The invasive hornet Vespa velutina affects pollination of a wild plant through changes in abundance and behaviour of floral visitors

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ORIGINAL PAPER

The invasive hornet *Vespa velutina* affects pollination of a wild plant through changes in abundance and behaviour of floral visitors

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Abstract The Asian hornet (*Vespa velutina*) is a keen predator of honeybees and other insects. Its success as invasive species threatens the stability of ecosystem services provided by them, such as pollination of wild plants and crops. In this study we analyse their impact as hunters of flower visitors on plant pollination, by studying the hunting behaviour of *V. velutina* in flower surroundings, the effect of *V. velutina* hunting on the floral visiting behaviour by insects and its impact on the amount of conspecific pollen deposited on the stigmas of *Mentha suaveolens*, a native and common herb to southern and western Europe. We made observations and video-recordings in blooming patches with and without the presence of *V. velutina* and quantified the deposition of conspecific pollen on stigmas in those same patches. Asian hornets were frequent and successful hunters of flower visitors of *Mentha suaveolens*, inducing changes in the foraging behaviour of several groups of pollinators. The patch visitation rate of European honeybees (*Apis mellifera*), the flower visitation rate of small hymenopterans and the flower visitation time of bumblebees (*Bombus* sp.) and syrphids was significantly reduced in patches with presence of the predator. As a consequence, the quantity of conspecific pollen on stigmas of the studied native plant decreased in patches with *V. velutina*. In this study we demonstrate the negative impact of the invasive hornet *V. velutina* on pollination services in invaded areas as a consequence of their hunting of pollinators in flower patches.

Keywords Behaviour of pollinators · Invasive hornet · Pollination services · Predation

Introduction

The sexual reproduction of most angiosperms is mediated by pollinators, currently threatened by stressors including the invasion of alien species (Potts et al. 2010; Ollerton et al. 2011). Invasive alien species (IAS onwards) can modify pollination interactions, producing cascade effects with severe consequences, from genetic structure of populations to eco-evolutionary dynamics at the ecosystem level (Traveset and Richardson 2006; Schweiger et al. 2010). The routes, direction and magnitude of the changes induced by IAS are diverse and highly context dependent (Pyšek et al. 2012). Commonly, the success of these species as competitors, herbivores or predators, leads to a re-shape of plant-pollinator networks with ultimate detrimental consequences for the fitness of plants or pollinators (Aizen et al. 2008; Russo et al. 2014). IAS

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behaving as predators may cause direct changes in the structure of populations of native pollinators, indirectly affecting the pollination services they provide (Vanbergen et al. 2018).

Several social vespids have been introduced into new regions, becoming invasive species of concern due to their impacts on native populations and ecosystem services (Beggs et al. 2011). Their success colonizing new regions is related to several factors, such as a generalistic-opportunistic behaviour and plasticity in life history traits, which provide great adaptability to new environments, and high reproductive rates, allowing significant increases in abundance in a short time (Richter 2000; Wilson et al. 2009). Vespids require water, carbohydrates and proteins for metabolic processes of larvae and adults (Richter 2000). Water is also required for temperature regulation and plant fibers for nest construction (Richter 2000). They obtain sugars from mature fruit, sap, honeydew and nectar (Beggs et al. 2011), while proteins are obtained from active hunting or scavenging. Pieces of prey or carrion are carried to the colony to feed the developing brood. Social vespids have an annual cycle and a monogynous colony starts with the formation of a small nest build by a single queen, which takes care of the brood during the first phase (Matsuura and Yamane 1990). After the first adults emerge, the queen remains at the nest and workers are responsible for foraging, building, cooling and defence of the colony, among other tasks. The colony and the nest grow throughout the season, with a consequent increase in resource requirements, as well as in specialization of tasks (Jeanne 1980). Sociality enhances the development of a variety of techniques used to locate prey and profitable hunting sites by memorizing, associative learning and communication (Richter 2000). Flowers constitute an ideal hunting arena for different kinds of insect predators because they attract visitors on a regular basis (Dukas 2005).

