Not just honeybees: predatory habits of Vespa velutina (Hymenoptera: Vespidae) in France

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19 **Not just honeybees: predatory habits of *Vespa velutina* (Hymenoptera:**

20 **Vespidae) in France**

21   **Abstract.** Understanding the impact of a predatory invasive alien species requires data on

22   its diet. *Vespa velutina* Lepeletier, 1836, is a notorious bee-hawking hornet accidentally

23   introduced in France before 2004 which spread across the European continent. Despite

24   numerous studies and the impact on beekeeping activities, there are very few data on the

25   diet of this species in its invaded range in Europe. To fill this knowledge gap, we studied

26   sixteen nests in Southwest of France between 2008 and 2010. Using a combination of

27   morphological and barcoding approaches, we identified 2151 prey pellets showing that *V.*

28   *velutina* acts as a generalist predator, preying on honeybees (38.1 %), flies (29.9 %) and

29   social wasps (19.7 %), as well as a wide spectrum of animal organisms (no less than 159

30   species identified). The prey spectrum is influenced by the nest surroundings, urban

31   colonies preying more on honeybees and forest ones preying more on social wasps. The

32   predation intensity reaches its peak in early October. By comparing the dry weight of prey

33   pellets to that of *V. velutina* larvae and considering the colony dynamics, we estimated that

34   a single hornet nest can consume on average 11.32 kg of insect biomass in one season.

35   Overall, our results suggest that *V. velutina* is a generalist opportunistic predator targeting

36   mostly locally abundant prey. While the species may have an impact on honeybees, its

37   generalist, opportunistic behaviour on abundant insects suggests a minor impact on wild

38   species. Instead, attempts to manage this species using non-selective traps have a much

39   greater impact on wild and domesticated entomofauna than the hornet itself.

40   **Résumé. Le spectre de proies du frelon asiatique (*Vespa velutina*) en France ne se**

41   **limite pas aux abeilles.**

42   Pour comprendre l'impact d'une espèce prédatrice exotique et envahissante, il faut disposer

43   de données sur son régime alimentaire. *Vespa velutina* Lepeletier, 1836 est un prédateur

44   bien connu des abeilles domestiques, accidentellement introduit en France avant 2004 et qui

45   colonise, depuis, le continent européen. Malgré de nombreuses études et son impact

46   reconnu sur les activités apicoles, il existe très peu de données sur son régime alimentaire

47   dans les régions envahies d'Europe. Pour combler cette lacune, nous avons suivi seize nids

48   dans le sud-ouest de la France entre 2008 et 2010. En combinant des approches

49   morphologiques et moléculaires (barcode), nous avons identifié 2151 boulettes de proies et

50   démontré que *V. velutina* se comporte comme un prédateur généraliste, chassant des

51 abeilles domestiques (38.1 %), des mouches (29.9 %) et des guêpes sociales (19.7 %), ainsi

52 qu'un large spectre d'autres animaux (pas moins de 159 espèces identifiées). Le spectre de

53 proies varie selon l'environnement du nid ; les colonies urbaines chassant plus d'abeilles

54 domestiques et les forestières plus de guêpes sociales. L'intensité de la prédation atteint son

55 maximum début octobre. En comparant le poids sec des boulettes de proies avec celui des

56 larves de *V. velutina* et en tenant compte de la dynamique de la colonie, nous avons estimé

57 qu'une seule colonie de frelon pouvait consommer en moyenne 11,32 kg de biomasse

58 d'insectes en une saison. Dans l'ensemble, nos résultats suggèrent que *V. velutina* est un

59 prédateur opportuniste, ciblant surtout les proies localement abondantes. Bien que cette

60 espèce puisse avoir un impact sur les abeilles domestiques, son comportement généraliste et

61 opportuniste sur les insectes abondants suggère un impact limité sur les espèces sauvages.

62 Alors que, par ailleurs, les tentatives de gestion de cette espèce à l'aide de pièges non

63 sélectifs ont un impact beaucoup plus important sur l'entomofaune sauvage et domestiquée

64 que le frelon lui-même.

65 Keywords: yellow-legged hornet; invasive alien species; predation; honeybees; diet

66 **Introduction**

67 Predation underlies the most spectacular damages induced by invasive alien species in invaded

68 ecosystems, sometimes cascading down to primary producers (Bruno et al. 2005; David et al.

69 2017; Graham et al. 2018). Intensification of human transport and commerce around the world

70 has led to widespread movement of species outside of their native range (Hulme 2009; Frost et al.

71 2019), including many arthropod generalist predators that feed not only upon herbivores but also

72 upon other predators and detritivores. Due to their complex trophic role, these invaders can have

73 particularly widespread impacts on the communities they invade (Snyder & Evans 2006).

74 Documenting dietary spectrum of invasive predators is not only necessary to assess their direct

75 impact on prey but also to better define their niche width and understand how they might alter

76 ecosystem services such as biological control or pollination.

