of workers manipulating it. At the same time, even if the rejection response is not automatic, the absence of elaiosome increases the probability for a seed to be rejected (**Chapter 2**).

We isolated the desiccation parameter in order to measure its influence on seed removal rates. Progressive seed desiccation reduced *Myrmica rubra* removal rates. Rehydration of the same seeds restored their attractiveness, thereby prolonging their functional life. *Viola odorata* seeds lost almost all their attractiveness after 4 days of desiccation, and rehydration only restored a reduced part of their attractiveness. On the contrary, *Chelidonium majus* seeds kept one third of their attractiveness after one month of desiccation, and recovered almost all their attractiveness after rehydration (**Chapter 3**).

The bibliographic review allowed us to compile an outline of the main features of seed-disperser ants. Some generalist features highly increase the probability that ants encounter seeds: their ubiquity and taxonomic diversity, omnivorous diet and their opportunistic “diffuse” foraging. Among insects, ants have unique traits that make them broad dispsersers: ground foraging, the ability to transport items, and nesting behaviour. Some other traits have a great influence on the seed dispersal system: the ant body size, their diet preferences, the phenology of the colony, the learning, and the frequence of nest relocation. We also develop the hypothesis according to which, handling efficiency of ants is a byproduct of hygienic behaviour of carnivorous oriented species, since they are used to manage perishable preys (**Chapter 4**).

We compiled the first list of myrmecochorous and potentially myrmecochorous plants species of European temperate regions (260 spp.). We show that these plants are mainly herbaceous. They also tend to flower earlier than the whole flora. The proportions of myrmecochorous seeds having a size range between 1 and 3 mm, and a weight range between 0.6 and 10 mg are higher than in the rest of the flora (**Chapter 5**).

Keywords: myrmecochory, elaiosome, seed dispersal, ants, seed desiccation, nest, hygienic behaviour, *Lasius niger, Myrmica rubra, Viola odorata, Chelidonium majus*.

Remerciements

Après quelques mois de stage sur les fourmis en forêt amazonienne en 2002 et 2003, je pensais que la recherche, c'était dormir en hamac, avoir une machette, chercher des nids de fourmis dans les arbres, boire du rhum et faire un peu de statistiques sur Excel.

Bon, je me suis un peu trompé, sauf pour le dernier truc.

Quand il a fallut trouver un financement de thèse, j'ai mis entre parenthèses ma tropicalité et j'ai atterri dans un labo à Bruxelles. Je me suis dit que c'était l'occasion d'apprendre un peu la « rigueur », ce concept un peu vague que les profs vous reprochent de ne pas avoir, en rouge sur la marge, et que j'avais jamais vraiment bien compris. Heureusement, dans le nouveau labo, y'avait quelqu'un qui étudiait les relations plante-fourmi. Alors on a fait un projet ensemble. Et puis la rigueur, j'ai compris ce que c'était. Mais j'ai mis le temps.

La thèse est un parcours initiatique et au début on ne vous le dit pas. Ou ceux qui vous le disent, on ne les écoute pas. D'ailleurs, quand on arrive dans un labo, les vieux doctorants mal rasés qu'on croise dans les couloirs vous déconseillent de faire une thèse. Et on ne les écoute pas non plus. On se dit qu'on ne sera pas pareils. Et puis au fil des mois, à force d'avoir plein de problèmes, on apprend. Lentement évidemment, parce qu'on a des années devant nous. Malheureusement, comme dit le poète, « *le temps d'apprendre à vivre, il est déjà trop tard.* » Alors on se dépêche de vite finir et dans l'urgence on se rend compte que les vieux doctorants avaient raison. Pire, on ne se rase plus et on décourage les nouveaux…

Enfin et surtout, on se rend compte qu'il y a les copains, c'est-à-dire ceux qui comprennent pas exactement ce qu'on fait depuis des années, et les collègues, ceux qui comprennent. Parce que malgré les apparences, une thèse, ça s'fait pas tout seul.

La seule personne qui ait été là du début à la fin et qui non seulement a réussi à tout lire, mais a en plus participé activement à la conception de cette thèse, c'est Claire Detrain. En me proposant le sujet, en m'accueillant dans son équipe, puis dans son labo, en me laissant pas mal de champ libre et en me montrant régulièrement et de très très … très près ce que voulait dire le mot « rigueur », elle m'a permis de venir à bout de cette épreuve. C'était dur, et parfois désagréable pour quelqu'un comme moi qui prend plaisir à se disperser, mais je dois avouer que j'ai beaucoup appris. Merci !