Successful hunting by predators around flowers has an obvious direct impact in reducing the abundance of pollinators. In addition, unsuccessful predation events and the sole presence of predators can have similar or even higher effects on pollination interactions, as mediated by fear (Jones and Dornhaus 2011). Because pollinators constantly face trade-offs between the benefits of feeding and the risk of predation, different anti-predatory behaviours are displayed. Most pollinator taxa respond to the risk of predation by reducing flower visitation rates and time spent at the flower, which ultimately translates in decreasing plant fitness (Romero and Koricheva 2011; Romero et al. 2011). Thus, invasive vespids may constitute important disruptors of mutualistic pollination interactions in invaded areas as a consequence of their hunting efficiency (Wilson et al. 2009).

The Yellow-legged hornet, *Vespa velutina,* is an invasive species of concern for European countries, North Korea and Japan, where it is nowadays well established (Choi et al. 2012; EU Regulation EU Regulation 1143/2014; Ueno 2014). After 15 years of its first detection in France (Haxaire et al. 2006), it has been recorded in several mainland and insular countries in Europe and its expansion continues (INPN 2018), naturally spreading at a speed of 100 km/year (Rome et al. 2015). The introduction into new regions has probably been mediated by human transport as well (Budge et al. 2017). An 8-year monitoring revealed that the number of nests within a particular area increased, with the carrying capacity threshold being unknown (Monceau and Thiéry 2017).

Vespa velutina is especially notorious for causing important losses in the beekeeping sector. They often hunt honeybees in the apiaries (Tan et al. 2007), where their most frequent hunting technique consists in hovering in front of the hives for workers coming back from foraging (Monceau et al. 2013), capturing the prey in flight (Tan et al. 2007) and carrying the thorax to the nest. *Vespa velutina* also hunts on a diversity of arthropods including native wasps, halictids, syrphids, and other dipterans (Perrard et al. 2009; Villemant et al. 2011), some of which are important pollinators of crops and wild plants (Carvalheiro et al. 2013; Rader et al. 2016). In their native area, Asian hornet workers hunt pollinators when visiting flowers, causing a reduction in the number of visits and the time spent in the flowers by the Asian honeybee (*Apis cerana*) (Tan et al. 2013). In invaded areas, however, this behaviour has not been recorded to date, nor its consequences for pollination services.

Due to the great success of *V. velutina* as IAS and as a predator, it is broadly believed that this species might act as a disruptor of pollination services in invaded areas (Beggs et al. 2011; Monceau et al. 2014; Vanbergen et al. 2018). Nevertheless, its impact on pollination processes has not been evaluated so far. In this work we aim (1) to study the hunting behaviour of *V. velutina* in flowers of a common herb (*Mentha* *suaveolens*), present in invaded areas of northwest Spain, (2) to understand the pathways through which *V. velutina* affects pollination services of native plants by studying the groups of pollinators affected by predation and their behavioural responses, and (3) to assess the consequences of predation for the pollination of the plant.

Methods

Study sites

The study was made during 5 weeks from 22th September to 30th October of 2017 in the municipalities of Baiona, Gondomar and Nigrán, a southern coastal area of the province of Pontevedra, Northwest Spain. *Vespa velutina* was recorded for the first time in this area in 2012 (Xunta de Galicia 2016) and the population has reached high abundances since then, with approx. 6700 nests being detected and destroyed by the local administration during 2017 in this province, and trapping campaigns being promoted each year by several institutions and organizations, which result also in substantial losses of other insects (Rojas-Nossa et al. 2018).

We selected 20 patches of *Mentha suaveolens*, in 10 of them *V. velutina* was present and behaved as predator of flower visitors (patches with *V. velutina*) while in the other 10 patches *V. velutina* was absent. The presence or absence of *V. velutina* was confirmed by direct observations carried out by one observer during 20 min in each patch and over three consecutive days before the trials. These observations were made from 10:00 to 14:00 h, randomly assigning the start time to each patch each day of observation. A mean of 4.1 (± 1.8 SD) hornets / 10 min / patch were observed hunting in patches with *V. velutina* during the trials. The patches with presence and absence of *V. velutina* were selected in similar conditions of flowering period and environment, to avoid differences in the behaviour of flower visitors caused by differences in the number of flowers per patch or the quality of the rewards. Trials were made when > 70% of inflorescences in the patch had fresh open flowers. We counted the number of inflorescences with open flowers per patch to incorporate this information in statistical models (see Data analysis section). The number of inflorescences per patch averaged 1657.2 (SD = 1235.5) in patches with *V. velutina* and 1538.4 (SD = 849.6) in patches without *V. velutina*. Patches were located in meadows, small orchards and gardens, with surrounding native herbaceous vegetation and at 8 to 125 m.a.s.l. Trials were made in sunny days from 10:00 to17:00 h. To avoid biases due to temporal effects, patch types (with presence *vs.* absence of *V. velutina*) were alternated and their order along the day changed in consecutive days. Besides, to reduce overlapping of foraging ranges of individuals, patches were placed at a minimum distance of 0.8 km to the nearest patch.