77   The recent introduction of the Yellow-legged Asian hornet *Vespa velutina* Lepeletier,

78   1836, in France was the first successful invasion of an exotic Vespidae in Europe (Rasplus et al.

79   2010; Beggs et al. 2011). This species is of great concern among public authorities and

80   beekeepers because of its rapid multiplication and high impact on beekeeping due to its strong

81   predation on honeybees (Perrard et al. 2009) and its hawking behaviour that disrupts bee colony

82   foraging (Rortais et al. 2010; Monceau et al. 2013; Arca et al. 2014; Requier, Rome, Chiron, et

83   al. 2019). The species was observed for the first time in 2004 in Southwest France and then it

84   rapidly spread across most of French districts. Between 2010 and 2017, it successively

85   established in seven neighbouring countries: Spain, Portugal, Italy, Germany, Belgium, UK and

86   the Netherlands, and adults have also been observed in Switzerland (Rome & Villemant 2015;

87   Burri-Schmassmann et al. 2017; Barbet-Massin et al. 2018). Climatic niche modelling suggests

88   that *Vespa velutina* could spread throughout Europe (Villemant et al. 2011; Fournier et al. 2017).

89   Taking into account recent climate change scenarios and the observed enlargement of its climatic

90   niche, future range expansion may even be more rapid than expected (Barbet-Massin et al. 2013;

91   Barbet-Massin et al. 2018).

92   As for other *Vespa* species, *V. velutina* is a generalist predator that attacks a wide range of

93   insects and spiders (Van der Vecht 1957). *Vespa velutina* generally catches its prey in flight and

94   immediately hangs on a support to process it, most often by removing all parts except the thorax

95   which contains the nutritious flight muscles. This flesh pellet is then brought back to the nest and

96   chewed to feed larvae with proteins. Adults only consume sugar-rich liquids and an energetic

97   protein-rich liquid regurgitated by the larvae (Matsuura & Yamane 1990). During its

98   development, the hornet larva does not produce faeces. The gut content is only eliminated during

99   the prepupal stage when the larva wove a cocoon with an operculum, which closes its cell. The

4

100   mass of released faeces is called meconium and will remain at the bottom of the cell after the

101   adult emerges (Rome et al. 2015).

102   While *V. velutina* arrived in Europe more than a decade ago, our knowledge of its diet

103   spectrum still relies on very limited data: anecdotal reports from its original range (Williams

104   1988; Abrol 1994) and only preliminary data in France (Perrard et al. 2009; Quentin Rome et al.

105   2011; Villemant et al. 2014). These studies suggest that social hymenopterans and brachyceran

106   flies are its main prey, although its scavenging behaviour on dead vertebrates and shrimps, in the

107   field or in street markets have been reported (Williams 1988). Studies quantifying its diet in the

108   invaded range are required to estimate the potential impact of this species on the local fauna. In

109   addition to its prey spectrum, the pressure of a colony of *V. velutina* on European honeybees is

110   also under-studied, considering its reputation as a bee-hawking predator in Asia and in Europe

111   (Abrol 1994; Monceau et al. 2013). Beehive mortality have helped to estimate such an impact

112   (Requier, Rome, Chiron, et al. 2019), but no real quantification is available from the literature

113   (Villemant et al. 2014).

114   In this paper, we assessed the diet of *V. velutina* in different environments in

115   Southwestern France to estimate its predation pressure on the local entomofauna. We first

116   estimated the diversity of prey predated by the hornet using a diversity index taking sampling

117   biases into account. Second, we explored how the landscape around the nests may have influence

118   the prey choices using a corresponding analysis. We then analyzed the variation in predation

119   activities across the season and during the day with field observation data. Finally, we used our

120   data to estimate the consumption of an average-size nest in one season.

Material and methods

121   **Material and methods**

122   *Collecting sites*

123   The study was performed over 3 years (2008-2010) from August to October/November, in the

124   Dordogne district, Southwest of France. These years, the colonized area was restricted to this

125   region. This district is close to the point of introduction and had relatively high and stable nest

126   densities. Sixteen colonies were studied for 1 day to up to 4 months depending on the duration

127   the landowners accepted to keep a living nest on their land (Fig. 1, Table S1).

128   *Prey collection*

129   Once a nest was located, depending on the opportunity to access the nest and on its destruction

130   date, we sampled workers to rob their prey almost every two weeks, either until the nest

131   destruction or until the end of the season. Sampling sessions took place from 1 h after dawn to

132   1 h before dusk and lasted 90 min. Any two sessions were separated by at least a 30 min break to

133   reduce the stress of the colony. Due to weather conditions or a too strong disturbance of the

134   colony resulting in hornets stopping to forage, 14 sessions out of 138 did not last 90 minutes. As

135   a whole, we performed 138 sessions corresponding to 199 h 23 min of sampling (Table S1).

136   For each session, we tried to catch with a sweeping net a maximum of hornet workers

137   returning to their nest. The rate of failure to catch a hornet was estimated low and relatively

138   constant among sessions. When a worker carried a pellet in its mandibles, it was forced to

139   abandon it in the net before being released. Prey pellets were preserved in individual tubes

140   containing 95 % ethanol. Returning workers also carried wood pellets as material to build the

141   nest. Those pellets were preserved dry.

