AXE 2 : DE LA COLLECTE A L'UTILISATION DES RESSOURCES PAR LES COLONIES DE *VESPA VELUTINA*.

L'objectif des recherches présentées dans cet axe est de mieux comprendre l'organisation et le fonctionnement des colonies de *V. velutina*. Dans cet axe nous explorerons la manière dont s'organisent la collecte et la distribution des denrées alimentaires chez *V. velutina*, en nous intéressant à trois principaux leviers de connaissances. Tout d'abord nous étudierons **la manière dont les ouvrières choisissent leurs ressources** (sont-elles spécialisées dans la collecte de certaines ressources, ou bien opportunistes ?). Puis nous décrirons **la manière dont les ouvrières fourrageuses se déplacent autour de leur colonie**, en explorant en particulier leur rayon d'action et ses limites, ainsi que l'implication de facteurs climatiques dans la durée et la fréquence de leurs sorties du nid. Enfin, nous nous attacherons à étudier comment les ouvrières collectrices **distribuent la nourriture dans leur colonie**.

Photo de A. Girard INRA. Ouvrière de *V. velutina* rapportant une guêpe à sa colonie, où une ouvrière gardienne taguée l'accueille.

B.1 Nutrition de *Vespa velutina* et biologie des nids

B.1.1 Nutrition des adultes

B.1.1.1 Collecte de ressources chez les *Vespidae*

Les *Vespidae* sont majoritairement des insectes **généralistes**, capables de changer de sources alimentaires de manière opportuniste ; leur bol alimentaire varie ainsi suivant les années et les saisons (Jeanne & Taylor 2009). Pour leurs besoins personnels et celui des autres adultes de la colonie, les ouvrières collectent des ressources glucidiques et, pour la nutrition des larves de leur colonie, des ressources protéiques (Raveret Richter 2000, Spradbery 1973).

Les insectes sociaux comme les abeilles et les fourmis ont mis en place de nombreuses stratégies de communication pour optimiser leur collecte de ressource en partageant des informations sur leur localisation (danses (Dornhaus & Chittka 1999, Grüter & Farina 2009), phéromones de marquage de ressource (Beckers *et al.* 1989, Billen & Morgan 1988, Hölldobler 1978, Nicolis & Deneubourg 1999, Pankiw 2004)). Mais pour l'instant, aucun signal produit par une ouvrière de *Vespidae* ayant réussi à trouver une ressource intéressante n'a pu être mis en évidence, qui donnerait des indications sur la localisation de la ressource à ses consœurs (Jeanne & Taylor 2009). Ces auteurs proposent différentes hypothèses pour expliquer ce manque: (1) la taille de colonie chez les guêpes est trop petite pour que ce caractère évolutif se soit développé (Beckers *et al.* 1989), (2) les capacités de stockage des ressources dans les nids sont limitées (Portha *et al.* 2002, Dornhaus & Chittka 2004, Dechaume-Moncharmont *et al.* 2005) (3) les ressources recherchées sont souvent dispersées et éphémères, ce qui sélectionnerait des stratégies de collecte opportunistes opposées au recrutement (Raveret Richter 2000). La seule exception à cette règle connue étant *V. mandarinia*, où après plusieurs raids couronnés de succès, l'ouvrière marque la ruche ou le nid à attaquer avec une sécrétion de sa glande de van der Vecht,[1] ce qui va entrainer le recrutement de ses consœurs pour ataquer la colonie marquée (Matsuura 1984).

[1] Glande de van der Vecht : sixième glande sternale chez les *Vespidae* (Billen & Morgan 1998).

Pour autant, la plupart des *Vespidae* sont capables de fournir des éléments utiles pour guider la collecte de ressources de leurs consœurs autrement. Par exemple **(1) à la source de nourriture** grâce à la **facilitation sociale** (Wilson 1975) : la présence de consœurs ou d'espèces concurrentes sur un site de collecte peut mener, suivant les espèces, à une augmentation locale des collectes (Thorpe 1963) ou, au contraire, à une inhibition locale des collectes (Parrish & Fowler 1983, Raveret Richter & Tisch 1999). Ce mécanisme diffèrerait suivant la taille de la colonie et surtout celle de l'insecte, et serait adapté au **vol de nourriture** (Raveret Richter & Tish 1999), aux communautés locales d'espèces, et à l'apprentissage individuel. Des indices peuvent également être laissés **(2) au nid**: Les ouvrières naïves de *Vespula germanica* et *V. vulgaris* apprennent par exemple dans leur nid l'odeur de nourriture riche, et utilisent cette information pour parvenir à en localiser la source (Maschwitz *et al.* 1974). Pour *V. germanica*, le dépôt de gouttes de sirop odoriférant (fruit) dans le nid suffit à en faire sortir des fourrageuses pour rechercher cette odeur (Overmyer & Jeanne 1998, Jandt & Jeanne 2005). Beaucoup de guêpes sociales, qui rappelons-le, ne produisent pas de miel, sont capables de stocker du nectar sous la forme de gouttes de liquide sucré sur les bords de cellules vides ou contenant des œufs (Hunt *et al.* 1998). Les colonies de *Polistes annularis* au Texas arrivent même à stocker suffisamment de miel dans leur colonie pour assurer la survie d'adultes durant l'hiver, après la dissolution de la colonie (Strassmann 1979). Dans certains cas, même des protéines peuvent être stockées : plusieurs espèces d'*Epiponine* accumulent ainsi des centaines de corps de fourmis ou de termites dans leurs colonies (Richards 1978). Il semblerait qu'en Corée, des cas de stockage de miel dans des colonies de *V. velutina* (invasif dans cette région du globe depuis presque dix ans (Choi *et al.* 2012)) aient été observés (Choi, Workshop Coloss Mallorca, 2017, pers com), mais en Europe ce phénomène n'a pas encore été rapporté. En général les sites de collecte de nourriture sont partagés chez les *Vespinae*, mais pas ceux de collecte d'eau ou de fibres de bois (Greene 1991). Cependant chez *V. velutina*, un partage de site de collecte de fibres de bois par des ouvrières a été observé (A. Manon, apicultrice, 2016, pers com).

Les preuves de marquage du site de collecte de nourriture sont donc très minces, et non reproductibles, chez d'autres espèces que *V. mandarinia* (Jeanne & Taylor 2009). Mais cette dernière espèce de frelon

a besoin d'agir en coopération pour percer les défenses des colonies d'abeilles, de guêpes et de frelons dont il se nourrit. Pour les autres guêpes sociales décrites jusqu'à présent, la nature des proies n'entraine pas la nécessité d'une telle coopération, et le bénéfice d'un tel marquage pourrait être trop faible. Pour l'instant aucun marquage des ruches n'a pu être mis en évidence suite à des chasses fructueuses par *V. velutina*. **Cependant *V. velutina* a lui aussi tendance à particulièrement attaquer en groupe des colonies d'abeilles soit en ruches (Tan *et al.* 2007, Monceau *et al.* 2013b), soit lorsque la colonie d'abeille essaime (D. Thiéry pers obs.). Aucune forme de coopération lors de la chasse sur les ruchers n'a encore put être mise clairement en évidence, mais peut-être est-ce une forme intermédiaire de facilitation sociale ?**

B.1.1.2 Polyéthisme et spécialisation

Le polyéthisme est une des caractéristiques des animaux sociaux, il définit la **division du travail** (Gordon 2010). Cette organisation dépend de l'**espèce**, de différents paramètres individuels **physiques** (taille), **physiologiques** (âge, caste), de paramètres **coloniaux** (âge de la colonie, besoins) ou **climatiques** (température, luminosité, humidité) (Jeanne & Taylor 2009). Chez les espèces de *Vespidae* faisant des petites colonies, les ouvrières ont généralement une grande flexibilité comportementale, contrairement à celles des espèces à grandes colonies, qui ont généralement un large spectre d'exploration et d'exploitation de leur environnement et sont plus spécialisées (Gautrais *et al.* 2002, Perveen & Shah 2013). *Vespa velutina* faisant des colonies de tailles importantes comparées à celles d'autres frelons (Matsura & Yamane 1990), nous pourrions donc supposer une plus forte probabilité de spécialisation de ses ouvrières.

Chez les *Vespidae*, les ouvrières commencent leur vie adulte en assurant le soin au couvain et à la reine, l'entretien du nid, puis elles vont se consacrer aux tâches risquées externes au nid. Elles commenceront donc par collecter les matériaux de construction pour le nid (fibres de bois et substances végétales, eau), travailler à la maintenance extérieure du nid (réparation des trous, imperméabilisation) avant d'évoluer vers de la collecte de carbohydrates, et enfin de proies ; les ouvrières âgées se concentrant au final sur la collecte d'eau (Potter 1964, Akre *et al.* 1976). Cependant, même après avoir démarré la collecte de denrées, les ouvrières n'abandonnent pas les

travaux au nid : la spécialisation chez les *Vespidae* semble donc être assez rudimentaire en comparaison d'autres insectes sociaux. Chez les *Polistes sp.*, par exemple, le taux de collecte est étroitement corrélé au nombre de larves dans la colonie, ce qui suggère que cette activité soit majoritairement **dirigée par la demande de la colonie**, en plus de l'offre (West-Eberhard 1969, Howard & Jeanne 2005). **U**ne demande grandissante ou imprévue de matériel de construction, par exemple en cas de destruction d'une partie du nid, ne fait pas changer de rôle les collectrices de nourriture ou augmenter la cadence de collecte des collectrices de matériaux chez *P. occidentalis* ; dans ce cas, les ouvrières passent juste plus tôt du rôle de nourrices à celui de collectrices de matériaux (O'Donnell & Jeanne 1992a). De la même manière, chez *Polistes instabilis*, le retrait d'un groupe de collectrices de nectar prend quelques jours à être compensé par la colonie, non pas en augmentant la cadence de collecte des collectrices de nectar existantes, mais en les remplaçant par des ouvrières recrutées parmi les ouvrières collectrices de matériaux ou nourrices (O'Donnell 1999). Ces auteurs observèrent ainsi une augmentation d'interactions dominantes durant cette période, ce qui suggèrerait également un rôle de la dominance dans la régulation de la collecte de nectar.

De plus, la **structure de la colonie** va influer sur ses besoins, et donc fait varier le polyéthisme: chez certaines guêpes polistes par exemple, les premières cohortes d'ouvrières vont devenir collectrices beaucoup plus tôt dans leur vie que les ouvrières émergeant plus tardivement, qui resteront sur des travaux au nid bien plus longtemps (West-Eberhard 1969, Dew & Michener 1981, Post *et al.* 1988). Chez d'autres espèces comme *Polybia occidentalis* (O'Donnell & Jeanne 1990), *Metapolybia spp.* et *Protopolybia exigua* (Simões 1977, Forsyth 1978, Karsai & Wenzel 2000) il existe à la fois un polyéthisme très fort lié à l'âge, mais également à l'échelle des individus, qui développent une très forte tendance à se spécialiser pour une ou deux des quatre ressources collectées en groupes fonctionnels: proies ou nectar (nourriture), ou pulpes de bois et eau (matériaux pour le nid). Les colonies de *V.vulgaris* (Potter 1964, Roland 1976) et *P. occidentalis* (Jeanne, unpublished data) favorisent, quant à elles, la collecte de pulpe de bois et la construction du nid tôt le matin, l'humidité le rendant surement plus malléables. Au contraire la collecte de proies et de carbohydrates a tendance à se répartir plutôt dans les périodes les plus éclairées (Archer 2004). Volynchik *et al.* 2008 ont pu

corréler l'activité de vol de *V. orientalis* avec les radiations en UVB, la température et l'humidité relative, puis Plotkin *et al.* 2010 ont su rattacher ce potentiel d'activité à une emmagasinassions d'énergie par ces frelons *via* leur cuticule. Il semble donc logique dans ce cas que les activités demandant le plus d'énergie, comme la chasse, soient réalisées lors des périodes les plus éclairées.

D'un point de vue évolutif, Jeanne & Taylor 2009 ont posé l'hypothèse que les stratégies de collecte de ressource chez les *Vespidae* sont optimales pour répondre à au moins trois compromis : (1) la **quantité d'effort** consacré à la collecte ou à d'autres activités (construction du nid, soin au couvain, défense), (2) **la division des efforts** entre la collecte de **ressources alimentaires et de construction**, et (3) **l'allocation des efforts** des collectrices entre la **chasse et la collecte de carbohydrates**.

B.1.1.3 Sources de nourriture de *V. velutina*

Vespa velutina est un insecte généraliste, comme la plupart des *Vespidae* (Figure 19 A, B, C, D) (Spradbery 1973). Les imagos (ouvrières, reines et mâles) collectent des ressources dans des zones naturelles (carbohydrates - sève, fruits, miel, nectar[2]- ; protéines - insectes, cadavres) ou dans des endroits plus ou moins urbanisés (carbohydrates : confitures, sirops et autres produits sucrés ; protéines (poubelles, étals de marchés, zones d'affrètement de marchandises, pêcheries et production ostréicoles (Monceau & Thiéry 2017)). Les proies sont prémâchées en boulettes pour nourrir les larves (Figure 19.B).

[2] Les adultes de *V. velutina* peuvent être observés butinant sur différentes fleurs, comme celles entre autres du lierre, du bananier, du camélia et du cerisier.

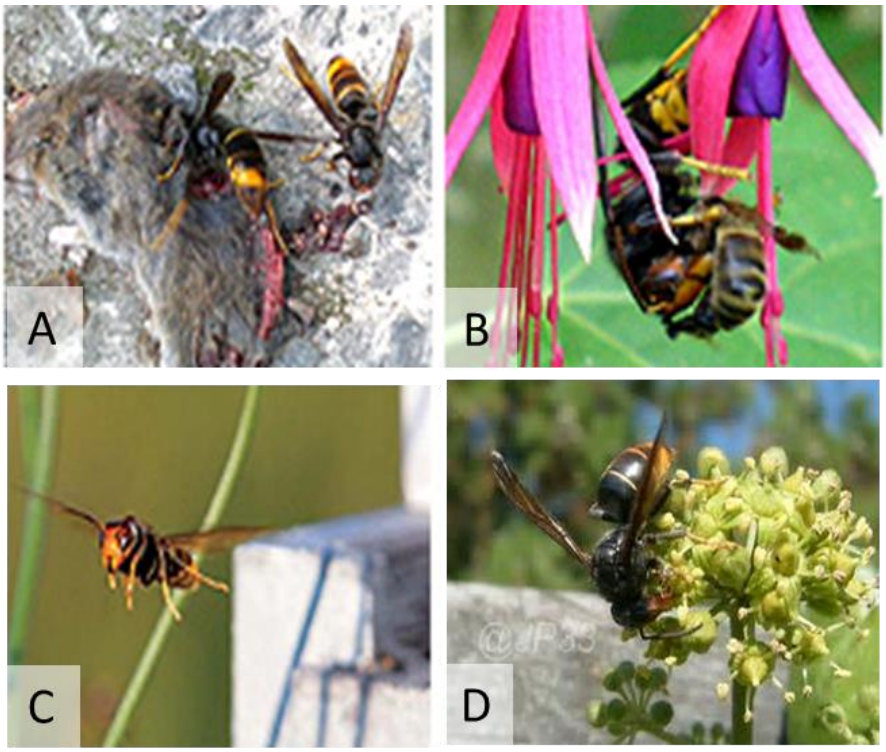


Figure 19 : A : Ouvrières de *Vespa velutina* se nourrissant de *via*nde sur une musaraigne morte, B : ouvrière de *V. velutina* découpant une abeille pour ne garder que le thorax et le donner, une fois mâché, aux larves de sa colonie, C : ouvrière de *V. velutina* en vol stationnaire devant une ruche pour y chasser des abeilles, D : *V. velutina* se nourrissant de pollen de lierre. (Photos : A, B, C Karine Monceau, D : Jean-Paul Cros).