Chaque année, un « comité d'accompagnement » jugeait l'avancée de mes travaux. C'est ainsi que j'ai pu bénéficier de l'expertise toujours pertinente et de la sympathie de Jean Louis Deneubourg et de Pierre Meerts, qui sont aussi peut être les seuls à être rentrés dans les rouages méthodologiques et à avoir mis les mains dans le cambouis. Pour avoir aussi fait partie du Jury de thèse, je les en remercie vivement. Le rôle de Jean-Louis n'a pas été limité à ce comité : sa présence quotidienne au laboratoire a été pour moi une grande source d'inspiration. Je tiens également à remercier chaleureusement Jérôme Orivel (Université Paul Sabatier, Toulouse) et Guy Josens (ULB) pour avoir accepté de faire partie du Jury.

Ah j'oubliais, il y a ceux qu'on appelle les « anonymous referees ». Ce sont des êtres surprenants, d'abord parce qu'on ne sait pas vraiment qui ils sont et puis parce qu'ils arrivent à lire les articles qu'on écrit en anglais, alors que je pensais que personne n'aurait le courage. Pire, ils trouvent les fautes. Moi, ça m'a toujours impressionné. Ils tomberont peut-être un jour sur ces remerciements. Je laisse donc des mercis sur la table et j'éteins la lumière, qu'ils se servent.

J'ai aussi rencontré la personne la plus motivée du monde. En tout cas en ce qui concerne les fourmis. Dominique Dewulf est impressionnant par sa culture myrmécologique, sa capacité de travail et sa bonne humeur. Je le remercie pour sa disponibilité (ainsi que toute sa famille) lors des petites virées dans la nature, à soulever des pierres et à aspirer des *Myrmica* en racontant des conneries.

Nous sommes peu nombreux dans l'univers à étudier la myrmécochorie, alors forcément, ça rapproche. Au cours de ces cinq années, j'ai pu bénéficier de la correspondance pointue et toujours agréable de Raphael Boulay (Estación Biológica de Doñana, Séville, Espagne), Nicola Gammans (NERC, Dorset, UK), Alan Andersen (CSIRO, Australie), Rob Dunn (Université de Caroline du Nord, USA) et Alain Lenoir (Université de Tours, France) (que j'ai eu la chance de côtoyer une semaine autour d'un GS-MS). Aussi à Tours, merci à Annie et Guy Le Roux pour l'identification des *Myrmica rubra*. Un immense merci avec mention spéciale à Veronika Mayer (Institut de Botanique, Vienne, Autriche) pour sa confiance, ses conseils, son accueil généreux, et pour m'avoir redonné le courage de continuer ma thèse à mi-parcours.

La motivation scientifique est assez capricieuse, mais elle sait comment vous prendre aux tripes. Si elle m'a suivie tout au long de ces années, c'est parce qu'elle était accompagnée de Nicolas Vereecken (merci pour le tsunami de pdf !), Denis Michez, Rachid Tahzima et Bruno Corbara. J'ai pensé à eux en écrivant la thèse. Celle-ci est entièrement de leur faute.

Au laboratoire, j'ai profité des conseils statistiques de Raphael Jeanson et Jesus Millor, de la tolérance des responsables des serres de botanique (Bat. B) de l'ULB et de la gentillesse des jardinier du Jardin Massart et du campus de La Plaine. J'ai aussi croisé le parcours académique d'Aurélie Motard et Virginie Fournel, dont les stages ont fait avancer mes expériences. Je les en remercie. En plus d'être toujours une source de bonne humeur, Anne-Cath a des enfants adorables… qui ont collecté des graines de Chélidoine pour moi dans leur jardin. Ça ne s'oublie pas ! Toujours au laboratoire d'Ecologie Sociale, et dans l'autre labo « en face », les collègues (et néanmoins amis) qui ont partagé ces milliers d'heures et participé indirectement à l'aventure. Mention spéciale à Stéphane "Zack" Canonge (mmmbig up !) pour avoir partagé l'exigüité du bureau, supporté mes goûts en musique, pour les téléstats, la biblio à la fin et pour les conversations qui servent à rien mais qui font du bien. C'est dommage qu'on ne puisse pas tout raconter, mais le cœur y est, alors je dénonce en vrac Etienne Toffin, Aurélie Buffin, Anne-Catherine Mailleux, Greg Sempo, Aina Astudillo Fernandez (merci pour la biblio et le résumé !), Damien Denis, Raphael Jeanson, Jean-Marc Amé, Philippe Rasse, Jesus Millor, Luc Dekelver, Françoise Detournay, Alex Dobly, Alex Campo, José Halloy, Rachid Hamidi, Laurent Cournault, Stéphane Portha, Chloé Yzoard, Bertrand Collignon, Fabien Reymond, Jean-Christophe de Biseau, Patricia Féron, Laurence Piraux et ceux que mon inconscient aurait oubliés.