142 ***Prey identification***

143 A first morphological identification of the prey pellets was made using a stereomicroscope

144 (Nikon SMZ 1000) and with the help of the insect collections from the Museum national

145 d'Histoire naturelle. Since many prey pellets were too strongly chewed by the hornets to be

146 reliably identified by their morphology alone, a molecular identification was also performed

147 when possible.

148 In the latter case, total genomic DNA was extracted from 50 mg of each pellet, using the

149 Macherey-Nagel NucleoSpin 96 Tissue Kit and following the manufacturer's protocol. The

150 mitochondrial cytochrome oxidase I (COI) gene was selected for PCR amplification and barcode

151 identification (Hebert et al. 2003).

152 The partial COI gene was amplified using the primers LepF1 and LepR1 (Hebert et al.

153 2004). Each PCRs contained 2 μl of 10 X PCR buffer, 13,94 μL of distilled water (DNAse free),

154 2.5 mM MgCl2, 5 % DMSO, 0.26 mM dNTPs, 0.3 μM of each primer, 1.5 units of Qiagen Taq

155 polymerase and 1 μl of DNA template, conducting to a final reaction volume of 20 μl. The PCR

156 thermal regime consisted of: one cycle of 1 min initial denaturation at 94 °C, 35 cycles of 30 s at

157 94 °C, 30 s hybridization at 47 °C, followed by an extension of 50 s at 72 °C and a final cycle of

158 5 min at 72 °C. PCR products were electrophoresed in 1 % agarose gel stained with ethidium

159 bromide and visualized under UV light. The positive PCRs were sequenced in both directions

160 using the Sanger method and the sequences were assembled with CodonCode Aligner

161 (CodonCode Corporation).

162 The molecular identification was performed comparing the COI sequences obtained from

163 the prey pellets with those available in Genbank and BOLD, using the BLAST and the

164 Identification Engine tools, respectively. In addition, we created our own barcode reference

165   library of local flies, since a great number of flies were identified among the pellets based on

166   morphology. We barcoded 104 flies identified at the species level by specialists: 78 hoverflies

167   (Syrphidae, 41 species) and 26 carrion and flesh flies (Muscidae, Calliphoridae, Sarcophagidae,

168   26 species). The extraction was carried out with the same protocol used for the pellets. For the

169   amplification we used either the primers described above or we amplified the COI gene in two

170   fragments, using the primer combinations Lep-F1/COI-intR1 and COI-intF7/Lep-R1 (Hebert et

171   al. 2004; Zuccon et al. 2012), with COI-intF7: 5'-GAAAGAGGAGTTGGAACAGGTTGAAC-

172   3'. The new fly sequences have been submitted to GenBank under the accession numbers

173   MW077745-MW077848(Table S2).

174   ***Analyses***

175   All analyses were performed using R (R Core Team 2018) except for the land-use estimation

176   around the nests for which we used QGIS (QGIS Development Team 2016).

177   *Prey spectrum*

178   The total number of prey species collected by *Vespa velutina* in the study area was estimated with

179   the ACE index using the 'vegan' R package (Oksanen et al. 2019).

180   *Landscape influence*

181   To investigate the influence of the landscape surrounding the nests on the diet of *V. velutina*, we

182   performed a correspondence analysis (CoA) of prey per land-use types. We computed the

183   proportion (%) of four main land-uses within buffers of 2 km radius centred on the nests (Fig. 1).

184   The choice of buffer size reflects the foraging range of workers reported from experimental and

185   field studies (Budge et al. 2017; Sauvard et al. 2018; Kennedy et al. 2018). The land-use

186 categories were extracted from the level one of the Corine Land Cover (CLC) categories:

187 artificial surfaces (CLC1); agricultural areas (CLC2); forest and semi natural areas (CLC3) and

188 water bodies (CLC 5) (Union Européenne - SOeS 2011).

189   Prey was grouped based on the abundance of the different taxa. Taxa with less than 10

190 individuals were grouped and analysed as a composite group, resulting in 17 prey groups (Table

191 1). Since prey from a nest could have been captured from areas of different CLC categories, prey

192 groups were attributed to the four CLC categories using a fuzzy coding of individual prey. Each

193 prey was not attributed to a single CLC category, but to each CLC category using a percentage

194 relative to the CLC category proportions in the nest surrounding. Prey group attribution was

195 computed using the sum of these CLC percentages across the different samples.