B.1.1.4 Tester le degré de spécialisation des ouvrières de *V. velutina*

Cette expérimentation fera éventuellement l'objet d'une soumission pour publication sous forme de courte note. Elle est donc présentée sous la forme de sa rédaction actuelle en Annexe 2.1.

Résumé

Les ouvrières chasseuses de *V. velutina* capturées devant les ruches sont attirées en laboratoire par les odeurs de la ruche (phéromones de reine ou d'agrégation d'abeille, cire, pollen) (Couto *et al.* 2014). Se pose donc alors la question de la spécialisation des ouvrières de frelon pour un type particulier de ressource : les *V. velutina* chasseuses d'abeilles sont-elles spécialisées dans la chasse d'abeilles sociales ou non sociales ? D'autres ouvrières capturées lors de la collecte d'autres denrées seraient-elles aussi attirées par les abeilles ? Comme cela a été dit précédemment, les ouvrières de *V.*

velutina collectent de nombreuses denrées glucidiques et protéiques. Afin de différencier une spécialisation d'un opportunisme dans la collecte de nourriture chez *V. velutina*, nous avons donc capturé dans cette expérimentation des ouvrières de frelon venant s'approvisionner sur divers appâts. Elles ont alors été soumises à un test de choix en laboratoire, afin de mesurer leur attirance pour différents items rappelant les sources de nourriture sur lesquelles elles avaient été capturées. Ainsi, 86 ouvrières de *V. velutina* ont été collectées sur 3 types de ressources de nourriture : des ressources glucidiques végétales (fleurs, fruits) ; des ressources protéiques type carcasses (poissons, crevettes, *via*ndes) ; et des ressources de type ruche (abeilles, cire, miel). Elles ont alors été soumises à un test de choix en laboratoire, dans des conditions similaires à celles de Couto *et al.* 2014, où différents items contenant des éléments des trois différentes sources et un témoin leur ont été proposés. Le temps passé sur chaque item par individu et le nombre de visites par item et par individu ont alors été analysés après un enregistrement vidéo.

Nous n'avons pas pu avoir suffisamment d'individus pour valider statistiquement les résultats de cette expérimentation pour les ressources poisson/crevette. De manière générale, nous observons que les ouvrières de *V. velutina* ont à la fois visité plus de fois et sont restées plus longtemps sur les ressources glucidiques végétales, ici fleurs, fruits et nectar, et un peu sur les ressources de la ruche. Nous ne pouvons pas différentier ici un comportement de collecte d'un ravitaillement, les glucides étant le carburant de base des adultes. De plus, des différences liées à la nature des appâts utilisés pour la capture des individus (site, manipulation) pourraient avoir impacté nos résultats. Pour conclure, nous proposons un protocole différent pour continuer ces investigations, qui se servirait des technologies RFID afin de suivre le comportement de collecte des individus tout au long de leur vie, avec des portails électroniques placés au nid et à l'entrée de différents nourrisseurs à l'extérieur (Pour plus de détails sur ce projet, voir la Discussion générale de cette thèse).

B.1.2 Nutrition de la colonie

Le transfert de nourriture entre les ouvrières et leurs jeunes est un élément central dans la vie de la colonie (Montagner 1963, Le Masne 1980), et a été étudié très tôt chez certains insectes sociaux comme les fourmis (Deby & Tschinkel 1986, Bonavita-Cougourdan & Passera 1978, Markin 1970, Cassill & Tschinkel 1995), les abeilles (Pershad 1967), chez certaines guêpes (chez *Polistes* par Pardi 1950, Morimoto 1960, Montagner 1963), et même chez un frelon (*V. orientalis* par Ishay & Ikan 1968). Il a été mis en évidence un fort effet de la **température**, du **type de nourriture** et de la **taille de la colonie** sur le nombre d'individus nourris par des ouvrières.

Rappelons ici que chez les *Vespidae*, contrairement aux abeilles, les larves ne baignent pas dans des réserves nutritives, et doivent donc être **régulièrement approvisionnées** de petites boulettes de nourriture et entretenues par les ouvrières. Le flux de nourrissage des larves est très important, par exemple chez *Ropalidia marginata*, une larve est nourrie en moyenne 0.21 ± 0.17 fois par heure (Gadakar & Joshi 1983). Chez les guêpes sociales les larves sont nourries proportionnellement à leur poids, les plus grosses recevant plus de nourriture (Montagner 1963). Lorsque l'automne arrive, des cellules plus grandes sont construites, dans lesquelles les futures reines seront produites : elles seront d'avantage nourries que les autres larves (Makino & Yamane 1997). La vitesse de développement des larves de frelon *V. affinis* dépend du nombre de larves dans la colonie, avec les premières générations qui se développent plus vite (Martin 1992).

Les **régurgitas** que produisent les larves lorsqu'elles sont stimulées par les adultes, le plus souvent avant nutrition, ont une composition très riche en sucres et en acides aminés (Maschwitz 1965, Takashi *et al.* 1991), et contiennent des enzymes sans lesquelles les adultes seraient incapables de digérer certaines protéines (Ishay & Ikan 1967). Les enzymes en question sont des endopeptidases proches de trypsines (chez *V. orientalis* et *V. crabro*, Jany *et al.* 1978). Cette récompense inciterait les adultes à nourrir plus abondamment les larves produisant ces récompenses. En 1968, Ishay & Ikan ont démontré l'incapacité des reines de *V. orientalis* de survivre sans couvain, à partir du moment où elles sont entrées dans une phase de ponte très importante : la quantité d'énergie nécessaire pour maintenir

le rythme de ponte serait trop forte sans l'apport énergétique permis par la consommation par la reine de régurgitas larvaires.

Vespula squamosal, *V. germanica* et *V. vulgaris* peuvent partager leurs proies au nid avec leurs consœurs, parfois systématiquement s'il s'agit de nourriture de type carbohydrate (Jeanne 1991). Mais ce partage peut évoluer avec la taille de la colonie, par exemple chez *V. pensylvanica* et *V. atropilosa*, c'est seulement dans le cas de vieilles colonies que les ressources en carbohydrates seront complètement réparties (Akre *et al.* 1976).

L'objectif de l'étude présentée ci-après est d'analyser les transferts de nourriture entre une ouvrière et le reste de la colonie suivant sa structure. Nous avons donc sélectionné deux types de nourriture, glucidique (de l'eau sucrée), et protéique (jus de mouche), que nous avons fait distribuer par deux ouvrières en 24h à 23°C dans des colonies de différentes structures (ratio mâles / larves / ouvrières / reines variable). L'objectif étant de mieux comprendre comment une potentielle substance perturbatrice pourrait être distribuée suivant le type de vecteur choisi, la taille et la structure de la colonie.

Nous avons pour cela utilisé des marquages par éléments traces (métaux lourds non radioactifs), et en particulier deux éléments (Césium et Rubidium) permettant de marquer deux types différents de nourriture.

Manuscrit 3: Studying food distribution in Vespa velutina nests using heavy metal tracers.

Juliette Poidatz, Olivier Bonnard, Denis Thiéry

*Manuscrit en cours de préparation, pour soumission potentielle dans **Animal Behaviour**.*

Abstract

Vespa velutina is an invasive hornet predator of bees which has dramatically expanded across Europe. Studying the food distribution by *V. velutina* workers among nestmates will guide future colony disruption control methods. Protein and sucrose solutions were labelled with non-radioactive heavy metals, rubidium and caesium, respectively. How individual workers, returning to their nests with labelled food, distributed this food amongst colony members was measured 24 hours later using ICP-M analysis. This was repeated for each of seven wild colonies, differing in size and structure. Caesium labelled sucrose was preferentially distributed to adults (workers, males and the queen), while rubidium labelled proteins were distributed more to larvae. Considering all individuals, the lightest larvae received labelled proteins more often. A small proportion of larvae also received sucrose, but in those the quantity of received food was inversely correlated with larval weight. Amongst workers that received labelled food, the quantity of received sugar was also inversely correlated with worker weight, while the quantity of received proteins increased with worker weight. The number of fed larvae and workers was not correlated with the total number of workers and larvae in the colony. Males only received food when there were no larvae in the colony.

These results improve our knowledge of hornet biology, and especially this methodology will prove itself useful in determining the amount of food entering a nest and the number of larvae fed by a single worker.

Keywords: colony, rubidium, caesium, yellow legged hornet, social insect

Highlights

- A description of food distribution inside a hornet nest using heavy metal labelling is presented.
- Labelled sugar was distributed preferentially to adults, while labelled protein was distributed more to eggs and larvae.
- The number of fed individuals with labelled sugar increased with the total number of larvae in each of the colonies, while the number of larvae fed with labelled protein increased the total number of individuals in the colony.
- Considering all individuals, the lightest larvae received labelled protein more often.
- Amongst larvae that received labelled sugar, the quantity of received food was inversely correlated with larval weight.
- Amongst workers that received labelled food, the quantity of received sugar was inversely correlated with worker weight, while the quantity of received protein increased with worker weight.

Introduction

In social insects, the nest is the central place in which the brood is located and in which most of the interactions between individuals occur (Oster & Wilson 1978, Theralauz *et al.* 1998, Pacala *et al.* 1996; Gordon 1996). Inside the colony, food is thus transferred by trophallaxis between adults and larvae and between adults (workers, males and queen) (e.g. in *V. orientalis* in Montagner 1963 and Ishay & Ikan 1968). The magnitude of these exchanges depends on the type of food, the colony size, the temperature, the amount of food entering the nest per unit of time and the hunger level of the colony (for ants : Cassill & Tschinkel 1995, Markin 1970 ; for bees : Galiot & Azoeuf 1979, Galliot et al. 1982, Nixon & Ribbands 1952, Pershad 1967 and for wasps : Montagner 1963). For example, Markin showed in 1970 that workers of the wasp *Tridomyrex humilis*, distributed sugar to 90% of the colony workers in a hungry colony, this proportion decreasing to 30% of colony workers in a well fed colony. While for protein, the opposite was found.

Compared to other social hymenoptera, vespids have one characteristic: Larvae are fed with proteins while adults feed mostly on sugars or sugar derivatives (fruits, honeydew produced by aphids...), occasionally on proteinaceous juices coming from the prey they had chewed for the larvae (Montagner 1964, Spradberry 1973, Edwards 1980, Raveret Richter 2000). Thus workers are mainly hunters which invest most of their foraging time in collecting protein for the larvae, and the rest of the time in collecting other resources for colony maintenance (sucrose food, water, nest materials) (Cane 1983, Matsuura & Yamane 1990, Raveret Richter 2000). When fed, larvae produce saliva drops rich in amino acids, to attract and reward their food provider (Hunt et al. 1982, Maschwitz 1965, Takashi et al. 1991). These secretions are very important for colonial cohesion, as they contain constituents essential for adult protein digestion (Ishay & Ikan 1967, Jany et al. 1978), and queen nutrition (Ishay & Ikan 1968). Males seem to be only receivers of food in the colony (Montagner 1963).

The development cycle of vespid colonies can be summarized as follows, and implies changes in the colony structure, i.e. its composition in term of castes and stages present : (1) **initiation period**: a foundress (or a few, given the species) initiates the building of a nest in which she raises the first cohort of workers ; (2) **growing phase**: the foundress stops foraging, letting the workers do this job

while she stays in the nest laying eggs, reusing cells several times for egg-laying for this purpose; (3) **reproductive phase**: the foundress concentrates her egg laying in the bigger bottom combs and starts producing reproductive (males, gynes); (4) **decline phase** : reproductives emerge, stay a while in the nest monopolizing the workers to be fed, and then mate before or after leaving the nest; in temperate climates, only the gynes survive hibernation, the rest of the colony declines (Matsuura & Yamane 1990).

Vespa velutina var. nigrithorax is a hornet from East China (Arca et al. 2015) accidentally introduced in France in 2004 and currently expanding across Europe (see Monceau et al. 2014 and Monceau & Thiéry 2017 for a review). *Vespa velutina* is, as other hornets, a monogenic species. It builds very prolific colonies, producing 15 000 individuals per year (Rome et al. 2015). The nests of *V. velutina* are made of paper, with one unique small entrance hole in the external envelope, located at the bottom of the nest in the early nest stages, and later on its side. Its shape is first round, but then expands mostly at the bottom, taking on an "egg" shape. Nests of *V. velutina* are mostly located in open areas, amongst trees, bushes, and more rarely in cavities in the ground, or in buildings *etc*… (Monceau *et al.* 2014a). *V. velutina* is a generalist predator of arthropods (dipteran, lepidopteran and other apidae) and a scavenger (Monceau *et al.* 2014a). Its workers predate on honeybees in huge amounts in front of their hives from July to November (Matsuura 1988, Tan *et al.* 2012, Monceau *et al.* 2013b). The invasion of *V. velutina* in Europe is thus a direct threat to the global local biodiversity (Monceau & Thiéry 2017).

Currently no efficient control practice can be applied against *V. velutina*, except its nest destruction, which is time consuming and rather expensive (Monceau *et al.* 2014a). Studying the food distribution among the colony members of *V. velutina* could thus allow further application, for example in helping develop Trojan Horse strategies, which consists of offering foragers food as bait, contaminated with either biological control agents (e.g. entomopathogenic fungi, biological toxins, or parasites (Naug & Camazine 2001)), or insecticidal agents (e.g. growth regulators, chitin synthesis inhibitors...), though such strategies would have to be first checked for the absence of unacceptable side effects to the

environment. Such a strategy was proposed against the invasive *Vespula germanica* in New Zealand, and to date is the only effective one (Beggs et al. 2011).

In the present work, we analysed how workers distributed the food they gathered amongst their colony members, utilising different colonies of variable size and structure, by using two types of non-radioactive food labelling: rubidium (Rb) for proteins and caesium (Cs) for sugars. These two elements are very stable which ensure their reliability over time for marking. Rubidium is classically used for labelling insects, and has the advantage of being metabolized in place of potassium. It was for example used for studying several phytophagous pests (Berry *et al.* 1972), impact natural enemies in biological control programmes (Cohen 1989), movement of insect communities in the field (Long *et al.* 1998, Prasifka *et al.* 2001), and amongst trophic webs constitution (Stimman 1974).

The present study had two objectives: 1- Describe how fed sugars and proteins were distributed inside the colony; and 2- Evaluate if this pattern of food distribution varied according to colony structure. To answer these questions, the distribution of rare elements, Rb and Cs, was analysed 24 hours after a worker returned with liquid labelled food, amongst seven wild *V. velutina* colonies of different size, under controlled conditions.

Material and methods

- *Baseline measurement of the natural Rb and Cs levels inside V. velutina*

To determine a baseline (i.e. average amount of Rb and Cs in individual hornets), we analysed 10 larvae, 5 pupae and 10 adults of *V. velutina* from one colony collected in the wild in Bordeaux (C1), and 10 adults from another C2 collected immediately before the marking experiments.