Enfin vient la très capricieuse et non moins importante motivation non-scientifique, entretenue en grande partie par les amis. D'abord par le courage d'Alba, qui a commencé *ma* thèse en même temps que moi et qui a eu la délicatesse de ne pas en faire une ennemie. Parce que j'ai bien plus appris à son contact qu'en douze ans d'université, je sais que l'aboutissement de ce travail est dû en grande partie à son soutien. Ma gratitude ne trouve pas assez de place dans des mots. De même, je ne pense pas pouvoir un jour évaluer le sentiment de reconnaissance que j'ai pour mes parents et pour mon frère Alex, toujours là quoi qu'il arrive. J'ai juste l'impression que rien n'aurait été possible sans eux. En Espagne, lors de la rédaction, Belen y Vicente m'ont hébergé à plusieurs reprises dans leur pension 5 étoiles avec une gentillesse à toute épreuve. Un clin d'œil à Ferdinand, l'ami de toujours, un peu délaissé ces derniers temps, mais qui comprend parce que lui aussi a passé des années à faire une thèse.

A Bruxelles, ce qu'il restera au bout du compte (en plus d'un manuscrit avec mon nom perdu dans une bibliothèque universitaire), ce sont des amitiés. Pour les projets futurs et pour les projets accomplis, les copains aux « foutues idées » : Hertje, Nico, Eric, Andreas, Ophélie, Alexis, Renaud, Jacob, et les autres qui de toute façon ne liront jamais ça. La présence rassurante et enrichissante de Jacques, Annick et leurs enfants, et aussi Mathieu & Elise. Aux copains « utopico-semenciers » -parce qu'ils ont raison, Kinder pour les extraordinaires et inoubliables virées nocturnes punko-pataphysico-surréalistes, Seb Denys pour ses cours de philosophie appliquée, Leopold pour les retraites rabelaisiennes à la campagne, et la bande d'Erasmus espagnols… qui parlent toujours fort dans le métro (Emilio, Orlando, Brijeton, Irenilla, Iria, José, Yoli, etc.). Enfin, les indispensables collocs, qui sont en réalité beaucoup plus que cela : Marie, Edu et grodidi. Dans la dernière ligne droite de la rédaction (5 mois !), le commensalisme immobilier (i.e. squat) a été pour moi non seulement salvateur mais inoubliable. Je ne trouve plus de mots, et j'espère pouvoir trouver assez de bière pour exprimer ma gratitude à Léopold, Zack & Ben, Mat & Elise, Matthieu S., Kari & Co, Nico et surtout Jean-Paul (pour la mimolette).

Il va sans dire que personne n'aurait pu lire ces lignes sans la contribution sans faille, généreuse et très précisément chiffrée des fonds publics belges du FRIA (Fonds pour la Formation à la Recherche dans l'Industrie et dans l'Agriculture) qui m'ont été attribués pendant quatre ans. Cet organisme, et plus particulièrement les personnes qui répondent au téléphone, m'ont particulièrement impressionné par leur efficacité et leur gentillesse (sic), qui ferait pâlir toute administration fédérale, communale, postale, fiscale ou syndicale. Je remercie également le baron Jaumotte de la Fondation Van Buuren pour le prix qui m'a permis de transformer l'essai en prolongeant l'aventure d'un an. Cette dernière année a aussi trouvé un soutien logistique auprès de l'Etat belge, par l'intermédiaire d'une allocation chômage, mais je ne sais pas très bien qui remercier…

Finalement, au cours de ces années, j'ai gâché la vie de milliers de fourmis en les gavant de graines et en les plaçant dans un environnement extrêmement peu stimulant (pouvant certainement rappeler les locaux d'interrogatoire de la Stasi) : du plâtre blanc à perte de vue, des murs lisses et froids, une température constante, la lumière froide des néons et toujours la même bouffe dans la gamelle. Certes j'ai souvent partagé avec elles cet environnement austère. Mais j'avais la chance de pouvoir m'en échapper le soir venu ! En fin de compte, je m'amuse encore à croire que certaines fourmis ont eu le courage de déserter, préférant le dérisoire destin de la fourmi seule mais libre, voire celui d'un suicide digne. Les autres, soumises, moins courageuses ou plus sensibles à la propagande rampante de leurs sœurs « jaunes », ont travaillé des mois durant afin que leur exploiteur puisse s'enrichir de quelques obscures publications en anglais, et sont mortes lamentablement atrophiées par une vie de travail absurde et monotone, avec pour toute reconnaissance ce ridicule paragraphe. Quelle pitoyable médaille du travail ! S'il fallait dresser un petit monument aux morts, on pourrait y voir l'inscription suivante: « Aux fourmis mortes *à cause de* la Science. »

Servigne P. 2008. Etude expérimentale et comparative de la myrmécochorie : le cas des fourmis dispersatrices *Lasius niger* et *Myrmica rubra*. Thèse de doctorat, Université libre de Bruxelles.