Video recordings of the behaviour of flower visitors and V. velutina

In each patch (20 patches), six plots of 0.5 × 0.5 m were randomly selected. A GoPro camera was placed in each plot to record floral visits during 7–8 min. In the laboratory, we discarded the first and last minute of each video record, to minimize the effect of the presence of the researcher near the plot on the behaviour of pollinators, keeping 5–6 consecutive minutes of each video record. Five of the 120 samples had to be discarded for the analysis because of the low quality of images. Thus, we finally analysed 115 samples and 653 min of video recordings using the software VLC for Windows.

In these video recordings, we identified the floral visitors as belonging to the following functional groups, based on taxonomy, size and behaviour (*i.e.* in groups comprising different taxa, these showed a consistent behaviour in response to *V. velutina*): *Apis mellifera*, *Bombus* sp., small Apidae (belonging to the families Megachilidae, Halictidae and Colletidae, < 8 mm in length), Vespidae (native wasps of the genera *Polistes* and *Vespula*), small Lepidoptera (family Lycaenidae, < 36 mm of wingspan), large Lepidoptera (families Nymphalidae, Pieridae and Sphingidae, > 36 mm of wingspan), small Syrphidae (syrphids < 8 mm in length), large Syrphidae (syrphids, > 8 mm in length), small non-syrphid dipterans (dipterans < 8 mm in length, mostly belonging to the families Ephydridae and Muscidae), and large non-syrphid dipterans (dipterans > 8 mm in length, mostly Tachinidae).

We counted the number of individuals of each functional group per 5 min (patch visitation rate), the number of flowers visited by each individual per 5 min

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(flower visitation rate), and the number of inflorescences per plot. We also measured, for each individual, the time spent visiting a flower (flower visitation time, *i.e.* from the moment of head or proboscis introduction into the flower mouth until its removal), by analysing the videos at 0.5 × speed.

In patches with presence of *V. velutina*, we also analysed their hunting behaviour in 2 min observation periods. We identified the insects under attack (according to the pollinator groups previously described) and counted the number of successful (prey captured) and unsuccessful (prey not captured) attacks.

Impacts on the quantity of pollen deposited on stigmas

To quantify pollen deposition on stigmas, we collected flowers from 10 randomly selected ramets in each patch. Two flower morphs occurred in most patches, one with long anthers and another with short anthers. Since this morphological difference in the length of the anthers can translate into different chances of receiving pollen on the stigmatic surface by selfing, morphs were sampled separately, with 10 flowers per morph per patch. To avoid effects of flower position on the plant, we collected flowers only from apical inflorescences. We discarded plants with spiders or spider webs, to avoid the effect of the presence of these predators. At the laboratory, we mounted stigmas on microscope slides with fuchsin glycerine jelly (Kearns and Inouye 1993) and counted the number of conspecific pollen grains deposited on stigmatic surfaces.

Data analysis

We fitted Generalized Linear Mixed Models (GLMMs) to analyse differences in patch visitation rates, flower visitation rates, flower visitation time and pollen deposition on stigmas. The predictor variable (fixed effect) was whether *V. velutina* was present or absent. Patch visitation rate models were fitted with Zero-inflated Poisson distribution functions, including the patch identity as random effect. The significance level was established at 0.05 in all tests, and was also corrected applying the Holm-Bonferroni (sequential Bonferroni) method (Holm 1979), accounting for the 30 tests performed. This correction is less conservative than the Bonferroni method. As floral display size (*i.e.* the number of flowers in a plant or patch) can affect flower visitor abundances in flower patches (Ohashi and Yahara 2001), the total number of inflorescences with open flowers per patch was included as an offset term. The models of flower visitation rate were fitted with the Poisson distribution, with the patch identity and the plot identity included as random effects and the total number of inflorescences per plot as an offset term. To fit the models of flower visitation time, data were first log-transformed and the models fitted with the Gaussian distribution function, with the patch identity and the individual included as random effects. To analyse differences in pollen deposition on flower stigmas between patches with and without *V. velutina* we fitted GLMMs with the Poisson distribution. The number of pollen grains was included as response variable, the presence/absence of *V. velutina* as a fixed effect and the patch identity as a random effect. For these analyses we used packages Lme4 (Bates 2010), car (Fox et al. 2013) and blmeco (Korner-Nievergelt et al. 2015). The analyses were performed using R software version 3.5.1.