- *Collection of colonies & maintenance for the marking experiments*

Seven wild colonies of *V. velutina* were collected in the field; the characteristics of the tested colonies and their origins are provided in table 1. Nests were collected early in the morning (between 5 and 8

am), and we captured as many workers defending the nest as possible with an entomological net. Nests were brought to the laboratory, and after a cooling period of 24h at 4°C, the nests were hung in a cage, using metal strings, placed inside a climatic room (23±1°C, LD 12/12). The cage was made of a mahogany frame, metal mesh and Plexiglas sides, equipped with two secured apertures and one secured coppered sliding plate, for food and water supply as described earlier (Monceau *et al.* 2013a, Couto *et al.* 2014, Poidatz *et al.* 2017). *Via* a drawer, hornets had *ad libitum* access to water, honey, fruit syrup and cat food. The nest was kept for at least one week under observation before conducting the tests, always under the same conditions, to be sure that the colony was active and not anarchic. To avoid the degeneration of captive colonies due to overpopulation in a confined space, we artificially removed some of the workers of populous colonies during their adaptation week (nest 2: 35 workers, nest 5: 40 workers). We intentionally collected "small" *V. velutina* colonies, the cage dimensions not being adapted to house massive nests (Monceau *et al.* 2013). In nest 7, one of the two control individuals that were released into the colony was missing when we collected the individuals, possibly due to cannibalism.

As mentioned in the introduction, the production of different castes changes with the season in *V. velutina* colonies, which explains the differences in the colony structure of the different wild colonies observed here. For example, nest 1 was at the beginning of its cycle ("initiation period"), with all the cells being full with larvae or eggs, while nest 7 was at the end of its cycle ("decline period"), with no larvae or eggs left in the cells, and males waiting for their nuptial flight.

- *Preparation of the labelled solutions*

Two feeding solutions were made, one with proteins and the other with sucrose, respectivelly labelled with rubidium (Rb) and caesium (Cs).

- **Protein labelled solution**: 1ml of 521 rubidium solution ([Rb]= 10g/L RbNO3, (Rb from rubidium ICP standard, RbNO3 in HNO32-3%, Certipur®, 1000mg/L Rb, Merck, Darmstadt, Germany)) + 1ml of fly solution. The fly solution was made by crushing flies (Blue bottle fly,

Calliphora vomitoria) for 10 minutes in a ball mills (TissueLyser II, Qiagen) in water, and then filtering the mix. All tests used the same initial protein solution, which was aliquoted in several Eppendorf tubes conserved in the freezer.

- **Sugar labelled solution:** 1ml of 438 caesium solution ([Cs]= 10g/L CsCl, (Cs from caesium ICP standard, CsNO3 in HNO3 0.5mol/L, Certipur®, 1000mg/L Cs, Merck, Darmstadt, Germany)) + 1ml of dissolved sugar in water ([sugar]=30g/L).

- *Experimental design*

After the colony observation period, at 10:00 pm on day 0, we selected 4 workers in the foraging drawer, later called “positive controls”, labelled them on the thorax using a paint pen (DECOpainter, Marabu), and put them in pairs in two aerated plastic boxes (10x10x10cm) without water or food for 4 hours (Figure 1). At 14:00, one 3cm diameter Petri dish containing either of the labelled solutions described before was introduced in each box. The positive controls fed for 2 hours in their respective boxes (Figure 1), then at 16:00 we froze one of each pair (-20°C, positive controls), and released the two remaining workers in the cage containing the colony (Figure 1). We let the positive controls transfer food to the colony for 24h, before we then froze the nest (-20°C). A picture of the nest combs was taken, and each larval coordinates was referenced and localized in the combs. All individuals of all stages (except pupae, which could not have been fed by the control because of their cocoon), were put in labelled Eppendorf tubes. Eggs and first stage larvae, being too slight and small for the analysis, were pooled in groups of 10 to 15 eggs/larvae.

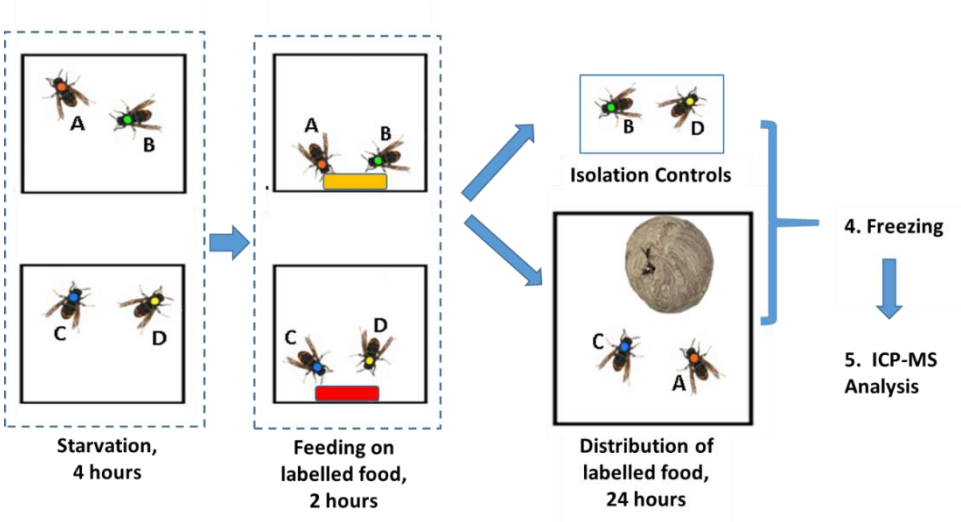


Figure 1: Experimental principle. From left to right: Positive control workers starved for 4 hours, then fed on labelled food for 2 hours, before one of each box is released at its nest while the other two are directly frozen. The whole colony is frozen 24h after. Then ICP-MS analysis took place.

- *Rubidium and caesium analysis*

The samples were weighed using an electronic high precision balance (Radwag AS110/X, e=1 mg, d=0.1 mg, [110-10 mg]), transferred in a silicone capsule, dried in a heat chamber (Memmert) at 103 °C for 12 hours, then cooked in a muffle oven (Thermolyne) at 480 °C for 5 hours. Samples were then solubilized in 2.5 ml citric acid (Baker Analyzed, 65% HNO_3) placed on a heating plate regulated at 120°C. After evaporation, 2.5 ml of miliQwater was added, and after evaporation, 5 ml of citric acid at 32.5% HNO_3 was added. The solution was then rinsed with hot miliQ water. Once the solution was cool, the analysis with an ICP-MS took place (Agilent technologies, 7700 series, plasma power: 1.55 KW, room temperature: 2 °C), equipped with a canister (Micromist, 1.03L/min gaz). For the ICP-MS calibration, we used solutions of Rb and Cs at five concentrations in HNO_3 (5%) being 0,15,30, 60, 120 ppb for the Rb (rubidium ICP standard, $RbNO_3$ in HNO_3 2-3%, Certipur®, 1000 mg/L Rb, Merck, Darmstadt, Germany); and 0,7.5, 15, 30, 60 ppb for the Cs (caesium ICP standard, $CsNO_3$ in HNO_3 0.5 mol/L, Certipur®, 1000 mg/L Cs, Merck, Darmstadt, Germany).

Positive controls that received labelled food from the other positive control were considered as other workers in the analysis.

1 *Table 1: Date of test, origin and structure of the studied colonies. T=control labelled workers*

	Nest 1	*Nest 2*	*Nest 3*	*Nest 4*	*Nest 5*	*Nest 6*	*Nest 7*
Date of collection	28/06/15	21/07/15	12/08/15	25/11/15	15/06/16	21/06/16	12/11/16
Date of test	15/07/15	06/08/15	12/08/15	02/12/15	27/06/16	27/07/16	21/11/16
Origin of the nest	le Haillan	Les Salles de Carrignan	Bordeaux	Artigues	Pessac	le Haillan	St Medard en Jalles
GPS	44°52'28.2"N 0°40'47.7"W	44°48'23.4"N 0°27'52.7"W	44°48'59.9"N 0°34'08.4"W	44°51'46.4"N 0°28'39.5"W	44°47'58.0"N 0°37'35.6"W	44°51'50.1"N 0°40'37.2"W	44°52'45.8"N 0°42'21.8"W
Workers	15+4T	67+4T	41+4T	7+4T	59+4T	46+4T	29+3T
Males	0	0	0	30	0	0	49
Queen	1	0	1	0	1	1	5
Larvae	5	24	82	30	54	17	0
Eggs	1 group	0	7 groups	0	7 groups	6 groups	0

- *Statistical analysis*

All statistics were made using the statistical software R3.2.2. Results are presented as the means ± SD. A Shapiro test was used to assess the normality of the data. For comparing categories, a Student's t-test was used when the data were normally distributed; otherwise, the Kruskal–Wallis test was used. To examine the correlation between different parameters, we used the Pearson correlation test if the arguments were normally distributed, otherwise the Spearman rank correlation test.

The ICP-MS technique allows to accurately assess the concentration of Cs and Rb (µg/kg) (Berg *et al.* 1995, Vogl & Heumann 1997). To correct their level reading, we had to assess the background value for each element: for Rb=16 µg/g, and for Cs=2 µg/g. For nest 5, the background level was much higher at Rb=50 µg/g. An individual was considered as having received food when its Cs or Rb level was above the baseline levels described above. For the presentation of the results, we ranked the nests given their position in the development cycle of *V. velutina*: N1; N6; N2; N5; N3; N4 and N7.

Results

- *Rare element base levels in different V. velutina stages*

The basal levels of Rb and Cs in the different *V. velutina* stages are presented in table 2. The larvae tested here were final stage larvae that weighed more than 200 mg.

Table 2: Quantities of rubidium (RB) and caesium (CS) in hornets as mean +SD [min – max] per kg of fresh body weight.

	CS (µG/KG)	RB (µG/KG)
LARVAE (N=10)	41.9 ± 19.6 [20 – 90]	7455 ± 1224.8 [5590 – 9130]
NYMPHAE (N=5)	50 ± 7.1 [40 – 60]	8220 ± 713.1 [7240 – 9000]
ADULTS (N=10)	780.1 ± 586.1 [357-2000]	8254.4 ± 3626.5 [3690 – 15600]

- *Food distribution inside V. velutina colonies*

➢ *Evolution of the number of labelled individuals with colony structure*

We first looked at the number of workers and larvae receiving protein solution or sugar solution relative to the total number of workers, larvae or all individuals in the colonies. We found no correlations between the number of workers that received proteins (labelled by Rb) or sugar (labelled by Cs) and the number of larvae, workers or total individuals in the colonies (spearman tests, $p>0.05$). On the other hand, the number of fed larvae with labelled sugar was correlated with the total number of larvae in the colonies (Spearman correlation test, $p=0.035$), and the number of larvae fed with labelled proteins was correlated with the total number of individuals in the colony (all castes without eggs, Spearman correlation test, $p=0.037$).

There was no differences in the weight of workers receiving labelled food (t.test, for sugar: $p=0.09$, for proteins: $p=0.35$, $N=260$); the same for sugar in larvae (t.test, $p=0.85$, $N=223$). But labelled proteins were distributed to the lightest larvae in the colony (t.test, $p=0.003$, $N=223$).

The evolution of colony structure and marked individuals by the labelled food is represented in Figure 2.

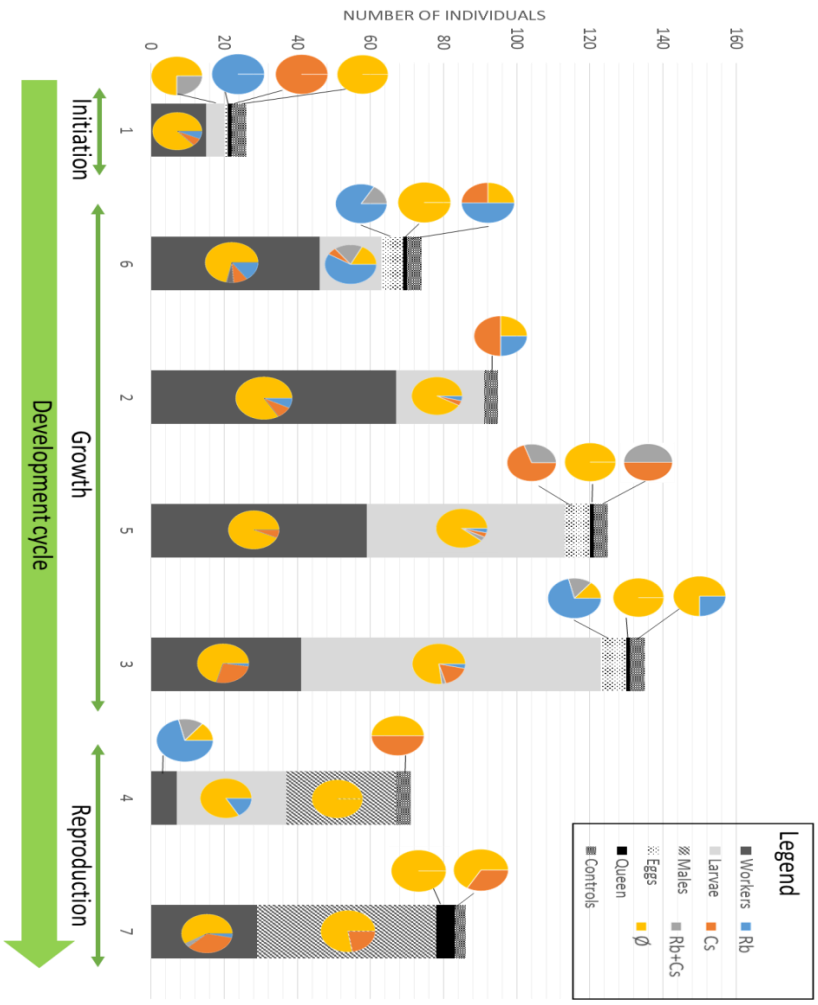


Figure 2: Number of individuals marked by received labelled food as a function of colony structure (Rb: protein solution labelled with rubidium, Cs: sugar solution labelled with caesium).

Each positive control worker, released in its nest, distributed food to a very variable number of individuals (considering all confounded castes, but without eggs), with a mean ± standard de*via*tion [range] of 7.85 ± 6.71 [2-21] individuals for labelled proteins, and 11 ± 9.73 [2-27] individuals for labelled sugar.

- *Number of Rb//Cs labelled individuals amongst each category of labelled individuals*

Larvae were more often receiving proteins (Rb label,) than sugar (Cs label, stats), when **workers** received more sugar (stats) than proteins (stats) (Figure 3). Considering queens and males, there was

only one case for each category where labelled sugar solution was received by one and 16 individuals respectively.

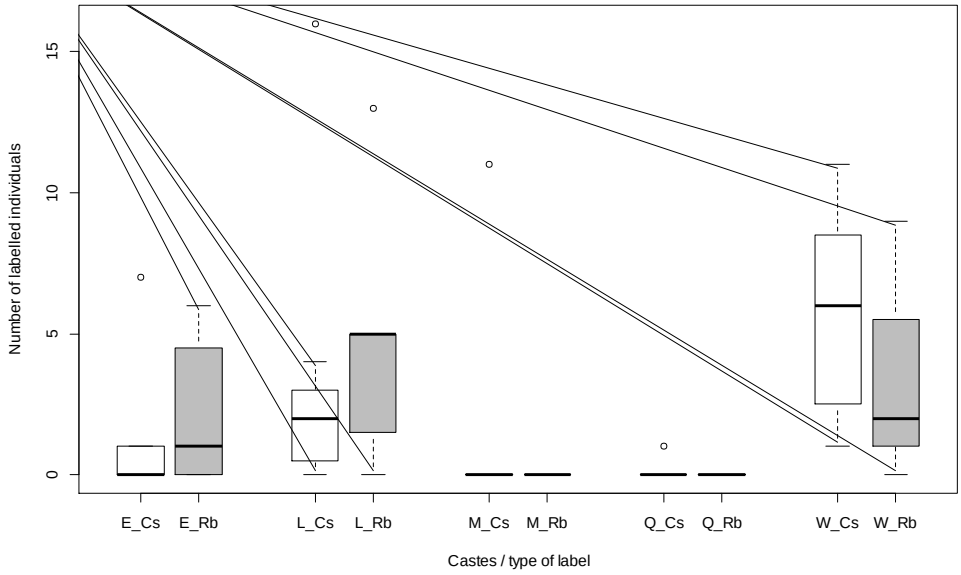


Figure 3: Boxplot of the number of individuals within each caste labelled with caesium (Cs, sugar, White) and rubidium (Rb, proteins, gray). E = Eggs, L = Larvae, M = Males, Q = Queens, W = Workers.