196 *Seasonal dynamics of predation*

197 In order to test for changes in the level of predation and in the content of the *Vespa velutina* diet

198 throughout the season, we analysed the total number of prey, as well as the percentages of either

199 honey bees, Vespidae or Diptera species, brought back to the nest per 90 minutes sessions using

200 generalized linear mixed models. We only included in the analysis data from nests sampled for at

201 least 5 sessions. Since the landscape diversity around the 8 remaining nests was very limited,

202 with only one nest in urban area and no nest in wet area, the land type was estimated using the

203 proportion of forest and semi-natural areas in a radius of 2 km around the nest.. Explanatory

204 variables were date and hour as well as their quadratic terms, land type as fixed effects, and nest

205 identity as random effect. Nine collecting sessions lasted less than 90 minutes, so the session

206 duration was also taken into account to model the number of prey and their overall diversity. All

207 variables were scaled beforehand. Poisson and binomial error distributions were used for the total

208 number of prey and the percentages of prey groups, respectively. Model simplification was

209   performed following the Akaike Information Criterion (AIC). Models were performed using the

210   'glmer' function of the package 'lme4' (Bates et al. 2015) and the effects tested using the

211   'Anova' function of the R package 'car' (Fox & Weisberg 2019).

212   *Prey consumption of a colony*

213   To estimate the total consumption, C_{tot}, of prey necessary for the development of a medium size

214   *V. velutina* colony, we use the following formula:

$$C_{tot} = \sum_{i=8}^{11} \frac{Wi}{Wp} \times Ni$$

215   In which i stands for month, W_i for the mean dry weigh of pupa+meconium+cocoon in each

216   month, W_p for the mean dry weight of a thorax prey and N_i for the mean number of hornets

217   produced each month by a colony.

218   To estimate the mean monthly pupa weight W_i, we sampled pupae from three nests, not

219   used for the observations, collected in July, September and October to account for increase in

220   mean hornet, and thus pupa, size along the life cycle of a colony (Rome et al. 2015). The pupa

221   weight of August was considered equivalent to that of September, and that of November

222   equivalent to that of October. Then the mean weight of a meconium and a cocoon was added to

223   the monthly pupa weight. Since there is no technique available today to properly breed a hornet

224   larva, we could not consider the energetic cost due to larvo-pupal respiration and the protein-rich

225   liquid regurgitated to adults. Note that, as previously said, meconium represents all the faeces

226   produced during the larva's life. Cocoon weight was also included because it is secreted by

227   larvae's silk-producing glands.

228   Having noted that all prey pellets brought back by *V. velutina* workers had

229   approximatively the same size, we assumed that they also have approximatively the same weight.

230   We estimated the mean dry weight W_p of a pellet by weighting together 30 thoraces of honeybees

231   dried in an oven at 57°C for 72 hours.

232   The number N_i of hornets produced per months follows Rome et al. (2015). On average, it

233   reached 630.5, 508.6, 739.9, 3441.3 and 831.2 hornets respectively from July to November.

234   Finally, to link these results to a potential impact of a hornet colony on beekeeping

235   activities, we compared the average number of bees potentially consumed to the average number

236   of bees produced in a beehive during the same period. If we consider a 30-days life-span of an

237   adult bee (Neukirch 1982), the number of bees produced by a hive during the foraging period of a

238   *V. velutina* colony would be the sum of its adult population in June, July, August, September and

239   October. Knowing that adults emerging in July partly come from larvae fed in June while those

240   emerging in November come from larvae fed in October. Based on data in the literature

241   (summarized in Becher et al. 2014), we can estimate a population of 10, 20, 30, 25 and 25

242   thousands of adult bees in June, July, August, September and October respectively for an average

243   hive in temperate region. Which amounts to 110,000 bees produced during the entire period.

244   **Results**

245   *Prey spectrum*

246   As a whole, from the 12,200 hornets captured, 2151 prey pellets and 1925 wood pellets have

247   been collected.

248   We identified 2151 prey pellets at least at the order level using morphological characters.

249   Among these, 2063 were selected for the molecular analysis and 1397 (67.7 %) COI sequences

250   were recovered. By comparison to Genbank, BOLD and/or our barcode library, it has been

251   possible to identify 1388 (99.2 %) prey to the species level, while the 9 other prey were identified

252   at higher level.

253   Morphological identification was confirmed by barcoding at 95 % for orders, 67.3 % for

254   families, 61.7 % for genera and 43 % for species, knowing that the number of specimens

255   morphologically unidentified greatly increased from order to species level. Barcode also showed

256   that 89.7 % of the prey pellets morphologically identified as *Apis mellifera* were correctly

257   recognized.

258   In our sample, prey collected by *V. velutina* include at least 141 species identified through

259   DNA barcode as well as 18 putative species identified at family or order levels (Table 1; S2).

260   This prey spectrum includes 11 orders and 43 families of insects, 3 families of spiders and 4

261   families of vertebrates. While our sample gathered 159 species, the ACE index suggested that

262   about 411.25 (se=13.51) different species were predated by *Vespa velutina* in the study area.

263   By number, the prey pellets are mainly composed of Hymenoptera (60.1 %), among

264   which *Apis mellifera* (38.1 %) and social wasps (19.7 %) dominate, and Diptera (29.9 %), with

265   Calliphoridae, Muscidae and Syrphidae each representing at least 5 % of total prey. Moreover,

266   dipteran prey pellets (102 spp) appear much more diverse than hymenopteran ones (14 spp).