Results

Hunting behaviour of Vespa velutina

The most frequent hunting strategy observed for *V. velutina* workers was to fly around *M. suaveolens* inflorescences (Fig. 1), where insect flower visitors

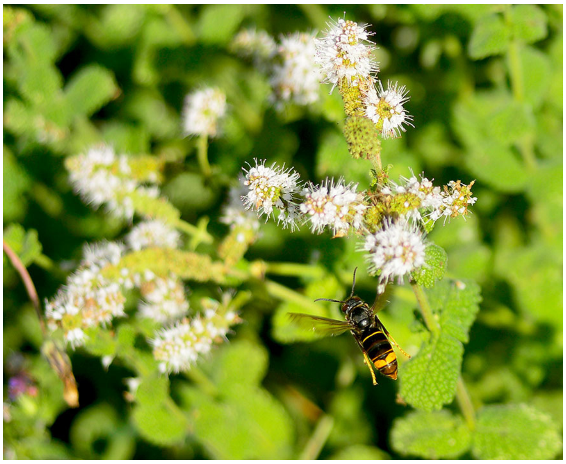

Fig. 1 *Vespa velutina* hunting flower visitors of *Mentha suaveolens*. Picture: S. Rojas-Nossa. Camera Nikon D3200 and manual lens Micro-Nikkor 55 mm

abound, increasing their speed when a potential prey was detected, and chasing and capturing insects usually in flight or, less often, on the flowers, when the insect was sitting on them. We observed attacks on Vespidae, *Bombus* sp., small Apidae, Lepidoptera, Syrphidae and other flies. We observed an 8% success in 25 hunting attempts. After catching a prey, the hornet usually perched on the vegetation on its hind legs (often on *M. suaveola*), while manipulating the prey with its front legs and tearing the prey apart with the mandible, discarding all but the thorax, which is carried to the nest to feed the larvae. Hornets often increased speed and landed shortly on dry inflorescences, as if taking it as a prey, but we did not observe nectar consumption by them in *M. suaveolens*.

Responses of pollinators

Patches with hunting *V. velutina* individuals had a mean of 6.3 (± 2.0 SD) species of pollinators, in comparison with 8.22 (± 2.3 SD) species present in patches without the predator. The abundance of floral visitors averaged 12.4 (± 7.6 SD) individuals in 10 min in patches with *V. velutina*, and 16.6 (± 6.1 SD) individuals in 10 min in patches without the hornet. Predation by *V. velutina* modified foraging behaviour of pollinators, with different effects depending on the pollinator group (Tables 1, 2 and 3). For instance, the patch visitation rate of *A. mellifera* was significantly reduced in flower patches with hunting *V. velutina* individuals (Table 1). Flower visitation rate was reduced in small hymenopterans (Table 2), while flower visits of *Bombus* sp. and small syrphids were shorter in presence of *V. velutina* (although the effect for *Bombus* became non-significant after applying the sequential Bonferroni correction; Table 3). On the contrary, native vespids made longer visits (Table 3).

Impacts on the quantity of pollen deposited on stigmas

The quantity of pollen deposited on flower stigmas was significantly reduced in patches of *M. suaveolens* with presence of *V. velutina* behaving as predators (Fig. 2). This occurred in both flower morphs ($\chi_2 = 6.57$, df = 1, $P = 0.01$ for the short anthers morph and $\chi_2 = 8.72$, df = 1, $P = 0.003$ for long anthers morph).

Discussion

Social wasps, and particularly hornets (*Vespa* sp.), are notorious predators of bees (De Jong 1990; Dukas 2005). The hunting behaviour of *V. velutina* has received major attention as a predator of managed honeybees in the hives, in both native and invaded areas (Tan et al. 2007, 2010; Monceau et al. 2013, 2014; Requier et al. 2019). In this study, we demonstrate that *V. velutina* is also an active hunter of other pollinators while they visit flowers.