➢ *Quantitative analysis of the amount of labelled food received by individuals*

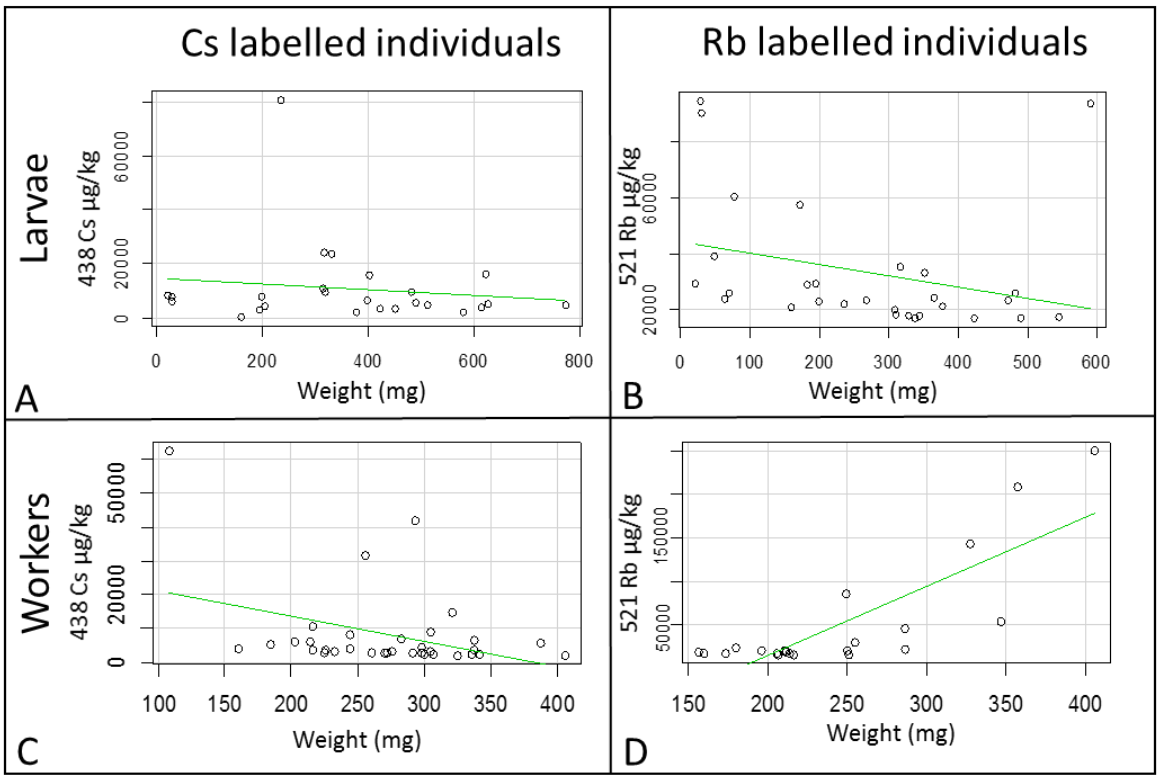


Figure 4 : Quantity of caesium (Cs) received by the larvae (A) and the workers (C) as a function of their weight (mg). Evolution of the quantity of rubidium (Rb) received by the Larvae (B) and the workers (D) as a function of their weight.

Considering all larvae that received labelled Cs sugar solution, no correlations could be found between larval weight and their amount of received sugar (Pearson test, p=0.92, N=25). On the other hand, considering all larvae that received labelled Rb protein solution, the amount of received protein decreased as larval weight increased (Spearman test, p=0.007, rho=0.48, N=29). Considering all the workers that received Cs labelled sugar, the amount of received sugar decreased as worker weight increased (Spearman test, p=0.001, rho=0.64, N=34**).** Considering all the workers that received Rb labelled proteins, the amount of received proteins increased as worker weight (Spearman test, p=0.029, rho=-0.37, N=22) (Figure 4).

Discussion

This study describes how workers distribute the food resources they gathered amongst their nestmates (larvae and adults) with special focus to proteins and sugar.

The Rb average background level observed in our *V. velutina* colonies was quite similar as the one observed in the study of Weeks *et al.* 2004 on ants, except for colony 5, which had a higher level.

The number of labelled individuals varied with the *V. velutina* colony structure, as the number of individuals fed with labelled sugar increased with the total number of larvae in the colonies, and the number of larvae fed with labelled proteins increased with the total number of individuals in the colony. Compared with ants, the number of feeded individuals by one worker seems to be very small both for sugar and proteins (to average 10 other workers in total in hornets, compared with average 100 and 32 workers for sugar and proteins respectively in ants) (Markin 1970). This may be due to the level of eusociality observed in the studied ant species (*Iridomyrmex humilis*) compared with our hornet.

Eggs and Larvae received proteins (Rb) more often than sugar (Cs), while workers received sugar more often than proteins, as is generally as expected (Montagner 1963).

In ants, some workers could redistribute part of the food they receive (Markin 1970), and we cannot thus distinguish labelled individuals that were directly fed by the control forager from the one fed by intermediaries.

- *Larvae and eggs*

The lightest larvae received labelled food more frequently than the heaviest, which could suggest that workers distribute food in priority to the youngest larvae, weight being connected with age in larvae (Dubuysson 1903, 1904). In our study, we also observed that the quantity of Rb labelled proteins received by the larvae decreased when their size increased. On the other hand, Montagner & Courtois (1963) showed in another vespid, *Paravespula germanica*, that the larvae were fed with

proteins in proportion of their size, and in their study on *Mischocyttarus drewseni*, da Silva *et al.* 2012 described that larger larvae received both more nectar and proteins. Such distinction in the results may be due to the studied model species, the experimental design, and studied timeframe. Indeed, these authors compared total cumulated food received by queen and worker larvae, while we focused on the precise distribution choices of single individuals during a day. Moreover, unlike in wasps, individual weight is probably not a caste criterion in *V. velutina* (Perrard *et al.* 2012).

Some labelled material was found in groups of eggs, and this raises an interesting question. This may be interpreted as due to small amounts of protein deposited as preparatory provisioning for neonate larvae, maybe by licking as in ants (Markin 1970) : this author hypothesized that the licking could be a way to transfer food to the egg. Though we cannot exclude that the high values determined here were due to an overestimate of marker concentration due to the very small weight of eggs (<100mg for the group). The results concerning this stage have thus to be interpreted with caution.

- *Workers*

In our study we cannot clearly determine the origin of the Rb labelled material found in adults. It could be the result of direct distribution of labelled proteins by the control forager, but could also be due to rewards received from labelled larvae who previously received such proteins from the control forager (Montagner 1963).

The proteins were preferentially distributed to heavier workers, and the quantity of proteins received increased with worker weight, while the quantity of Cs labelled sugar solution received decreased with their weight. In most vespids, the workers size enhances with the colony development, the older workers being thus the smallest. In *Polistes occidentalis*, (O'Donnell & Jeanne 1995) demonstrated that smaller workers, thus being the older, were more likely to be dominant in social interactions between workers (grooming and crop requesting). This was also the case in other *Polistes sp*. (Strassmann & Meyer 1983, Hughes & Strassmann 1988). We can thus hypothesize that concerning workers, the sugar is more "classically" requested between workers during dominance interactions,

while we may expect that Rb labelled proteins could come preferentialy from indirect sources as larvae that previously received labelled proteins.

- *Males and queens*

Considering queens and males, there was only one case for each category where labelled sugar solution was received by one and 16 individuals, respectively. This is congruent with the literature, knowing that in theory adults only need sugar for their own survival, while they are presumed to consume proteins only occasionally *via* prey remains intended for larvae (Montagner & Courtois 1963). In Iridomyrmex humilis, queens have been shown to be preferentially fed with sugar compared with workers (Markin 1970), but in our study it seemed that the new gynes did not received any food in the colony observed (colony 7). The differences can be due both to the methodology in the experiment, that let all the workers in the ant colony access to labelled food instead of one worker, and moreover this author had no males in the observed ant colonies.

In the two colonies in which they were present, males behaved completely different. None were labelled in nest 4 suggesting they had received no food from control foragers, while 16 of them received labelled sugar solution in nest 7. As larvae were absent in nest 7, we could thus hypothesize that the **males are neglected when larvae are in the colony** when sugar is distributed.

The background level of Rb in nest 5 was found to be much higher than in all other nests, which suggests that this nest could have been contaminated by the food present in its surroundings. Biomagnification can occur and for example, rubidium can be found in high and unexpected quantities in seafood like oysters (Campbell *et al*. 2011). This nest was found in a town (Pessac) in the vicinity of different market places that sell such seafood, hence such biomagnification could be hypothesized.

For technical reasons, we artificially regulated colonies' population sizes, and in autumn we worked on smaller colonies than the ones found in the wild (typically housing several thousand individuals

(Monceau *et al.* 2014a)). Even if the colony size could have an impact on the way the food was dispatched amongst colony members by workers, as demonstrated by Markin 1970 for ants, we can suppose that food distribution at the scale observed here, i.e. by individual workers, would not differ much between such colonies. Indeed, in our experiment, the control workers had limited access to labelled food, and had limited distribution capacities.

Even if ensured that the control workers with access to labelled food exhibited similar hunger levels and were already foragers, we cannot assess the quantity of labelled food that each one collected and distributed from its crop. Such quantities could depend for example on their size. In bees, such collection capacity is estimated between 50 and 70µL (Kacelnik *et al.* 1986) for average 50mg, we could estimate that hornets of average 400mg might have a crop load capacity of 400µL. To answer this question, multiple artificial feeders containing small amounts of nectar could be proposed, as done in Schmid-Hempel *et al.* 1985.

Invasive argentine ants are able to share food between colonies because their "supercolonies" are very close genetically. The method developed here could be a way to explore this behaviour in the invasive hornet *V. velutina*; such effort justified since this hornet is believed to stem from a single invasion event in Europe (Arca *et al.* 2015).

Conclusions

In this study we assessed for the first time the impact of colony structure on food distribution in a vespid species, the invasive Yellow-legged hornet *V. velutina*. We isolated global patterns of sugar and protein provisioning according to colony size and structure, and found links between size and caste of receiving individuals and the type and amount of food received.

Author Contributions

JP and DT conceived the experiments and the experimental design; JP did the experiment and analysed the data; JP and DT wrote the manuscript.

Acknowledgements

We thank Thiéry Dalix from the UMR USRAVE INRA for running the ICP-MS analysis. We thank the beekeeper union "Association Anti Frelon Asiatique" for collecting the nest. We thank Bayer Crop Science, represented by B. Laborie, for the first author's financial support. The authors declare no conflict of interest. The first author's funding sponsor had no role in any steps of the study (design, data collection, analyses, writing). We thank Dr Peter Kennedy for assistance with English revisions to the manuscript.

B.2 Rayon d'action de *Vespa velutina* autour de son nid

Connaitre le rayon d'action des individus qui collectent la nourriture est fondamental pour comprendre la zone écologique d'intervention d'une colonie d'insectes sociaux. La mise en place de stratégies de lutte efficaces dépend aussi de ces données comportementales. Le rayon d'action des ouvrières dépend de plusieurs facteurs, entre autres la disponibilité en ressources (nutritives ou matériaux d'entretien de la colonie) et la structure du paysage.

B.2.1 Rayon d'action et capacités de retour au nid

Les insectes sociaux sont des « Central Place Foragers », c'est-à-dire qu'ils évoluent autour de leur colonie, considérée comme élément central, pour collecter des ressources pouvant servir à sa nutrition, mais également à son entretien (eau, matériaux de construction) (Spradbery 1973, Bell 1990). Ils optimisent leurs trajets en effectuant des compromis entre la distance parcourue, la quantité et la qualité des ressources, tout cela en fonction des besoins de la colonie (Bell 1990).

Les insectes sociaux à nourrisseuses aériennes sont capables de naviguer entre leur nid et leurs sites de collecte grâce à différents mécanismes d'orientation comprenant par exemple un compas solaire, du magnétisme (démontré chez les abeilles, Gould 1990), des patterns visuels et des odeurs (pour plus de détails, voir **le manuscrit ci-dessous**). Au-delà de ces vols de routine, il a été montré que ces insectes étaient capables de retrouver leur colonie même après en avoir été déplacés artificiellement à plusieurs km (Fabre 1882). La distance maximale à laquelle des individus d'une espèce sont capables de revenir à leur colonie, le « homing », est ainsi un bon indicateur du rayon d'action maximum pouvant être parcouru pour collecter des ressources par une espèce donnée, et est généralement liée à la taille de l'insecte (démontré par exemple chez les abeilles par Greenleaf *et al.* 2007).

B.2.2 Techniques d'enregistrement et d'analyse des déplacements d'insectes

Etudier le comportement d'animaux et d'insectes dans leur environnement suppose soit **leur marquage par différentes techniques** (Osborne *et al.* 2002, Hagler *et al.* 2016), **soit l'enregistrement de leurs déplacements** (Bell 1990, Reynolds & Riley 2002). Dans cette partie nous n'entrerons pas plus dans les détails de m**arquages passifs** classiques tels que : les marquages physiques (morceau d'aile, de patte, encoche), marquages métaboliques (métaux lourds, radioactivité, marquages protéiques comme l'albumine), marquages colorés (peinture, poudres colorées) (Hagler *et al.* 2015). *(Pour plus d'informations sur ces techniques, voir l'introduction du manuscrit 3).*

Nous nous intéresserons ici uniquement aux techniques qui nous paraissent utiles à développer pour suivre les déplacements de *V. velutina* adultes. Celles-ci reposent principalement sur l'usage de radars harmoniques (B2.2.1), de caméras 3D (B2.2.2), d'étiquettes magnétiques (RFID) (B.2.2.3) ou d'émetteurs actifs miniaturisés (B.2.2.4).

B.2.2.1 Le radar harmonique

La technique du radar harmonique consiste en la détection par une antenne (Figure 20) d'un réflecteur positionné sur un insecte. Ce type de marquage reconstruit la trajectoire d'un insecte volant dans son ensemble. C'est un outil de premier ordre pour la recherche fonctionnelle et théorique, en milieu semi-contrôlé : il permet, dans des conditions optimales (terrain ouvert, plat), de détecter de manière très précise un insecte dans l'espace en temps réel. Les radars harmoniques ont beaucoup été utilisés pour l'étude de pollinisateurs (Riley *et al.* 1996, Osborne *et al.* 1999, Capaldi *et al.* 2000, Reynolds 2007, Wolf *et al.* 2014)

Figure 20 : Radar harmonique (à gauche) permettant de suivre automatiquement la trajectoire d'insectes portant un réflecteur, ici un bourdon (à droite). Source photo : http://www.rothamsted.ac.uk/news-views/radar-tracking-reveals-%E2%80%98life-stories%E2%80%99-bumble-bees

Cependant, plusieurs inconvénients à cette technique existent: une taille du radar souvent imposante (transport par véhicule obligatoire), un rayon de détection assez faible (<900m), un coût très élevé, une grande complexité de fonctionnement, des signaux non individualisables (impliquant la perte de chaque trajet dès que deux individus se croisent), et enfin la nécessité d'effectuer les détections dans des sites d'essais plats et vides (sans échos verticaux (arbres, bâtiments), creux, bosses *etc.*). Une telle technique est actuellement développée sur *V. velutina* en Italie du nord pour tenter de trouver les nids de frelons (Milanesio *et al.* 2016, 2017). Les principaux freins de ce prototype restent actuellement sa faible mobilité (système embarqué sur véhicule), un rayon de détection très faible (150m maximum, à cause de l'angle de vision du radar plus large (angle de 30°C), entrainant une perte de puissance du signal, mais permettant de détecter des signaux en milieux vallonnés), et toujours l'effet écran des échos verticaux (Porporato, résultats présentés au UE Coloss meeting 2016, Mallorca).