267   Other prey is represented by 3.1 % of vertebrates and 9.2 % of a wide spectrum of other

268   arthropods, each occurring at very low frequencies.

269   ***Landscape influence***

270   The foraging area of the 16 studied colonies globally comprised 48.35 % of forest and semi-

271   natural areas (CLC 3), 41.24 % of agricultural areas (CLC 2), 9.90 % of artificial surfaces

272   (CLC 1), and only 0.51 % of water bodies (CLC 5).

273   *Vespa velutina* prey were collected mainly in field and forest areas (Table S3). The main

274   axis of the CoA distinguished the prey spectrum of colonies found in fields and forests from

275   those found in urban and wet areas (Fig. 2). The latter, much less sampled, comprised relatively

276   more *Apis mellifera*, Mecoptera, Tachinidae flies and other (non-Vespidae) Hymenoptera than

277   the colonies from forest and field areas. The second axis of the CoA illustrates the less

278   pronounced diet difference between colonies from forest and field areas.

279   ***Temporal dynamics***

280   The variation in number of prey caught along the season was best modelled by taking quadratic

281   effects of dates and hours into account, but not the land types (Table S4; Fig. 3A). All remaining

282   effects were significant. This model suggests a peak of predation activity around the 4th of

283   October. Predation is also at its highest around mid-day. The diversity of captured species

284   followed a similar trend (Fig. S1).

285   Among the sampled prey, the proportion of *Apis mellifera* significantly decreased during

286   the season (Table S5, Fig. 3B). The best model included date and hour effects, both linear and

287   quadratic, and excluded the land type. With a similar model, the proportion of Vespid wasps

288   increased in early season before reducing in late season (Table S6, Fig. 3C). Diptera proportion in

289   the diet of *Vespa velutina* was best modelled by using only the date as fixed effect. The model

290   showed a significantly higher proportion of Diptera early (July) and especially late (November)

291   in the flight season of the hornet than during its peak of activity (Table S7, Fig. 3D). Diptera

292   seemed to make for most of the hornet diet from November onward, at which point the prey

293   diversity strongly decreases.

294   *Prey consumption of a colony*

295   The mean fresh prey pellet and mean dry prey pellet weights were 33.3 mg and 11.7 mg

296   respectively (N=30). Dry pupa weighed on average 159.5 mg (N=79, sd=25.1) in July, 174.6 mg

297   (N=55, sd=20.5) in September and 192.4 mg (N=66, sd=41.1) in October. So, the lowest estimate

298   of the mean consumption of one larva is 13.6 prey in July, 14.9 prey in September and 16.4 prey

299   in October. Combining these data with the mean number of individuals produced by a colony

300   over a season (Rome et al. 2015), we could estimate that a colony need on average 97,246.45

301   honeybee-like prey along its life cycle, which corresponds to a mean of 3.24 kg of prey's

302   thoraces. Assuming that each prey weights as much as a honeybee, and that one honeybee

303   weights 116.37 mg (N=165 SE=0.61 mg); (Bowen-Walker & Gunn 2001), an average colony

304   would consume on average 11.32 kg of insects.

305   **Discussion**

306   *Prey spectrum*

307   Before its introduction in Europe, *Vespa velutina* was perceived as a predator focusing its attacks

308   for honeybees and bumble bees (Williams 1988; Shah & Shah 1991; Abrol 1994). Its notorious

309   hovering behaviour in front of beehives in France emphasized its reputation of bee killer (*e.g.*

310   Monceau et al. 2014), although previous studies suggested that the species is preying on a wider

311   diversity of insects (Van der Vecht 1957; Perrard et al. 2009). With 159 prey species found in our

312   sample and with an estimated 411 species predated by the studied colonies, our results confirm

313   that this species is a generalist predator.

314   *Vespa velutina* seems nonetheless to favour social Hymenoptera: more than half of the

315   sampled prey is honeybees and social wasps other than hornets. There was also a non-negligible

14

316 amount of flies captured by the hornets. These abundances suggest that *Vespa velutina* would

317 prey following an opportunistic pattern, attacking species of the right size that are abundant and

318 with a high local density such as bees in front of a hive or flies around carrions or cattles (Perrard

319 et al. 2011).

320   For the majority of the 22 known hornet species, the predation behaviour seems to match

321 this description of opportunistic predators (Matsuura & Yamane 1990). Preference for

322 brachyceran flies of *V. velutina* seems to be shared with a closely related species: *Vespa simillima*

323 Smith, 1868. In the latter, flies make up to 60 % of its diet. This preference may be related to the

324 similar size of both *Vespa* species, which is on the lower side of size-range in hornets.