Hunting behaviour of Vespa velutina in flowers

In contrast with other predators of floral visitors, such as crab spiders that perform ambush attacks sitting on the flowers (Brechbühl et al., 2010), *V. velutina* workers fly actively between inflorescences and attack their prey mostly in flight, which constitute an energetically expensive strategy.

We recorded attacks to different groups of insects, including hymenopterans, lepidopterans and dipterans, when visiting the flowers of *M. suaveolens*. Similar groups were also detected by Villemant et al. (2011) in the pellets carried by workers to nests. Despite differences in approach, region and study period, both studies reflect that *V. velutina* is a successful and frequent predator of pollinators in invaded areas in Europe.

The hunting behaviour of *V. velutina* in flowers of *M. suaveolens* differs from the behaviour shown in honeybee hives. In hives, the most common strategy is to 'wait' for returning honeybees in suspended hovering flight, and then chasing its prey (Tan et al. 2007; Monceau et al. 2013). In flowers, hornets are permanently moving in flight around the flowers and attack pollinators in flight or when sitting on inflorescences. This difference is related to the spatial disposition of resources (*i.e.* insect prey). Returning or departing domesticated honeybees are compelled to pass through the entrance of the hive. Thus, prey is concentrated in a small area, slightly elevated from the ground. In flowers of *M. suaveolens*, however, the distribution of flowers and prey is not concentrated, but extended and closer to the ground. The behaviour of increasing speed and shortly landing on dry inflorescences during hunting bouts suggests that they recognize them as potential prey. Hornet hunting success is favoured by a high capacity to increase speed rapidly when chasing a prey.

Table 1 Results of the GLMM models for the effects of *Vespa velutina* on patch visitation rate (number of individuals per plot in 5 min) for each pollinator group

Pollinator group	Estimate ± SE	z value	n	χ^2	*P*
Hymenoptera					
Vespidae	1.28 ± 0.88	0.69	114	0.47	0.494
Apidae					
Apis mellifera	**2.45 ± 1.03**	**2.37**	**114**	**5.64**	**0.018**
Bombus	−3.07 ± 4.26	− 0.72	114	0.52	0.472
Apidae small	1.28 ± 0.88	1.45	114	2.09	0.148
Lepidoptera					
Lepidoptera-small	1.04 ± 1.06	0.98	114	0.97	0.325
Lepidoptera-large	2.36 ± 1.28	1.84	114	0.391	0.66
Diptera					
Syrphidae-small	2.17 ± 1.11	1.96	114	3.82	0.051
Syrphidae-large	0.43 ± 0.38	1.13	114	1.27	0.260
Other flies-small	0.83 ± 0.72	1.15	114	1.33	0.249
Other flies-large	0.40 ± 1.09	0.37	114	0.136	0.713

Significant results ($p < 0.05$) are indicated in bold, and remained significant after applying the sequential Bonferroni correction, considering the 30 tests performed, in Table 1–3). The sign of the estimate indicate the direction of the effect, with positive estimates indicating a decrease in patch visitation rate caused by the predator

Table 2 Results of the GLMM models for the effects of *Vespa velutina* on flower visitation rate (flowers visited per plot in 5 min) for each pollinator group

Pollinator group	Estimate ± SE	z value	n	X_2	*P*
Hymenoptera					
Vespidae	−0.17 ± 0.24	− 0.71	27	0.51	0.476
Apidae					
Apis mellifera	0.52 ± 0.77	0.68	28	0.46	0.499
Bombus	−0.31 ± 0.55	0.58	15	0.31	0.578
Apidae small	**1.60 ± 0.57**	**2.77**	**27**	**7.68**	**0.006**
Lepidoptera					
Lepidoptera-small	0.91 ± 1.13	0.80	25	0.64	0.423
Lepidoptera-large	−0.66 ± 0.87	− 0.75	21	0.57	0.451
Diptera					
Syrphidae-small	−0.82 ± 0.94	− 0.86	50	0.75	0.387
Syrphidae-large	−0.39 ± 0.35	− 1.12	26	1.26	0.261
Other flies-small	1.45 ± 1.26	1.15	23	1.32	0.251
Other flies-large	1.25 ± 0.67	1.85	27	3.43	0.064

Significant results ($p < 0.05$) are indicated in bold, and remained significant after applying the sequential Bonferroni correction, considering the 30 tests performed, in Table 1–3). The sign of the estimate indicate the direction of the effect, with positive estimates indicating a decrease in flower visitation rate caused by the predator