B.2.2.2 Les caméras 3D

Associées avec des logiciels complexes de reconstruction des trajectoires, elles permettent, sur une distance assez faible (1-2m max), d'obtenir des informations ultra-précises sur la vitesse, la trajectométrie des insectes, et de décrire des comportements bien spécifiques. Par exemple, en 2016, Stürlz *et al.* ont décrit avec une grande précision la manière dont les guêpes acquièrent des

informations visuelles durant leur vol d'apprentissage, et l'usage qu'elles en font afin de retrouver leur nid (Figure 21).

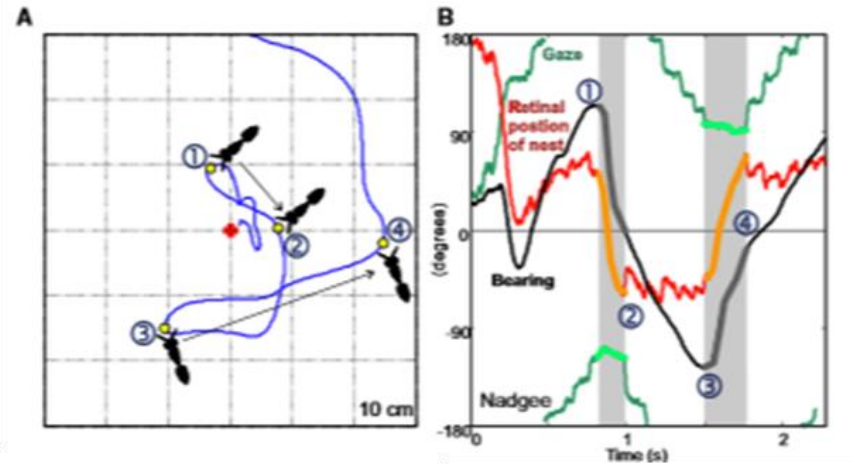


Figure 21 : exemple de résultats d'analyse de trajectoires avec des caméras 3D, Stürzl *et al.*, How Wasps Acquire and Use Views for Homing, Current Biology (2016).

B.2.2.3 Emetteurs actifs

Cette technique a été largement utilisée pour l'étude de mammifères et d'oiseaux. Elle repose sur l'usage d'une balise émettrice embarquée par l'animal. La miniaturisation de l'électronique embarquée et des batteries a permis son usage chez les gros insectes, principalement marcheurs (Kennedy & Young 1993), et avec la miniaturisation des batteries, certains coléoptères volants et libellules ont pu être suivies sur plusieurs km (Chapman *et al.* 2004). Nous avons commencé à l'été 2017 une collaboration avec une équipe de chercheurs Anglais, spécialisée dans ce genre de travaux (Peter Kennedy et Juliette Osborne, University of Exteter, projet : Evaluation of technologies for their potential to track Asian hornets (*Vespa velutina*)). Ce projet étant dans la phase de mise au point, nous n'avons pas encore de résultats à présenter dans la thèse. Cependant, des éléments supplémentaires sur ce projet sont présentés dans la discussion de cette thèse.

B.2.2.4 La technique du RFID (Radio-Frequency Identification)

La technique du RFID est un système de marquage passif assez classiquement utilisée en entomologie, et particulièrement pour l'étude d'insectes sociaux, grâce entre autres (1) au suivi individualisé et synchronisé de plusieurs insectes qu'elle permet (un tag = un code = un individu), (2) aux biais très réduits liés à la manipulation des individus ou au poids du système, même chez des insectes volants (Boiteau *et al.* 2009). Un tag est positionné de manière équilibrée et non gênante sur un insecte (en général sur le thorax ou l'abdomen), le code du tag pourra alors être lu lors du passage de l'insecte tagué près d'antennes réceptrices, pouvant être classiquement des portails (Henry *et al.* 2012, Ohashi *et al.* 2010 par exemple), ou des détecteurs mobiles portatifs (permettant par exemple de suivre les déplacements d'insectes de surface ou souterrains (Silcox *et al.* 2011)). Certains modèles de tags ultra miniaturisés font moins d'un demi-millimètre de côté, et peuvent être utilisés pour l'étude de drosophiles ou de fourmis (Moreau *et al.* 2011). Ainsi, les progrès dans **la taille des tags**, dans **le périmètre de détection** des tags par les détecteurs ainsi que dans leur **précision de lecture** ont popularisé l'usage de cet outil pour les expérimentations sur insectes (Figure 22). Dans la recherche sur les pollinisateurs, l'usage de cette technique a permis (1) d'étudier le comportement des insectes : comportement de collecte (Stelzer *et al.* 2010, Ohashi *et al.* 2010, Stelzer & Chitka 2010 (*Bombus sp.*), Rodet & Henry 2014, Tenczar *et al.* 2014 (*Apis melifera*)), la capacité de retour à la colonie (He *et al.* 2012, Pahl *et al.* 2011), la dérive d'une colonie à l'autre (Sumner *et al.* 2007), les vols nuptiaux (Heidinger *et al.* 2014) *etc.* mais également (2) d'évaluer l'impact de traitements phytosanitaires sur les colonies d'abeilles (Decourtye *et al.* 2011, Feltham *et al.* 2014, Gill & Raine 2014, Henry *et al.* 2012, Schneider *et al.* 2012, Henry *et al.* 2015).

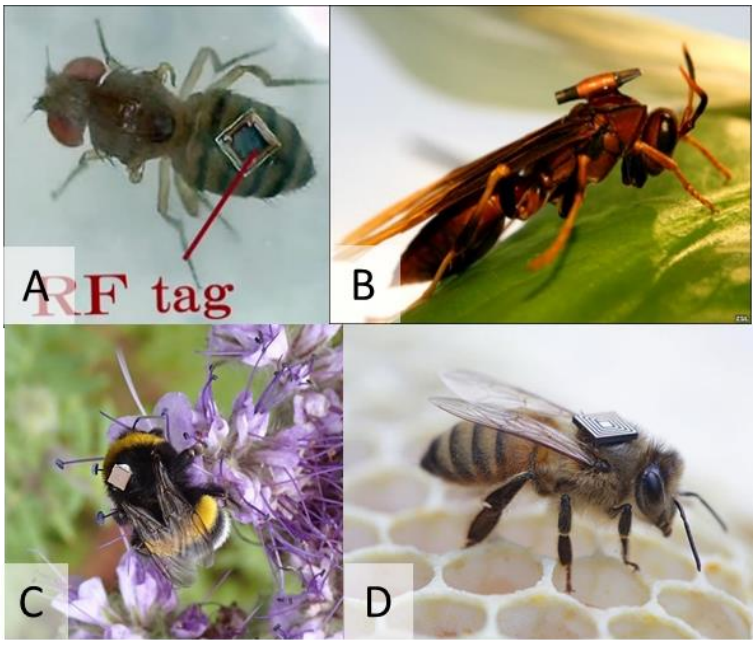


Figure 22 : Exemples d'usages de tags RFID pour le suivi d'insectes. A : sur drosophile (tag de 0.46 mm x 0.48 mm) (Photo SK-Electronics®' Fine Tag) ; B : sur guêpe ([Zoological Society of London] Photo : Queen Mary, University of London); C : sur bourdon (Photo Sigrun Bocksch ©Eurofins Agroscience Services Ecotox GmbH) ; D : sur abeille (Photo CSIRO).

L'ensemble de ces avantages nous ont conduit à choisir cette technique de marquage pour étudier le rayon d'action et l'activité des ouvrières de frelon asiatique, en adaptant le protocole utilisé sur les abeilles par Pahl *et al.* 2011.

B.2.3 Application de la technique de RFID au frelon asiatique à pattes jaunes *V. velutina*

Cette partie est soumise pour publication à Journal of Pest Science (sept 2017) « Homing behavior and activity of Vespa velutina workers ». (cf Manuscrit 4). Une description résumée axée sur la mise en place technique de l'expérimentation est néanmoins produite ici.

Pour que la technique RFID fonctionne, il faut que les insectes marqués traversent un portail de détection. Chez les frelons, cela revient à positionner le détecteur soit sur les sites de chasse soit au

nid. Pour pallier à l'accès difficile aux nids et au très important comportement de défense du nid par les frelons *in natura*, nous avons encagé un nid, limité les entrées et les sorties à un tunnel et placé les détecteurs RFID à la sortie de ce tunnel. Les ouvrières du frelon asiatique *V. velutina* sont 5 à 8 fois plus grosses qu'une abeille (~50mg *vs* 250 -400mg), les tags sont donc d'autant mieux adaptés à l'étude de ces insectes. L'objectif de l'étude présentée dans l'article ci-après est de collecter à la fois (1) des données de **capacité de retour à la colonie**, qui permettront d'avoir une idée du **rayon d'action maximum du frelon asiatique autour de son nid** ; mais également (2) des **données de biologie sur le fonctionnement du nid**, grâce au rythme d'activité des différents individus tagués qui s'y trouvent. Nous comparerons cette activité à différents critères à la fois intrinsèques aux frelons tagués (morphologiques), temporels (heure de la journée), mais également à des paramètres climatiques connus pour jouer un rôle dans la navigation des insectes (température, rayonnement, vent, pluie *etc*…).

- *Installation du nid encagé*

Un nid de *V. velutina* de 15cm de diamètre a été collecté à St Médard-en-Jalles (Aquitaine, France) le 28/04/2016. Après l'avoir endormi en le plaçant 24h à 4°C, le nid a été suspendu avec du fil de fer au toit d'une cage sécurisée placée à l'intérieur d'une cabine en fer grillagée de 2m x 1.5m x 2m dans une zone balisée du parc de l'INRA de Bordeaux, sans accès extérieur. Des matériaux de construction pour le nid (carton, bois, écorces) ont été placés dans le fond de la cage, et de la nourriture (miel, pâtée pour chat, sirop) et de l'eau, à volonté dans le plateau d'alimentation. Une semaine plus tard, après une surveillance régulière de l'activité de la colonie, de la reconstruction de l'enveloppe du nid, et du nombre de morts, nous avons estimé qu'une délocalisation du nid n'était plus à craindre, et le tunnel d'accès (10cm de diamètre) reliant la cage à l'extérieur a alors été installé. Le plateau d'alimentation a été fermé, pour inciter les collectrices à sortir de la cage. Les ouvrières ne parvenant pas à trouver la sortie à cause de la transparence de la cage pour l'observation des nids, nous l'avons donc recouverte de carton. Dans ces conditions, le tunnel a été très rapidement adopté et exploré par de nombreuses ouvrières (Figure 23). Deux portails de détection des puces RFID ont alors

été installés (MAJA® reader module 4.2, Mycrosensys) l'un derrière l'autre à l'entrée du tunnel, afin d'obtenir une lecture séquentielle des passages de puces et donc de pouvoir discriminer les entrées des sorties par leur ordre d'activation lors du passage d'un frelon marqué.

Figure 23: (A) Installation en semi-field vu de l'intérieur de la cabine grillagée dans laquelle est installée la cage sécurisée recouverte de cartons contenant le nid (B). (Photos J. Poidatz)

- *Marquage RFID des ouvrières de V. velutina*

Les ouvrières de *V. velutina* ont été capturées au filet entomologique devant l'entrée du nid, puis placées chacune dans un tube Falcon (50ml) avant d'être endormies par hypothermie en plaçant le tube dans de la glace durant 20 min. Les insectes ont alors été pesés (balance radwag, e=0.001g), et leur largeur de tête mesurée avec un pied à coulisse électronique (1-150mm, e=0.01mm). Puis ils ont été placés dans une boite à poussoir grillagée (Figure 24 A, B), permettant de leur appliquer sur le thorax une goutte de ciment dentaire (sans odeur, non toxique, séchage très rapide, Henry *et al.* 2012), où le microtag (MAJA, Mycrosensys) a ensuite été fixé. Les frelons ont alors été placés dans des cages à température ambiante, avec de l'eau et du miel, pour surveiller leur réanimation.

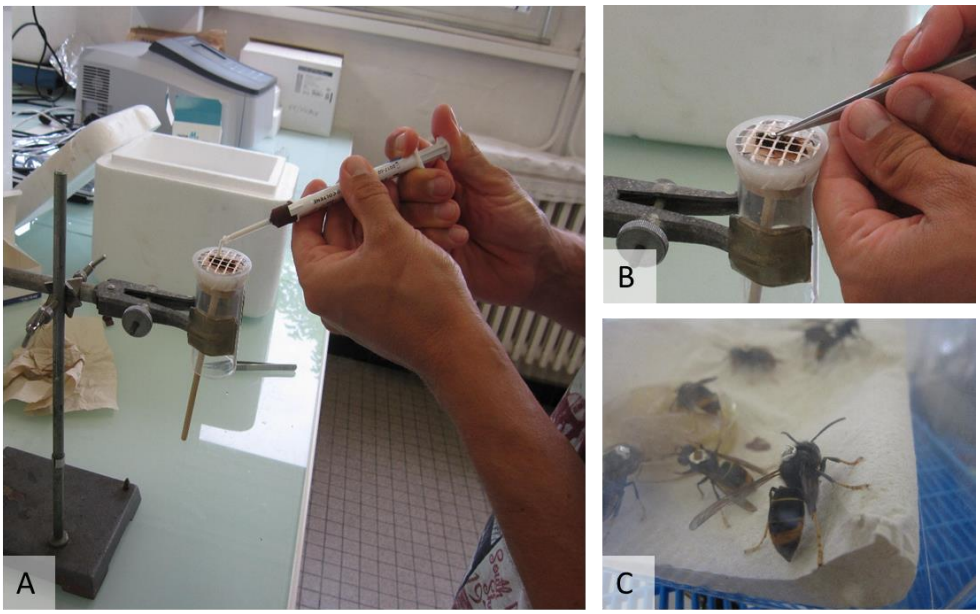


Figure 24 : Marquage de frelons asiatiques avec des puces RFID. A. application d'une goutte de ciment sur le thorax d'un frelon endormi placé dans une boite à poussoir. B. Placement à la pince fine du microtag sur la goutte de ciment encore fraiche. C. les frelons tagués sont placés dans une cage avec eau et nourriture à température ambiante pour se remettre de l'opération. (Photos J. Poidatz).

Manuscrit 4: Homing behaviour in an invasive honeybee predator

Juliette Poidatz, Karine Monceau, Olivier Bonnard, Denis Thiéry

*Soumis à **Journal of Pest Science** (sept 2017), under review.*

Abstract

The homing ability is an intrinsic parameter of a species, corresponding to the maximum distance it can travel to return to its nest. It is a good proxy of a species maximum foraging distance. In the context of invasive species monitoring, such parameter is of first interest. *Vespa velutina* is an invasive predator of honeybee extending through Europe. The foraging range of workers around the nest is still unknown and their foraging activity is poorly known. Using RFID technic, the homing ability of *V. velutina* workers was assessed, by releasing 359 individuals at different distance from their colony. Then, daily activity was also monitored.