325   Nonetheless, the peculiar behaviour of *V. velutina* attacking honeybees in front of hives

326 and the high proportion of honeybees in its diet suggest some kind of specialization. Other hornet

327 species present some degree of diet specialization depending on the season or the locality: the

328 European hornet *Vespa crabro* L., 1758 feeds mostly on cicadas in Japan (Matsuura 1984) and

329 the great-banded hornet *Vespa ducalis* (L., 1758) attacks mostly smaller social wasps (Sakagami

330 & Fukushima 1957; Matsuura, Makoto 1991). Specialization towards exploiting honeybees is

331 well documented in another species: the giant hornet *Vespa mandarinia* Smith, 1852. This

332 species has a unique way to exploit colonies of social Hymenoptera, including honeybees, using

333 group predation. Workers attack the colonies as a group to annihilate the adults, then they collect

334 the brood and resources (Matsuura & Sakagami 1973; Matsuura & Yamane 1990). This tendency

335 of semi-specialization of hornets towards locally abundant prey could increase their foraging

336 efficiency. It would be a strong evolutionary advantage for social wasps since their colonies

337 require proteins in large quantities to feed the multitude of larvae in a growing nest. However,

338 only *V. ducalis* has been recorded as an obligatory specialist towards social wasp prey (Matsuura

15

339   1984). Other hornet species, including *V. velutina*, retained enough plasticity in their behaviour to

340   exploit a wide range of protein sources.

341   ***Landscape influence***

342   The influence of the environment on the prey spectrum suggested by our data reinforce the idea

343   of an opportunistic and generalist behaviour of *Vespa velutina*. While some prey such as *Apis*

344   *mellifera* is part of every colony's diet, we found prey specific to colonies located in forest and in

345   field areas. The diet was mainly characterized by its high proportion of social wasps and meat-

346   flies, while colonies in open areas such as fields and cities captured more flower visitors such as

347   bees and hoverflies, as well as spiders. These data further suggest that *V. velutina* preys mostly

348   on species that it can find in abundance in the surroundings of the nest. Our results also show that

349   there was no significant effect of the proportion of forest or semi-natural area in the nest

350   surroundings on the number of prey, their diversity or the proportion of honeybees, hoverflies or

351   social wasps captured. This result may in part be related to the limited number of nests that we

352   could study long enough to include in the analyses. Further analyses to test the difference in

353   predation between rural, urban and wet areas would be required, but getting authorizations to

354   keep a nest alive long enough is often difficult, especially in urban areas.

355   ***Temporal dynamics***

356   The predation dynamics suggest a peak of activity around late September and October (Fig. 3A).

357   Surprisingly, the proportion of honeybees in *V. velutina*'s diet seems to diminish along the late

358   season, partly due to an increase in dipteran prey. Such a shift in diet may be related to the impact

359   of *V. velutina*'s predation on beehives (Requier, Rome, Chiron, et al. 2019). This predation

360   reduces the activity of honeybee foragers, which may in turn reduce their attractiveness as a prey

361   source along the season. The number of available flies may also increase along the season

362   relative to the number of available honeybees. An opposite trend was observed in a diet survey of

363   *Vespula germanica* (Fabricius, 1793) from New Zealand (Harris 1996), in which dipteran prey

364   was reduced in winter while lepidopteran prey increased. This trend was related to seasonal

365   changes in prey abundance, which further emphasizes the impact of prey availability on the diet

366   (Edwards 1980).

367   ***Prey consumption of a colony and its impact on the entomofauna***

368   The opportunistic nature of *V. velutina*, which preys mostly on abundant species, suggests that

369   this species has a milder impact on the entomofauna than its predation on honeybees could

370   suggest. However, even a generalist invasive predator can have an impact on its environment,

371   depending on the level of predation pressure it exerts on the local entomofauna (Snyder & Evans

372   2006).

373   Social wasps, like other social insects, have an especially strong impact on their direct

374   surrounding (Beggs et al. 2011). Harris & Oliver (1993) estimated that a colony of *Vespula*

375   *germanica* can predate around 1.8 kg of prey per season in New-Zealand, which corresponds to

376   236,842 prey. In some special cases, wasp nests can become enormous and their colony consume

377   more than 200 kg of prey (Pickett et al. 2001). While the predation of *Vespa velutina* does not

378   reach such extreme values, it seems to have on average a higher impact than its smaller relatives

379   of the genus *Vespula*. By focusing on larger prey, a colony of *V. velutina* may require less prey,

380   but seems to consume a higher biomass of insects, with a mean of about 97,000 prey (11.31 kg)

381   per season of equivalent honeybees (Bowen-Walker & Gunn 2001). It should be noted again that

382   this impact is underestimated as metabolic losses due to larvo-pupal respiration and the protein-

383   rich liquid regurgitated to adults were not considered. Moreover, the largest colonies are about

384 twice as populous as the average and could therefore have twice the impact on the surrounding

385 insects (Rome et al. 2015).