Ce travail porte sur la dispersion des graines par les fourmis et se divise en deux parties : l'une expérimentale (Chapitres 1, 2 & 3) et l'autre synthétique (Chapitres 4 & 5).

L'approche expérimentale a consisté en une exploration en conditions de laboratoire des comportements des fourmis à deux étapes du processus de myrmécochorie: à la source de graines et dans le nid. Des graines des plantes myrmécochores *Viola odorata* et *Chelidonium majus* ont été présentées aux fourmis *Lasius niger* et *Myrmica rubra.* Chaque étape de la séquence myrmécochorique a généré une variabilité des comportements propre à chacun des quatre couples fourmis-graines.

L'élaiosome n'attire pas les fourmis à distance. Les fourmis suivent toujours la même séquence de comportements : antennation, manipulation et prise des graines. Le nombre d'antennations et de manipulations avant la prise de graines peut être considéré comme un indicateur de l'« hésitation » des fourmis à prendre les graines. L'espèce à tendance carnivore *Myrmica rubra* a été plus rapide et efficace dans la prise de graines que l'espèce éleveuse de pucerons *Lasius niger.* Parallèlement, les fourmis ont moins antenné, moins manipulé et plus pris de graines de *Chelidonium majus*, ce qui montre un intérêt particulier pour cette espèce. Un jour après l'expérience, toutes les graines des deux espèces se trouvaient dans les déchets à l'extérieur du nid, avec au moins la moitié des élaiosomes consommés **(Chapitre 1)**.

Lors du passage des graines à l'intérieur du nid, les fourmis *Myrmica rubra* ont également montré une capacité à traiter les graines rapidement, en montrant une dynamique d'arrachage de l'élaiosome et de rejet des graines hors du nid plus rapide. Le taux d'arrachage de l'élaiosome a été influencé par l'espèce de graine, plus important pour les graines de *Chelidonium majus.* Nous avons montré qu'une proportion variable de graines rapportées au nid (moins de la moitié) étaient déposées directement au contact des larves, les autres étant traitées ailleurs par les ouvrières ou laissées temporairement à l'abandon dans le nid. Par ailleurs, les dynamiques de rejet des items hors du nid ont curieusement été peu influencées par l'espèce de graine. Pour une graine, le fait de ne plus avoir d'élaiosome diminue le nombre moyen d'ouvrières qui la contacte simultanément. Parallèlement, même si la réponse n'est pas de type « tout ou rien », l'absence d'élaiosome accroit aussi la probabilité qu'une graine a d'être rejetée. **(Chapitre 2).**

Nous avons isolé expérimentalement le paramètre de dessiccation des graines afin mesurer son influence sur le taux de prises. La dessiccation progressive des graines réduit les taux de prises par les fourmis *Myrmica rubra.* La réhydratation des mêmes graines leur permet de retrouver une attractivité et donc une valeur fonctionnelle prolongée. Les graines de *Viola odorata* perdent presque toute attractivité après 4 jours de dessiccation et leur réhydratation ne rétabli que faiblement leur attractivité. A l'inverse, les graines de *Chelidonium majus* gardent un tiers de leur attractivité après un mois de dessiccation et leur réhydratation restaure presque entièrement leur attractivité **(Chapitre 3)**.

La synthèse bibliographique a permis de dresser un aperçu des principales caractéristiques des fourmis dispersatrices de graines myrmécochores. Certains traits « généralistes » rendent les rencontres entre fourmis et graines très probables : leur ubiquité et diversité taxonomique, leur régime alimentaire omnivore, et leur fourragement « diffus » et opportuniste. Les fourmis possèdent des traits qui les rendent uniques par rapport aux autres insectes : le fourragement au sol, la capacité à transporter de la nourriture, ainsi que la nidification. Certains traits des fourmis ont une influence considérable sur la dispersion des graines : leur taille, les préférences de régime alimentaire, la phénologie, la capacité d'apprentissage et la fréquence de déménagement des nids. Nous développons également l'hypothèse que la rapidité et l'efficacité du traitement des graines par les fourmis seraient une conséquence d'un comportement hygiénique des fourmis à tendance carnivore, habituées à gérer des proies périssables **(Chapitre 4)**.

Nous avons dressé pour la première fois une liste des espèces de plantes myrmécochores et potentiellement myrmécochores des régions d'Europe tempérée (260 spp.). Nous montrons que ces dernières sont majoritairement herbacées et ont tendance à fleurir plus précocement que les autres espèces. La proportion de graines myrmécochores comprises entre 1 et 3 mm et entre 0,6 et 10 mg est plus importante que dans le reste de la flore **(Chapitre 5).**

Mots-clés: myrmécochorie, élaiosome, dispersion des graines, dessiccation des graines, nid, comportement hygiénique, fourmis, *Lasius niger, Myrmica rubra, Viola odorata, Chelidonium majus.*