The homing ability of *V. velutina* was evaluated up to 5000 m and was not affected by the cardinal orientation of release point. The lag time to return to the nest increased with the distance of release. Most of the flight activity took place between 07:00am and 08:00pm, hornets doing principally short flights of less than an hour. Foraging range was thus estimated ca. 1000m around the nest. This study of *V. velutina* assisted by RFID tags provides for the first time a baseline for its potential foraging distance that increase our knowledge of this species to i) refine more accurately models for risk assessment and ii) define security parameter for early detection of predation on invasion front.

Key words: Asian yellow-legged hornet, central place foraging, early detection, RFID, invasive species, radio tracking, *Vespidae*

Key message

- The homing ability and foraging range of the invasive bee predator *V. velutina* are unknown.
- As such information are key both for impact prediction and for management, *V. velutina* homing abilities were assessed using RFID technic.
- *V. velutina* maximum homing distance was assessed at 5000m from its nest, and its classic foraging range between 500 and 1000m.
- Homing ability in *V. velutina* was not affected by the cardinal orientation of release, but the distance of release increased its lag time.

Introduction

Central place foraging is largely represented in animals in both vertebrates and invertebrates (Bell 1990). It implies that individuals are able to return to their nest after foraging for resources, to store and share it with the members of the group family (Orians & Pearson 1979, Houston & McNamara 1985, Bell 1990). Nesting site choice results from the trade-off among the habitability of the location, its safety from predators and the distance to resources (Pyke *et al.* 1977, Osborne *et al.* 1999, Williams & Kremen 2007, Osborne *et al.* 2008). To limit foraging costs, individuals optimize different parameters linked to foraging such as the distance they travel (Pyke 1984, Bell 1990). For example, bumblebees are able to adjust their traplines linking different flowers in few foraging boots to reduce the duration of the nectar collection (Lihoreau *et al.* 2012). One limiting key parameter is however the maximal distance an individual is able to travel going back home, called homing ability (Van Nieuwstadt & Iraheta 1996). Homing ability is an intrinsic parameter of a species, while its actual foraging range depends on the resource distributions, abundance and quality (Bacon *et*

al. 1965, Ricketts 2001), the individual capacities (Greenleaf *et al.* 2007), the landscape context (Southwick & Buchmann 1995; Steffan-Dewenter & Kuhn 2003) and climatic parameters. Additionally, homing ability is also closely related to orientation. Studies on homing abilities of diverse organisms (insects and birds for instance) allowed the discovery of compass systems, and include the use of the sun, the stars or geomagnetic fields (Gould 1986, Wehner & Menzel 1990, Goulson & Stout 2001, Collett & Collett 2002).

Homing ability and orientation have been extensively studied in social insects, mostly in pollinators such as honeybees (Abrol & Kapil 1994, Van Nieuwstadt & Iraheta 1996, Pahl *et al.* 2011, He *et al.* 2012) and bumblebees (Goulson & Stout 2001). For example, *Bombus terrestris* workers basically forage in a 1000 m range, but may travel up to 4300 m from their colony to collect valuable resources (Osborne *et al.* 1999, Goulson & Stout 2001, Wolf & Moritz 2008, Goulson & Osborne 2009). They are however able to find their way home up to ca. 10 000 m, what is twice their maximal foraging range (Goulson & Stout 2001). Foraging behaviour being critical to ensure colony survival, understanding how central place foragers control and exploit their environment is a key topic to better understand their ecology that is of special importance for pollinators considering their actual decline (Goulson *et al.* 2015). Interestingly, homing behaviour and orientation have been slightly investigated in Vespid species probably because their impact on ecosystems is less important than the pollination service (see Schöne *et al.* 1993a, b on diggerwasp, Stürzl *et al.* 2016 on groundwaps, Ugolini 1985, 1986, 1987 on *Polistes* sp. and *Vespa orientalis*). Nevertheless, in the last centuries, several species of *Vespidae* invaded a wide range worldwide with different levels of impact on their new ecosystems (Beggs *et al.* 2011).

The Asian yellow-legged hornet, *Vespa velutina var nigrithorax* (Lepelletier 1835) was accidentally introduced into Europe around 2004 from eastern China (Arca *et al.* 2015, see Monceau, Bonnard and Thiéry 2014a for a review). Since its introduction, it has spread

through Europe in Spain (López *et al.* 2011), Italy (Demichelis *et al.* 2013), Portugal (Grosso-Silva & Maia 2012), Germany (Witt 2015), and more recently in UK, Belgium (2016) and Switzerland (2017). *V. velutina* is a generalist predator of arthropods mostly known for its damages on honeybee hives (Abrol 1994, Tan *et al.* 2007, Shah & Shah 1991). This predation pressure can directly and indirectly, by reducing the beehive overwintering abilities, enhance the colony loss risks (Monceau *et al.* 2014a). Thus, *V. velutina* predation is an additional pressure that contributes to bee decline. Furthermore, the action range is of first importance for the monitoring and the potential management of invasive species (Holway & Suarez 1999). This information could help finding colonies, and give a scale for potential control methods application. Moreover, to date only models concerning the nest distribution were done (Villemant *et al.* 2011, Bessa *et al.* 2016, Robinet *et al.* 2016, Franklin *et al.* 2017, Monceau & Thiéry 2017) while risk assessment on apiaries is still missing. Such a model would require estimating the foraging range of *V. velutina* but this later is still unknown.

In order to accurately record the rhythm of entries and exits from the nest of several *V. velutina* workers at the same time over a long period of time, a *V. velutina* colony, maintained in semi-field conditions was equipped with Radio-Frequency Identification (RFID) device. Multiple release of tagged hornet allowed (i) evaluating the homing ability of *Vespa velutina*, and the part of cardinal orientation of the release points and body condition in this behaviour, (ii) describing the activity of the hornets at the individual level within the colony. Two main experiments were thus realized in parallel: (1) the release of individually tagged workers at increasing distance from the nest to measure their homing ability and (2) the daily individual activity for the workers release at the vicinity of the nest.

Material and methods

Biological model and annual life cycle

The life cycle of *Vespa velutina* is annual. During spring, a single gyne (foundress) initiates a nest and lays her eggs. Once the first workers emerge, they quickly replace the queen for all activities except egg laying. The colony grows through the months and the need for proteins to feed the larvae increases too, resulting in an increase of the predation on honeybee hives during summer and fall (Monceau *et al.* 2013c). In mid-September-early October, males and gynes emerge, leave the nest and mate. Only gynes (mostly mated, Poidatz *et al.* unpublished data) hibernate during the winter, while the rest of the colony (males, workers and the old queen) dies (Monceau *et al.* 2014a). The nests of *V. velutina* can be found from underground to the top of the trees. They are paper made, often water hose shaped or spherical, with one unique small entrance.

Nest installation

A 15cm large diameter *V. velutina* wild colony was collected in St Médard-en-Jalles (Aquitaine, France, GPS coordinates: 44°53'35.8"N 0°44'51.4"W) on the 28th April 2016. After a 24hours cooling period at 4°C, the nest was carefully fixed with iron strings inside a cage (Appendix S1), made of mahogany and stainless steel grid and Plexiglas (see Monceau *et al.* 2013a, Couto *et al.* 2014). The cage was then transported inside a 2m x 1.5m x 2m stainless steel grid cabin with a corrugated plastic roof in the INRA de Bordeaux site (La Grande Ferrade, Aquitaine, France, 44°47'30.4"N 0°34'36.9"W). The nest was first installed with no possible outlet from the cage, with food, water and nest construction material (wood, leaves, bark) provided *ad libitum*, to prevent the colony from relocation. After a one-week acclimatization period, a tunnel was installed to connect the cage to the outside (Appendix S1). The inner cage was covered with opaque cardboard sheets, to provide a single light source from the tunnel outlet, and help hornets to find the exit. At the same time, food

previously provided inside the cage was removed. The colony could then grow freely for a week before the installation of the RFID system.

RFID-system

As compared to other techniques such as radio tracking, the RFID technic has several main advantages: it is cheap, allows tagging individuals with a unique combination and limits handling (Boiteau *et al.* 2009, Kissling *et al.* 2014). It was already used for homing studies especially in honeybees (Kissling *et al.* 2014). Two RFID portals A and B (MAJA® reader module 4.2, Mycrosensys) were placed in series on a wood support at the entrance of the tunnel on the outside (Appendix S2) thus recording AB sequence or BA sequence for on- or outgoing movements respectively that were recorded by a RFID HOST controller iID® HOST MAJA (Mycrosensys) (see Henry *et al.* (2012) and He *et al.* (2012) for details).

Hornet tagging

V. velutina workers were collected at their nest entry to be equipped with RFID micro TAG (mic3®-TAG 16Kbit, iID-2000-G, 2.0x1.7x0.5mm). The captured hornets were gently isolated in a falcon tube (50ml) and then anesthetized by keeping the tube on ice for 15-20min. Back to the laboratory, each hornet was immediately weighted (AS 220/C/2, Radwag 2011, precision ± 0.0001g). The largest distance between the eyes was used as for a measurement of head width and obtained with an electronic calliper (precision ± 0.01mm). Prior to fixation, the RFID micro tag was activated and then fixed on the dorsal side of the hornet thorax by using temporary cement (TempoSIL2, Coltène). The tagged hornets were allowed to recover in groups of height individuals on different meshed boxes (10 x 20 x 10 cm), with water and honey *ad libitum* during a maximum of 3 hours before their release,

either next to the nest or farther for the homing experiment (see below). The monitoring of these tagged hornets was realized from the 8th August 2016 to the 11th November 2016.

Hornets release

To test homing abilities, 318 workers were released at different dates between 2 pm and 5 pm at four different places corresponding to the four cardinal points for each distance from their nest: being at 0, 500, 1000, 2000, 3000, 4000 and 5000 m (Figure 1, Apendice S3). Traveling boxes with hornets were placed in an opaque plastic crate both to protect the hornets from heat and also to prevent them from getting any guiding visual information before release. To confirm first results, another release session was done for the distances of 3000, 4000 and 5000m. Each release was realized in similar weather conditions (no rain, sunny days).

To observe hornet activity, an additional batch of 41 workers was released near the nest on three consecutive days (8th, 9th and 10th August 2016) between 2pm and 5 pm.

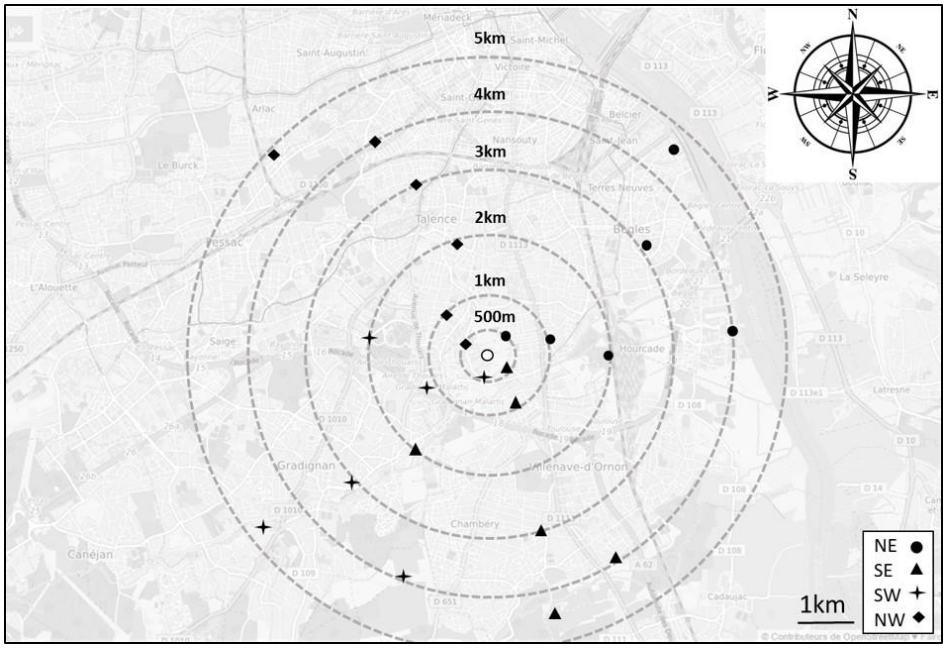


Figure 1. Map of the different release points of the hornet batches. The white dot in the centre stands for the position of the nest. Rounds: NE = North-East; Triangles: SE = South-East; Crosses: SW = South-West; Diamonds: NW = North-West. GPS coordinate are provided in Appendice S3. Background map Openstreetmap©.

Data analysis

Statistical analysis was done using R software (v.3.3.0., R Core Team 2016). First, the sessions 1 and 2 for the release distances of 3000, 4000 and 5000 m were compared using either Fisher's exact test (3000 and 5000 m) or Pearson's χ^2 test (4000 m). The standardized mass (i.e., the body condition) of the hornets was assessed with the scale mass index developed by Peig & Green (2009) based on standardised major axis regression using '*smart'* package (Warton *et al.* 2012). The effect of the release distance on the probability of returning to the nest was tested using a Cox proportional hazards regression model from '*survival'* package (Therneau 2014). The significance of the overall model including the standardized mass of the hornets and the cardinal point of release was tested using log-likelihood ratio test. As expected in homing experiments, some individuals (n = 205) were still missing at the end of the experiment (minimum time since their release = 320h) and were thus included as censored data. A Tukey post hoc test was used to test the differences among groups (distance and/or cardinal points). The difference in body condition between the hornets that returned to the nest and those that did not come back was tested with Wilcoxon rank sum test.

Individual activity was then analysed based on the individuals, which were released under the nest (N = 71, Appendice S3) to avoid potential confounding effect of the consequences of flying over long distance. The influence of the weather conditions (temperature, wind and humidity, obtained by the platform INRA CLIMATIK) on the number of trips per day per individual was assessed using a Negative Binomial Generalized Linear Mixed Effects Model (NBGLMM). First, a synthetic variable including the mean daily temperature (mean ± sd during the experiment: 26.11 ± 4.35°C), humidity (46.94 ± 13.80%) and wind speed (4.26 ± 1.07 m/sec) was computed with Principal Component Analysis (PCA). The first axis of the PCA (PC1) accounting for 60.71% of the total variance (eigenvalue >1) was therefore used to describe the daily weather conditions (factors loadings:

temperature: 0. 70; humidity = -0.70; wind speed: 0.14); positive values of PC1 correspond to warm dry and windy days while positive ones correspond to mild and humid days. The NBGLMM included the identity of the individual as random effect. The number of trips per day and hour was also compared among individuals using Poisson Generalized Linear Model (GLM) including quadratic effects for days and hours (see Monceau *et al.* 2013c). For GLM and NBGLMM, the statistical significance of each parameter was tested with likelihood ratio-based χ^2 statistics and Wald test respectively for unbalanced design (Fox & Weisberg 2011).

The length of each trip was extracted by the automated analysis of RFID tracking data Track-a-forager software (v 1.0, Van Geystelen *et al.* 2016). Different parameters were selected: (1) "natural foraging", because no food source was installed outside, (2) "shared access" for in and out access, (3) and "two" portals. Trips shorter than 20 seconds and longer than 86 400 seconds (i.e. 24h) were not included and the minimal threshold length was fixed to 60 seconds. The effect of individual body condition on the trip length was tested using Linear Mixed Effects Models (LMM) based on rank transformation, associated with *F*-ratio statistics. This procedure was preferred to the classical non-parametric Friedman tests because the data did not meet the conditions of normality and homoscedasticity (Baguley 2012).