Another relevant aspect of the hunting strategies of *V. velutina* is the number of individuals hunting at the same place simultaneously. In patches of flowers of *M. suaveolens* this number was relatively low when compared with the number of hornets hunting in apiaries or even in single hives (Monceau et al. 2013;

Table 3 Results of the GLMM models for the effects of *Vespa velutina* on flower visitation time for each pollinator group

Pollinator group	Estimate ± SE	z value	n	χ^2	*P*
Hymenoptera					
Vespidae	**-0.42 ± 0.09**	**− 4.51**	**64**	**20.33**	**< 0.001**
Apidae					
Apis mellifera	−0.22 ± 0.32	− 0.69	107	0.47	0.493
Bombus	**0.289 ± 0.14**	**2.01**	**32**	**4.02**	**0.045**
Apidae small	−0.55 ± 0.37	− 1.49	27	2.24	0.135
Lepidoptera					
Lepidoptera-small	−0.71 ± 0.39	− 1.83	145	3.36	0.067
Lepidoptera-large	−0.75 ± 0.65	− 1.16	11	1.33	0.248
Diptera					
Syrphidae-small	**0.39 ± 0.14**	**2.71**	**49**	**7.33**	**0.007**
Syrphidae-large	0.06 ± 0.18	0.34	26	0.11	0.737
Other flies-small	−0.004 ± 0.17	− 0.02	90	0.002	0.980
Other flies-large	−0.02 ± 0.08	− 0.27	104	0.07	0.791

Significant results ($p < 0.05$) are indicated in bold; all but the test for *Bombus* remained significant after applying the sequential Bonferroni correction, considering the 30 tests performed, in Table 1–3). The sign of the estimate indicate the direction of the effect, with positive estimates indicating a decrease in flower visitation time caused by the predator for that group of pollinators

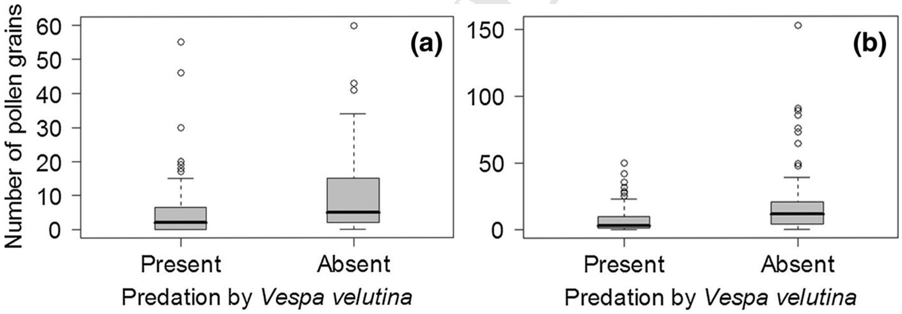

Fig. 2 Pollen deposited on stigmas of two flower morphs with presence and absence of *Vespa velutina* behaving as predator of pollinators. **a** Morph with short anthers (χ^2 = 6.57, df = 1, *P* = 0.01) and **b** morph with long anthers (χ^2 = 8.72, df = 1, *P* = 0.003)

Requier et al. 2019). This difference is probably a response to the quantity of potential prey present in both environments, meaning that hornets have the ability to regulate hunting effort (*i. e.* number of recruited hunters at a single place) and adapt its hunting techniques according to the environmental conditions and the spatial distribution of prey. The success of *V. velutina* hunting feral and managed pollinators is high, and more studies are needed to understand the impact on native insect populations at the long term.

Effects of predation by Vespa velutina on the behaviour of pollinators

The responses we detected in different pollinators can be a consequence of responses of individuals after experiencing hornet attacks or induced by the information transmitted by other individuals. We found negative responses to the presence of hornet hunters especially in social hymenopterans. Their complex social behaviour, which include learning, memorizing, deciphering chemical compounds and communication of resources' suitability, can contribute to improve their response against *V. velutina*, reducing predation risks in flowers. This can help them to cope, in some manner, with the stress that this new predator exerts on native pollinators in invaded areas (Leza et al. 2019).