386   When comparing these results to the number of bees produced by a beehive in the same

387 period, it appears that an average colony of *V. velutina* could consume about as many bees as the

388 ones produced by a single hive. Of course, this comparison only aims at scaling the impact of a

389 colony on the surrounding fauna: indeed one colony of *V. velutina* never consumes an entire hive

390 since its predation pressure is not focused on a single hive of an apiary (Monceau et al. 2014), nor

391 on the honeybees only as shown by our results. However, as honeybees represent in our results

392 39 % of its diet, an average hornet colony could prey on about 40 % of the individuals produced

393 by one hive, which is non-negligible. While the greatest impact of the hornets is due to their

394 presence hovering in front of beehives, which results in the disruption of the foraging activity

395 (called "foraging paralysis") of the bee colonies, the predation *per se* appears to mainly threaten

396 isolated or poorly populated and unhealthy beehives (Requier, Rome, Chiron, et al. 2019).

397 Foraging paralysis increases the risk for a bee colony to die after wintering but the hornet impact

398 can be significantly reduced by adding a simple protective wire mesh to the hive (Requier, Rome,

399 Villemant, et al. 2019).

400   On the other hand, while the majority of preyed insects are pollinators with variable

401 efficiency, wild bees (bumblebees and solitary bees, excluding wild honeybee colonies) represent

402 only 0.02 % of the *V. velutina*'s prey recorded in this study. The predation impact on these main

403 pollinators appears therefore very low. However, a long-term monitoring of pollination success in

404 presence or absence of *V. velutina* would be required before any conclusion on the actual impact

405 of the hornet on pollination services.

406   As we currently lack data about the state of insect populations other than honeybees

407 before the invasion, we cannot assess whether *V. velutina*'s predation may have had an impact on

408   them. However, the mainly opportunistic nature of *V. velutina*'s predation behaviour suggests

409   that the hornet has a limited impact on endangered entomofauna. If *V. velutina* catches non-

410   abundant species by chance, it could be expected that few specimens of rare and endangered

411   species are caught by the hornet, since they are locally scarce. Further studies are therefore

412   required to compare *V. velutina*'s preying behaviour to local prey abundances, in order to clarify

413   whether it preys randomly or could focus occasionally on some rare species and thus impact their

414   populations. Particular attention should be paid to insects nesting in aggregation during the fall,

415   like autumnal *Colletes* bees, which are sometimes actively predated by *V. velutina* (J. Raingeard,

416   com. pers.). A strong impact of the hornet could also be expected through competition with other

417   predators of similar arthropods (Snyder & Evans 2006; Choi et al. 2012; Cini et al. 2018; Ikegami

418   et al. 2020).

419   Since its introduction in Europe, the development of methods to control *V. velutina*

420   without scientific evaluation is thriving; they range from poison baiting to rifle shooting on nests

421   (Turchi & Derijard 2018). The most widely used method is sugar-beer trapping although less

422   than 1 % of the total catches are hornets and composed a wide diversity of other insects (Dauphin

423   & Thomas 2009; Demichelis et al. 2014; Rojas-Nossa et al. 2018). A 1 liter trap would catch

424   around 30,000 non targeted insects, and around 20,000 if they are placed after June and in the

425   vicinity of beehives (Q. Rome et al. 2011). Although biomass was not estimated, and most of the

426   caught insects were of small sizes (see the regularly updated list for French territories:

427   https://inpn.mnhn.fr/espece/jeudonnees/22213), four to six small traps would catch as many

428   insects as a *V. velutina* colony could prey. Control methods thus seem to be a greater threat to

429   insect biodiversity than *V. velutina* predation. The effectiveness of most of these methods has not

430   been demonstrated so far (Edwards 1980; Beggs et al. 2011; Monceau et al. 2012; Turchi &

431   Derijard 2018), so that their impact on biodiversity probably adds up to that of *V. velutina*. The

432 hornet is mostly present in degraded environments, while natural ones are mostly unsuitable

433 (Fournier et al. 2017), suggesting that its impact on rare species is probably low. Instead of using

434 trapping methods that negatively affect wild population of insects, it would be better to try to

435 control the invasive hornet, whose eradication is illusory, by using only scientifically validated

436 methods and developing protective or repulsing strategies to reduce its impact on beekeeping.

437 **Author contributions**

438 QR and AP contribute equally to the paper. FM, QR, CV and AP conceived the idea, designed

439 methodology and collected the prey pellets; CV and FM made the morphological identifications;

440 FM, AQ, DZ and QR made the molecular identifications; AP, CF, QR and CV analysed the data,

441 and AP, QR and CV wrote the first version of the manuscript; all authors contributed critically to

442 the drafts and gave final approval for publication.

452

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633

634 Table 1. Diversity of *Vespa velutina* prey. Family and species number represent the minimum

635 number of families or species for each order, whether they were identified by morphological or

636 molecular criteria. Data for Diptera, Hymenoptera and Other Insecta are the sums of the different

637 prey groups they encompass. The numbers in front of prey group names correspond to the

638 different prey groups considered in the correspondence analysis.