Results

Homing ability of V. velutina workers

No difference between the two replicates of the 3000, 4000 and 5000 m release distances in the number of individuals coming back to the nest was detected (Fisher's exact test for 3000 m: P = 1 and 5000 m P = 0.24; Pearson's χ2 for 4000 m: χ^2 = 0, df = 1, P = 1). Thus, the two sessions for each distance were pooled for subsequent analyses. All distances pooled, a total of 112 individuals over 318 released individuals were detected back at the nest (Table 1, Figure 2). However, four of them (released near the nest, i.e. 0 m) were excluded from the following analyses because their return was not recorded (only the first exit after the return). The probability of returning to the nest was affected by the distance of release (Cox proportional hazard model: χ^2 = 161.69, df = 6, P < 0.0001, Figure 2) but not the body condition of the hornets (χ^2 = 2.82, df = 1, P = 0.09) or by the orientation (cardinal points) of the release (χ^2 = 2.97, df = 3, P = 0.39). Three different groups based on the release distances did not differ: 0 and 500 m (Tukey test, P = 0.95), 1000 and 2000 m (P = 1) and 3000, 4000 and 5000 m (P > 0.15 in all cases). These groups differed from each other (P < 0.05) except in the case of 1000 vs. 4000 m that is marginally non-significant (P = 0.08). The homing rate decreased of ca. 50% from a group distance to the further one (Figure 2). Hornets coming back to the nest and those considered lost (i.e. that did not return to the nest during the experiment) differed in their body condition (Wilcoxon rank sum test: W = 12934.5, P = 0.01): the former were lighter (median [95%CI]: 284.1 [273.3; 294.1] mg) than the latter (295.8 [286.8; 299.9] mg).

Table 1. Homing rate, time to return and speed of *V. velutina* workers in function of their release distances, and the cardinal points of release (NE= North-East, NW= North-West, SE= South-East, SW=South-West). The sample size for each category is also given (N).

Release distance (m)	N	homing rate (%)					homing time (h) mean ± sd	homing speed ($m.h^{-1}$)
		NE	NW	SE	SW	Overall		
0	71	-	-	-	-	83.78	2.40 ± 2.01	-
500	32	100.00	75.00	12.5	100.00	90.91	3.91 ± 6.73	484.8 ± 596.64
1000	32	37.50	50.00	25.00	62.50	43.75	8.02 ± 19.17	862.17 ± 691.25
2000	32	62.50	37.5	62.50	37.50	50.00	16.75 ± 12.21	375.27 ± 451.97
3000	64	12.50	12.5	18.80	12.50	14.06	80.11 ± 53.23	56.60 ± 39.68
4000	64	18.80	25.00	25.00	18.80	21.88	77.53 ± 53.34	92.17 ± 73.03
5000	64	6.25	6.25	0.00	6.25	4.69	176.17 ± 118.3	36.53 ± 18.53

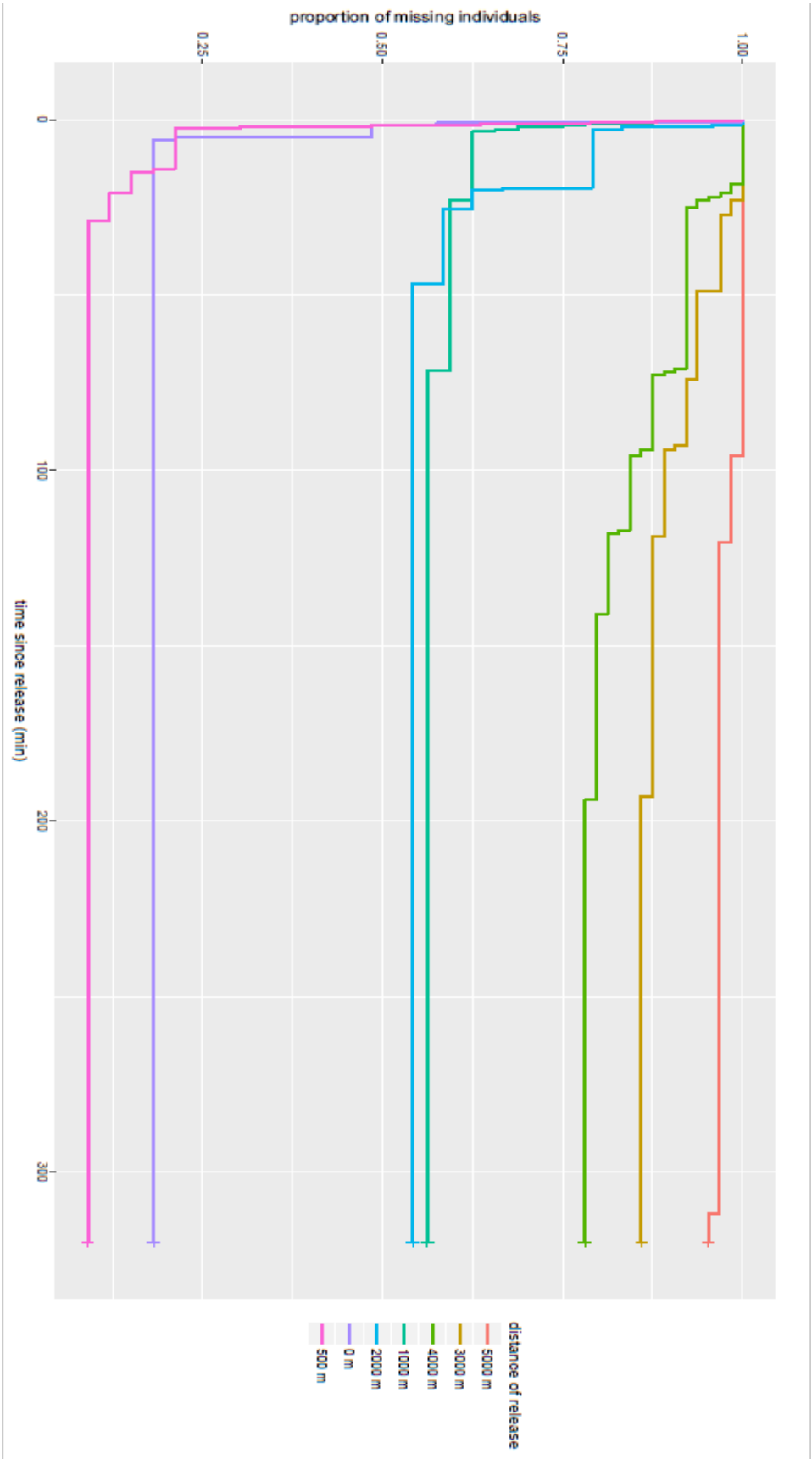


Figure 2. Homing time to nest of tagged workers of *V. velutina* in function of their release distance from nest.

Individual flight activity

Considering the 71 individuals released at the nest (4467 trips), the individual average duration period of activity lasted 4.98 ± 4.44 days (mean ± sd, range: 1-26 days of detection) with an average 12.62 ± 10.97 trips per day per individual. Ninety eight percent of the trips were recorded between 7:00am and 08:00pm; the remaining trips (72) made during the night were excluded from subsequent analyses.

The number of trips per day and individual was affected by the weather conditions (NBGLMM, estimates ± sd = 0.15 ± 0.04; Wald test: $\chi2$ = 13.15, df = 1, P < 0.001): the number of trips increased with higher temperature and lesser humidity. The number of trips differed among individuals with no clear pattern (Poisson GLM: $\chi2$ = 353.85, df = 70, P < 0.0001, Figure 3) and hours of the day (hours: $\chi2$ = 17.61, df = 1, P < 0.0001; hours^2 $\chi2$ = 15.94, df = 1, P < 0.0001) with a maximal number of trips was reached in early afternoon (02:00 pm – 03:00 pm, Figure 4). There was no difference among days (days: $\chi2$ = 2.53, df = 1, P = 0.11; days^2: $\chi2$ = 0.55, df = 1, P = 0.46) or their interactions (hours x individuals: $\chi2$ = 40.73, df = 61, P = 0.98; hours^2 x individuals: $\chi2$ = 39.55, df = 61, P = 0.98; days x individuals: $\chi2$ = 41.96, df = 48, P = 0.72; days^2 x individuals: $\chi2$ = 46.61, df = 48, P = 0.53).

Trip duration was divided into 2 samples: long trips that lasted more than 1 hour and short trips that lasted less than 1 hour (Figure 5). Long trips represented 3.60% of the trips and range more than 1 hour to ca. 22 hours. These trips were not considered in the following analyses. Most of the trips were thus short trips of ca. 949.7 ± 750.46s (mean ± sd, ca. 15 min 50 ± 12 min 30, range: 68s to 3597s). Trip duration was not influenced by body mass (F = 0.34, df = 1 and 24, P = 0.57).

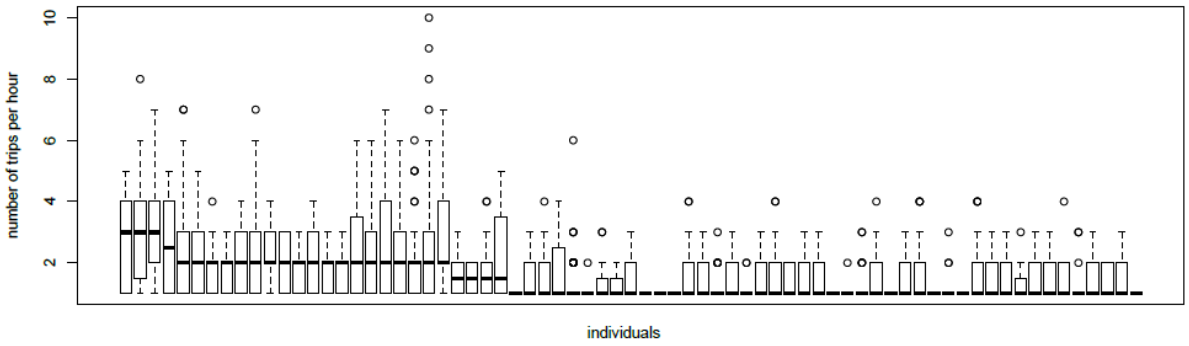


Figure 3: Number of trips per hour of the different *V. velutina* workers.

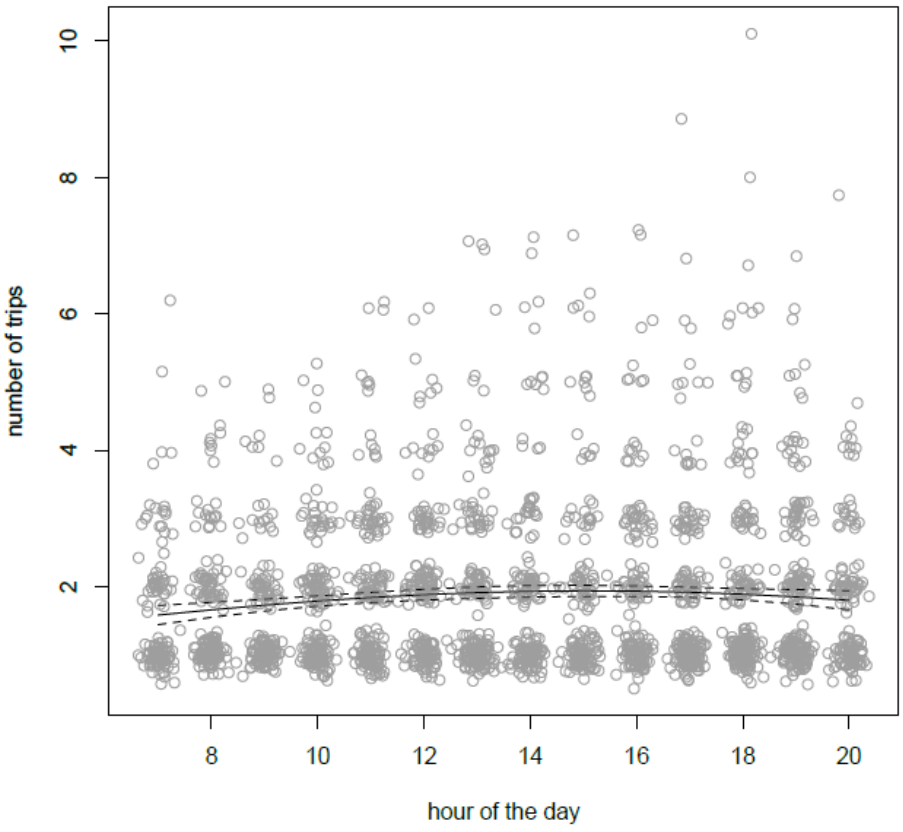


Figure 4: Number of trips of the different *V. velutina* workers in function of the hour in the day. The fitting of the corresponding GLM-Poisson is represented with the solid line, and the ±5% errors in dotted lines.

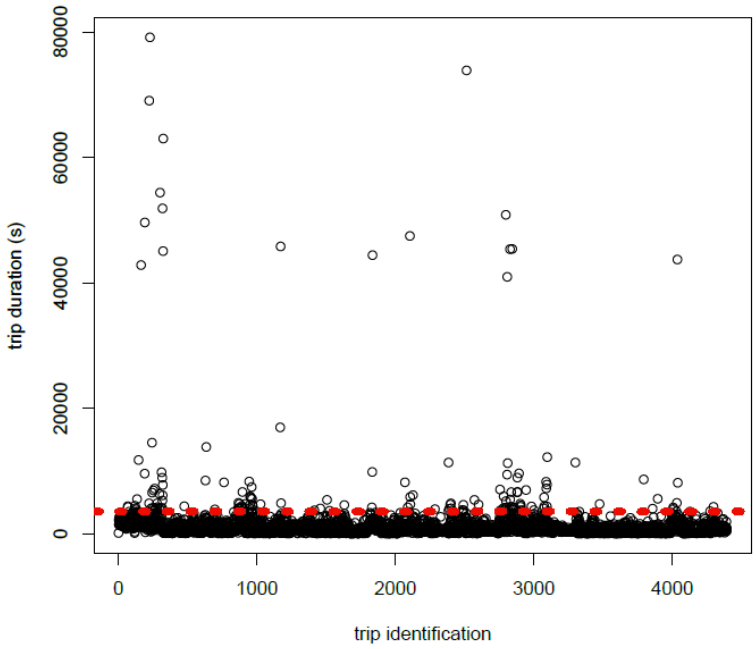


Figure 5: Global trips duration in *V. velutina* workers. The red dotted line at 3600 s = one hour, separates short trips from long trips in our analysis.

Discussion

Homing is a critical behaviour for central forager and represents an upper limit of the distance an individual is able to travel. In the case of invasive and pest species, this parameter should be taken into account in impact models. Homing ability and nest activity were here assessed for the first time on the hornet *V. velutina* using RFID technic on a semi-field nest.