European honeybees, which showed lower abundance in patches with the presence of hunting individuals of *V. velutina*, probably recognize *V. velutina* as an imminent danger of death, since they are under predation pressure in both flowers and hives. In addition, as other social bees, they are able to use olfactory cues to avoid flowers where other insects have been attacked (Abbott 2006; Llandres et al. 2013). Odours can be actively released, such as pheromones, or accidentally produced during the attack, such as body fluids (Llandres et al. 2013). Moreover, *A. mellifera* workers have complex communication systems. For instance, they communicate danger in flowers as part of the information provided about the quality of floral rewards during the waggle dance (Abbott and Dukas 2009). In addition, foraging honeybees experiencing attempted predation increase stop-signalling to inhibit waggling recruitment by other bees to the resource (Nieh 2010). All this information combined can help European honeybees to avoid dangerous flower patches, thus reducing mortality by hornet hunters. In the hives, European honeybees do not have efficient anti-predation strategies against *V. velutina* (Arca et al. 2014), in contrast with their Asian relatives (*A. ceranae*). Asian honeybees show both physiological and behavioural responses that contribute to diminish predation pressures by Asian hornets (Tan et al. 2013). For instance, the formation of ‘heat- balls’ that successfully kill *V. velutina*, the wing shimmering behaviour, the increase in flight speed when entering the hive or the increase in recruitment of bee guards (Tan et al. 2007, 2010).

Bumblebees were also observed being chased and hunted by *V. velutina*, despite their large body mass. They showed a reduction in the time spent in the flowers (although this effects became non-significant after the sequential Bonferroni correction), which can be related to the direct disturbance produced by the attacks and movements of hunters (Abbott 2006). This impact in flower visitation time was also observed in hoverflies, and has also been showed in other studies in response to the presence of ambush predators (Elliott and Elliott 1994; Suttle 2003; Muñoz and Arroyo 2004). On the contrary, native vespids increased flower visitation time in patches with the hunter. The observed increase of flower visitation time may be due to nectar stores in flowers being less depleted because of diminished visitation by other pollinators negatively affected by *V. velutina* presence. Native vespids seem to be less threatened by *V. velutina*, despite hunting attacks to them being also observed. Studies evaluating the possible interactions between the native hornet *Vespa crabro* and the invasive *V. velutina* suggest that trophic preferences are broadly similar (Cini et al. 2018) and temporal patterns of activity of these species partially overlap (Monceau et al. 2015), although some degree of niche differentiation was observed, which may limit the impact of competition (Monceau et al. 2015). It would be important to have a better understanding about the nature (namely competition, predation, facilitation, among others) and intensity of interactions between invasive and native vespids in order to have a more complete idea of the effects of this invasive species.

Detrimental effects on pollination services caused by Vespa velutina

Theoretical and empirical studies have revealed that predation of pollinators has a strong influence on abundance, density and behaviour of prey that, in turn, impacts the interactions and processes in which these organisms are involved (Barbosa and Castellanos 2005).

During this work, we could find patches in the same region with and without *V. velutina* hunters, allowing us to avoid important confounding effects, such as local differences in diversity of pollinators, which is a key and challenging aspect in this kind of studies. Thus, the differences in pollination levels observed here were mainly caused by the effect of current predation and the presence of the predator that affected pollinators’ abundances and behaviour.

Our results show that hunting by the invasive hornet *V. velutina* reduces patch and flower visitation rates and flower visit duration of native pollinators, with subsequent detrimental effects on *M. suaveolens* pollination. In addition, taking into account the wide hunting range of *V. velutina* and the important quantity of insects predated by colonies (Villemant et al. 2011; Poidatz et al. 2018), it is expected that pollinators populations are directly affected by hornet predation at different temporal and spatial scales, causing population decreases. Since the maximum population abundance of *V. velutina* occurs at late summer and early

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autumn (Monceau et al. 2014), detrimental effects for late blooming species, such as *M. suaveolens*, are likely to be higher than for early blooming plants.

In this study, we show a strong top-down negative impact of the presence of the invasive hunter *V. velutina* on the pollination of a common native plant. Predation by *V. velutina* affects pollination by direct consumption of flower visiting insects but also through changes in the foraging behaviour of pollinators. These effects translated in differences between patches with and without *V. velutina*, with a decrease in the patch visitation rate (as we observed for *A. mellifera*), in the number of flowers visited per bout (as occurred for small bees), or in the time spent to visit flowers (as recorded for bumblebees and small syrphids). We considered only short term effects of predation, but long term effects should also be evaluated in future studies to determine the incidence of the diverse impacts on species and communities, and the ultimate consequences on native ecosystems.

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