Prey group	Family Nb	Species Nb	Specimen Nb
1. Araneae	3	7	40
2. Coleoptera	4	3	10
Diptera	13	102	643
3. Calliphoridae	*1*	*22*	*147*
4. Muscidae	*1*	*25*	*125*
5. Sarcophagidae	*1*	*9*	*61*
6. Syrphidae	*1*	*18*	*108*
7. Tachinidae	*1*	*10*	*22*
8. Other Diptera	*8*	*18*	*180*
9. Hemiptera	5	5	31
Hymenoptera	8	14	1293
10. *Apis mellifera*	*1*	*1*	*820*
11. Vespidae	*1*	*4*	*428*
12. Other Hymenoptera	*7*	*9*	*45*
13. Lepidoptera	6	13	17
14. Mecoptera	1	3	14
15. Orthoptera	1	2	12
16. Vertebrata	4	4	67
17. Other Insecta	5	6	19
Dermaptera	*1*	*1*	*2*
Dictyoptera	*2*	*2*	*7*
Nevroptera	*1*	*1*	*2*
Trichoptera	*1*	*2*	*5*
Unidentified Insecta	*NA*	*NA*	*3*
Unidentified	NA	NA	5
Total	**50**	**159**	**2151**

639

640

Figure captions

642 Figure 1. A. Total number of prey pellets caught (size of red spot) for each 16 studied colonies

643 and CLC of the studied area. B. Detailed land use in foraging areas (blue circles) of 4 *V. velutina*

644 colonies near the city of Sarlat-la-Canéda (Dordogne). Corine Land Cover codes: CLC 1

645 Artificial surfaces, CLC 2 Agricultural areas, CLC 3 Forests and semi-natural areas, CLC 5

646 Water bodies.

647

648 Figure 2. Results of the Correspondence analysis on the prey groups relative to the land cover

649 types in which the prey was captured. Only the two first axes are represented. Percentages

650 indicate the amount of variation explained by each axis. Dot sizes illustrate the number of prey

651 sampled in each group.

652

653 Figure 3. Evolution of the prey captured along the year. The x-axis represents the ordinal date, in

654 days. A. Number of prey captured in a 90-minute session. The line models the evolution of these

655 captures according to a linear model including date and hour as covariates, as well as nest type as

656 random effect. The points illustrate the results of the 90-minutes collecting sessions, shorter

657 session results were not represented. B, C and D. Proportion of prey types along the year. The

658 line (red) illustrates the logistic regression of presence of a prey type among the prey, depending

659 on the date (B, C & D) and hour (B & D), with nest identity as random effect. Top bars (green)

660 indicate the number of prey of this type sampled per day. Bottom bars (blue) indicate the number

661 of other prey sampled per day. Prey types: B. *Apis mellifera*; C. Diptera; D. Vespidae (social

662 wasps).

663

664   **Supplementary materials**

665   Table S1. Sampling summary by month. Lines in bold represent respectively the total number of

666   nests sampled, of sampling days, of sessions and the total duration of sampling of each month.

667   Regular lines represent the median, minimum and maximum number of days, sessions or the

668   duration nests were sampled each month.

669

670   Table S2. List of prey collection reference. MNHN_CollectionID is the catalog number of the

671   voucher in the MNHN-Paris Collection

672   https://science.mnhn.fr/institution/mnhn/collection/ey/item/search/form?lang=en_US The

673   complete informations on the specimens is also available in GenBank under the accession

674   numbers MW077745-MW077848.

675

676   Table S3. List of prey pellets identification. CLC correspond to the percentage of each Corine

677   Land Cover code around the monitored nest. Number_Vespa_caught, PreyPellet and

678   WoodPellets correspond to the number of the total corresponding session of capture.

679   MNHN_CollectionID is the catalog number of the voucher in the MNHN-Paris Collection

680   https://science.mnhn.fr/institution/mnhn/collection/ey/item/search/form?lang=en_US The

681   complete and updated list is also available in the INPN website

682   https://inpn.mnhn.fr/espece/jeudonnees/40455

683

684   Table S4. Fixed effects of number of prey collected per 90 minutes according to a generalized

685   linear mixed model following a Poisson distribution, with the site as a random effect.

686

687   Table S5. Fixed effects of the proportion of *Apis mellifera* collected among the prey of *V.*

688   *velutina*, per 90 minutes, according to a generalized linear mixed model following a binomial

689   distribution, with the site as a random effect.

690

691   Table S6. Fixed effects of the proportion of vespid wasps collected among the prey of *V.*

692   *velutina*, per 90 minutes, according to a generalized linear mixed model following a binomial

693   distribution, with the site as a random effect.

694

695   Table S7. Fixed effects of number of prey collected per session. Fixed effects of the proportion of

696   Diptera collected among the prey of *V. velutina*, per 90 minutes, according to a generalized linear

697   mixed model following a binomial distribution, with the site as a random effect.

698

699   Figure S1. Evolution of the number of species captured per 90 minutes along the year. The x-axis

700   represents the ordinal date. Each dot represents the number of prey captured in a session this day.

701   The line models the evolution of these captures according to a linear model including date, hour

702   and area type as covariates, as well as nest location as random effect.

703