Homing ability of *V. velutina* decreases gradually with the distance of release: most of the individuals flew back when released up to 500 m, half of them returned to the nest when released up to 2000 m and less than a quarter were retrieved when released farther than 3000 m. These data show that *V. velutina* workers can find their way back over several kilometres. However, their foraging range is probably lower than 2000 m, probably in a radius around the

nest of 500 m. These results are congruent with the data available in other *Vespa* sp. Indeed, homing ability in *V. orientalis* is ca. 1000 m with a probable territory range of 500 m (Ugolini, Kessler & Ishay 1987). *V. orientalis* and *V. velutina* being of similar size, finding congruent capacity is not surprising. In the case of *V. velutina*, almost 5% of the individual released at 5000 m were able to find their nest. This long distance can be compared to the foraging range of the giant Japanese hornet *V. mandarinia*, is ca. 1000-2000m, with a maximal distance of 8000 m (Matsuura & Sakagami 1973). This experiment also shows that homing behaviour is not affected by the orientation of the release point. This suggests that compass information due to sun orientation or magnetic fields might not be involved in this homing behaviour, what is congruent with previous findings in *Vespidae*. Orientation system mostly relies on visual (Zeil 1993), olfactory (Takagi *et al.* 1980) and tactile cues (Jeanne & Taylor 2009). Vespids learn visual information during an orientation flight, to find their path to or back from foraging sites (Ugolini 1987, Raveret Richter & Jeanne 1991; see Raveret Richter (2000) for a review). In this orientation flight, the individual flies along ever increasing arcs around the nest that allow combining flight trajectory (arcs) and gaze orientation, to acquire sufficient visual cues for homing (Zeil 1993, Toh & Okamura 2003, Stürzl *et al.* 2016). One should however consider that workers' previous experience was not controlled in our experimental design so some individuals might have already experienced long trips from their nest that could have facilitated their return. Returning workers had a smaller body index (i.e. lower mass for a similar size) that could reflect their age. Indeed, worker body mass increases through the season probably because the consecutive cohorts of workers benefit from increasing food provided during larval stage (Matsuura & Yamane 1990, Monceau *et al.* 2013b). Individual with lower body mass could be older individuals, thus with more experience. Most of the tagged workers come back to the nest within the first 24 hours. However, some of them return to the nest more than four days (i.e., 100 hours) after

their release. Such duration was also observed in *Bombus terrestris* (Goulson & Stout 2001). This means that individuals may survive for several days outside their nest and thus questions their travel path during this episode. Considering that *V. velutina* nest density in Bordeaux suburbs is quite high, one hypothesis is that individuals may have wandered from colonies to colonies since several non-nest mates were observed as accepted by other colonies (K. Monceau and O. Bonnard, pers. obs.). It also questions the fate of those that never come back to the nest: lost, died or fully accepted by other colonies. However, this question cannot be answer with the RFID technique and other tracking devices are still not usable to cover such long distances (see Milanesio *et al.* 2016, 2017).

Up to date, only direct observations or video records have allowed monitoring the activity of *V. velutina* (Perrard *et al.* 2009, Monceau *et al.* 2013b, 2013c, 2017). Our results are in line with these previous studies. First, most of the activity is realized between 07:00 am and 08:00 pm confirming that *V. velutina* is diurnal; some individuals still have a nocturnal activity (only 2% of the activity). *V. crabro* is also active with low light intensity but in a higher propensity (Kelber *et al.* 2011). Second, the worker activity is driven by weather conditions that is quite classical in *Vespidae* (Cruz *et al.* 2006, da Rocha & Giannotti 2007, Kasper *et al.* 2008; Canevazzi & Noll 2011, de Castro *et al.* 2011). The observed enhancement of the hornet activity during the day with a maximum around noon, already observed by video analysis (Monceau *et al.* 2017), can be either attributed to an increase in temperature or in UVB solar irradiation. Indeed, *V. orientalis* is able to convert solar into metabolic energy with photovoltaic like cuticle cells (Ishay & Kirshboim 2000, Ishay 2004, Volynchik *et al.* 2008, Plotkin *et al.* 2010). Such a reaction has not been investigated in *V. velutina* for now but should receive attention as it would also explain his performance in hovering for preying honeybees (Monceau *et al.* 2013c).

Contrary to previous studies, RFID allows identifying unique individual behaviour. Thus, the duration of each trip can be accurately quantified with RFID: 95% of the flights lasted less than 1 hour. Flying speeds of *V. velutina* workers are so far unknown, but in *V. crabro*, it has been estimated at 1.86m.s^{-1} (i.e., 6.7 km.h^{-1}) (Spiewok & Schmolz 2005). If both species fly at a similar speed and considering the average trip duration being 15 min, *V. velutina* workers probably forage within less than 1000 m away from their nest. Moreover, predation includes catching and processing the prey and then coming back to the nest with an additional load that impacts the flying speed, thus they probably forage in a 500-800m diameter perimeter. This means that if predation is detected on hives, *V. velutina* nest should be searched within a radius of at least 1000 m. Although, this approximation is based on a specific area where resources (i.e. honeybee hives) are common and thus should be replicated in a different area. Nevertheless it is congruent with the homing behaviour experiment, which does not depend on resource richness.

This work explored for the first time homing abilities using RFID technic in an invasive hornet species threatening honeybees, and allowed us to evaluate the boundaries of its foraging range. Radio tracking allows tracking hornets only over short distances, and it is not accurate enough (Milanesio *et al.* 2016, 2017): the RFID provides the best compromise to acquire new information on workers flight behaviour that are of first interest for the monitoring and control of this special invasive hornet. Invasive social insects, especially Vespids, can deeply affect their environments (Beggs *et al.* 2011, Bradshaw *et al.* 2016), and their impact is obviously related to foraging range. How animals use their environment and their movements are key parameters in biological invasion (Holway & Suarez 1999) and such parameters should be implanted in future impact models.

Author Contributions

JP, OB and DT conceived the ideas and designed methodology; JP and OB collected the data; JP and KM analysed the data; JP, DT and KM wrote the manuscript.

Acknowledgements

We thank the bee team of the INRA Magneraud, P. Aupinel and J.-F. Odoux, for lending us components of the RFID material and helping us to use it. We thank the beekeeper union "Association Anti Frelon Asiatique" and especially J.-P. Croce for collecting the nest. We thank Bayer Crop Science, represented by B. Laborie, for the first author financial support. The authors declare no conflict of interest. The first author's funding sponsor had no role in any steps of the study (design, data collection, analyses, writing). We thank A. Foucard-Wellwood for English language proofreading.

Supplementary material

Appendice S1. Installation of the nest within the cage inside the cabin (side view).

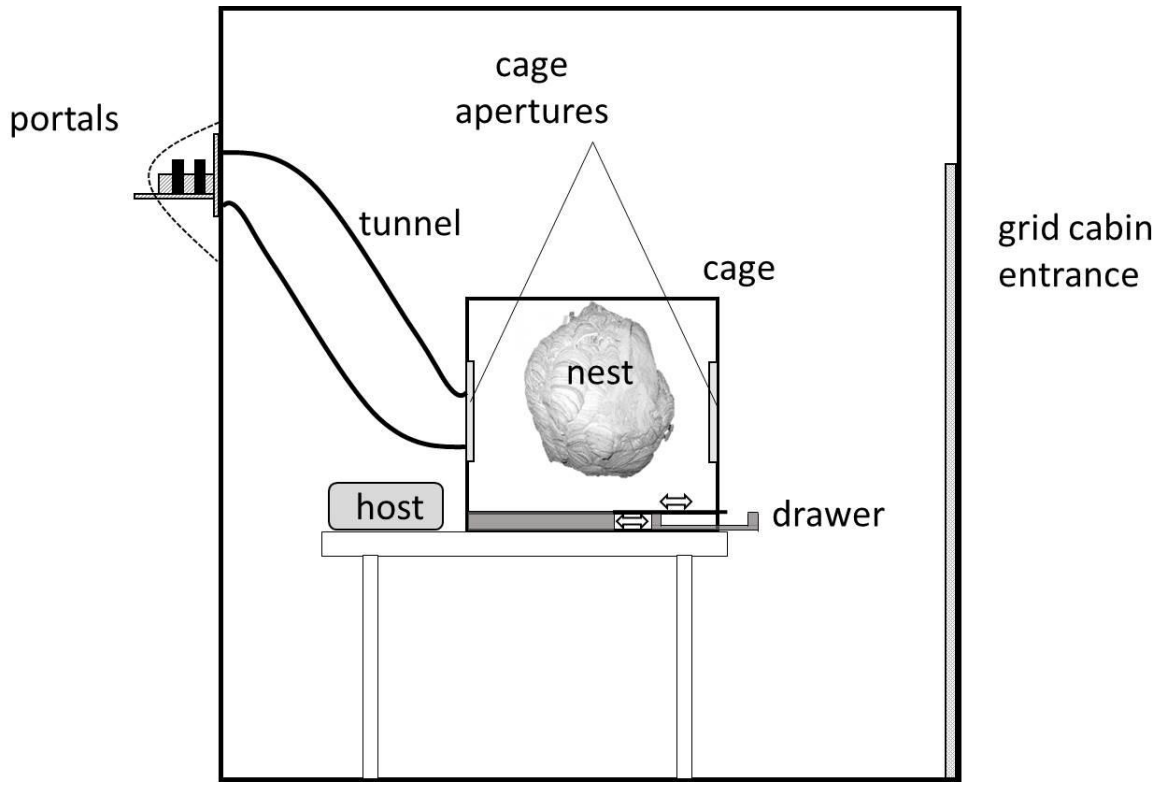
Appendice S2. RFID portal design.

Appendice S3. GPS coordinates of the different release points of the hornets at different distances from the nest.

Appendice S1. Installation of the nest within the cage inside the cabin (side view).

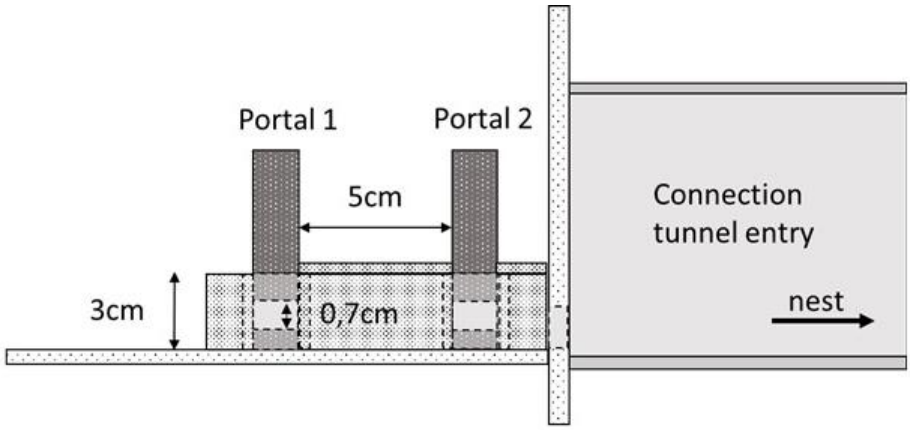
The nest is fixed with iron strings inside the cage made of mahogany stainless steel grid and Plexiglas. This cage was equipped with two secured apertures and one secured covered sliding plate and a mobile drawer for food and water supply. The cage is placed on a table inside a 2m x 1.5m x 2m stainless steel grid cabin with a corrugated plastic roof.

Once allowed to leave the cage, the hornets can access the outside or enter the nest *via* a 10 cm diameter transparent plastic tunnel connecting the cage to the outside. The RFID portals, covered with a plastic copper (see details in Appendice S2), are positioned at one extremity of the tunnel so the hornet must pass through the portals to go in and out. All movements are recorded by A RFID host controller iID® HOST MAJA (Mycrosensys).

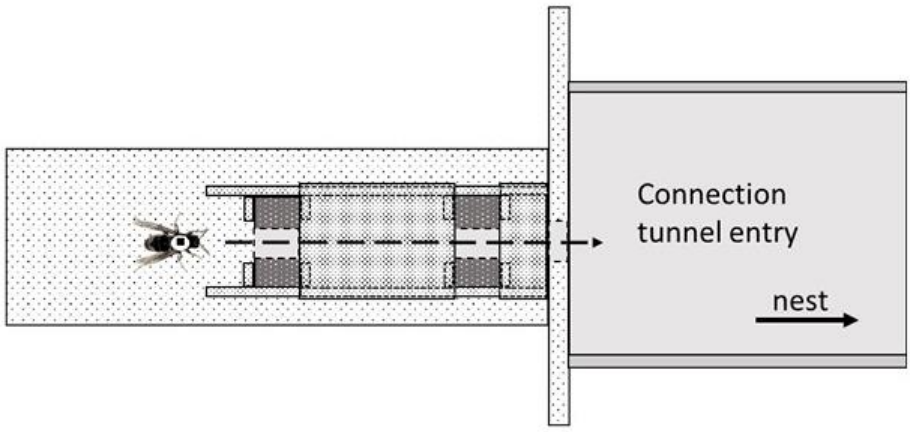


Appendice S2. RFID portal design. Two RFID portals A and B (MAJA® reader module 4.2, Mycrosensys) were placed on a 5 cm long wood support, one behind another at the entrance of the tunnel on the outside. Thus ongoing (AB sequence) and outgoing individuals (BA sequence) could be sorted out and are recorded by the RFID host controller. Hornets are tagged with RFID micro TAG (mic3®-TAG 16Kbit, iID-2000-G, 2.0x1.7x0.5mm).

From the side



From above



Appendice S3. GPS coordinates of the different release points of the hornets at different distances from the nest. For 3000, 4000 and 5000m distances of release, 2 replicates were made.

Distance (m)	Cardinal point	GPS coordinates	Date	Number of hornets
0	-	44°47'30.4"N 0°34'36.9"W	5 August 2016	5
			8-10 August 2016	36
			30 August 2016	30
500	NW	44°47'37.6"N 0°34'32.3"W	16 August 2016	8
	SE	44°47'16.2"N 0°34'24.8"W	16 August 2016	8
	SW	44°47'08.0"N 0°34'35.8"W	16 August 2016	8
	NE	44°47'33.2"N 0°34'55.6"W	16 August 2016	8
1000	NE	44°47'20.4"N 0°33'49.1"W	16 August 2016	8
	SE	44°46'55.5"N 0°34'16.2"W	17 August 2016	8
	NW	44°47'13.1"N 0°35'30.8"W	17 August 2016	8
	SW	44°47'49.3"N 0°35'13.2"W	17 August 2016	8
2000	NE	44°47'36.1"N 0°33'12.4"W	28 September 2016	8
	SE	44°46'42.4"N 0°35'12.1"W	28 September 2016	8
	SW	44°47'31.3"N 0°36'02.0"W	28 September 2016	8
	NW	44°48'38.7"N 0°35'04.7"W	28 September 2016	8
3000	NE	44°48'08.8"N 0°32'34.9"W	18 August 2016	8
			10 October 2016	8
	SE	44°45'50.4"N 0°34'03.7"W	18 August 2016	8
			10 October 2016	8
	SW	44°46'18.2"N 0°36'22.1"W	18 August 2016	8
			10 October 2016	8
	NW	44°48'47.4"N 0°34'58.3"W	18 August 2016	8
			10 October 2016	8
4000	NE	44°47'43.9"N 0°31'41.0"W	29 September 2016	8
			11 October 2016	8
	SE	44°45'38.1"N 0°33'03.7"W	29 September 2016	8
			10 October 2016	8
	SW	44°45'33.2"N 0°35'57.3"W	29 September 2016	8
			10 October 2016	8
	NW	44°48'53.0"N 0°36'32.8"W	29 September 2016	8
			11 October 2016	8
5000	NE	44°49'08.8"N 0°32'20.5"W	18 August 2016	8
			10 October 2016	8
	SE	44°44'51.7"N 0°33'53.2"W	18 August 2016	8
			10 October 2016	8
	SW	44°45'14.8"N 0°37'31.5"W	18 August 2016	8
			18 October 2016	8
	NW	44°48'39.9"N 0°37'42.7"W	18 August 2016	8
			18 October 2016	8