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RAPID COMMUNICATION

A biodiversity-friendly method to mitigate the invasive Asian hornet's impact on European honey bees

Fabrice Requier[1,2] · Quentin Rome[3,4] · Claire Villemant[4] · Mickaël Henry[2]

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Abstract

The Asian hornet is an invasive predator of honey bees in Western Europe. The Asian hornet-related risk of bee colony mortality has motivated the development of biological and physical control methods over the past years. Although the technical cost–benefit ratio has been established for most of these control methods, it is still unclear whether such methods can reduce the detrimental effects of the Asian hornet on European honey bees. In this study, we investigated the potential benefits of a biodiversity-friendly control method, the beehive muzzle. We observed the flight activity of bees and the predation behaviour of the Asian hornets at the beehive entrance of 22 pairs of honey bee colonies, each with one muzzle-equipped colony and one control colony without muzzle, in France. We measured HF (bee homing failure due to hornet predation of bees) and FP (foraging paralysis: the stop of flight activity in beehives due to hovering hornets), and estimated the mortality probability of the colonies using a mechanistic modelling approach. The beehive muzzle did not reduce the hornet-related HF, but drastically reduced FP. Moreover, the muzzle increased the survival probability of hornet-stressed colonies up to 51% in context of high abundance of Asian hornets based on theoretical simulations. These results suggest that installing beehive muzzles can mitigate the detrimental effect of the Asian hornet on European honey bees. This low-cost technique does not lead to any environmental impacts and could therefore be recommended to beekeepers as an effective biodiversity-friendly method of Asian hornet control.

Keywords *Apis mellifera* · Biological invasion · Control methods · Predator–prey interaction · Yellow-legged hornet

Key message

- The Asian hornet is an invasive threat for honey bees in Western Europe
- This predator affects bee foragers' homing failures, foraging paralysis, and colony survival
- We tested whether the beehive muzzle can mitigate the Asian hornet' impacts on honey bees
- Beehive muzzles are physical devices with biodiversity-friendly control method approach
- Beehive muzzles support the foraging activity and survival probability of hornet-stressed colonies

Introduction

In Western Europe, the invasion of *Vespa velutina nigrithorax* (called Asian hornet hereafter) greatly concerns beekeepers and governmental policies, as this predator of honey

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✉ Fabrice Requier
requierf@gmail.com

[1] Department of Animal Ecology and Tropical Biology, Biocenter, University of Würzburg, Am Hubland, 97074 Würzburg, Germany

[2] INRA, UR406 Abeilles et Environnement, 84914 Avignon, France

[3] UMS 2006 PatriNat – AFB, CNRS, MNHN, Muséum National d'Histoire Naturelle, CP 50, 45 rue Buffon, 75005 Paris, France

[4] Institut de Systématique, Evolution, Biodiversité (ISYEB), Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, CP 50, 57 rue Cuvier, 75005 Paris, France

bees (*Apis mellifera* L.) may represent an additional risk factor involved in the mortality of currently declining bee colonies (Leza et al. 2019; Requier et al. 2019). The Asian hornet was introduced in Southwest France in 2004 from China (Haxaire et al. 2006; Arca et al. 2015) and has rapidly spread across most of France (Robinet et al. 2017; Rome and Villemant 2018). Between 2010 and 2017, it also established successively in Spain, Portugal, Belgium, Italy, Germany, the UK, and the Netherlands (Grosso-Silva and Maia 2012; Witt 2015; Bertolino et al. 2016; Garigliany et al. 2017; Keeling et al. 2017; López et al. 2011; Rome and Villemant 2018). In these European regions, beekeepers already suffer high rates of honey bee colony mortality in winter (Neumann and Carreck 2010; Potts et al. 2010; van der Zee et al. 2012; Gray et al. 2019), likely related to a combination of multiple stressors including parasites, pesticides, and lack of flowers (Potts et al. 2010; 2016; Goulson et al. 2015; Steinhauer et al. 2018). The Asian hornet captures foraging bees at the entrance of the colony and affects the latter in two ways. (1) The predation of adult bees leads to homing failure (HF) of the bees (Monceau et al. 2013; Requier et al. 2019). Translated at colony level, this loss of bees by predation reduces the population size of the honey bee colonies (Requier et al. 2019). (2) The presence of Asian hornets hovering (for predation) in front of the beehives disturbs the flight activity of the bee colony (Monceau et al. 2018; Requier et al. 2019). In response to the presence of Asian hornets in front of beehives, bee colonies express a behavioural symptom of foraging paralysis (FP) consisting in the stop of the flight activity (Monceau et al. 2018; Requier et al. 2019). Given the predation activity of the Asian hornet occurs during the critical pre-wintering season for honey bee colonies in Western Europe, the hornet-related impacts decrease the winter survival probability of the colonies (Requier et al. 2019).

The Asian hornet-related risk of colony mortality has motivated the development of biological and physical control methods over the past years (Supporting Information ESM 1). The most widely applied technique consists in trapping hornets at the beehives location, using simple passive traps with homemade syrup or poisoned (with insecticide) baits (Turchi and Derijard 2018). However, this technique cannot sustainably reduce the populations of *V. velutina* (Beggs et al. 2011; Monceau et al. 2014; Turchi and Derijard 2018) and might not efficiently reduce the hornet-related impacts of HF and FP (Requier et al. 2019). Moreover, such traps have important environmental impacts due to their absence of species-specificity, remaining a threat to numerous species of the local entomofauna (Rome et al. 2011; Rojas-Nossa et al. 2018, Rodríguez-Flores et al. 2019). Thus, more species-specific trapping systems, based on sex pheromone attraction (Couto et al. 2014; Cheng et al. 2017; Gévar et al. 2017; Wen et al. 2017), are in the process of development, however not yet evaluated and available for stakeholders (Turchi and Derijard 2018). The location and destruction of the Asian hornet nests is also a well-established control technique applied in the field. Nests may be located by simple triangulation methods (Turchi and Derijard 2018) or more complex approaches using tracking devices such as harmonic radar and radio-telemetry (Milanesio et al. 2016; Kennedy et al. 2018) or drones (Reynaud and Guérin-Lassous 2016). While the technology-based methods are still under development and mostly dedicated to research purposes, traditional triangulation is very time-consuming for stakeholders and leads to rather inefficient results at large spatial scale (Turchi and Derijard 2018). Moreover, nest location per se is insufficient as a control method if not combined with nest destruction. The most established technique to destroy the nest is biocide injection, e.g. injecting permethrin (but also see nests gunshot destruction methods for a similar non-biodiversity-friendly method, Turchi and Derijard 2018). In France, authorities have implemented such action plans involving location and destruction of nests in several of the hornet-invaded departments, with very little success in terms of biological control and for an economic expense of 150 k€/year on average per department (Turchi and Derijard 2018). Thus, these field-applied techniques present well-known environmental, economic, and/or development time costs (ESM 1), and unfortunately, it is still unclear whether such methods can reduce the detrimental effects of the Asian hornet on European honey bees.

In this study, we tested whether the use of beehive muzzles can mitigate the Asian hornet's impacts on honey bees. A beehive muzzle is a low-cost (ESM 1) and non-lethal control method that does not aim to collect or poison any organism (Turchi and Derijard 2018). This thus called biodiversity-friendly control method consists in a mesh device placed around the entrance of the beehive that allows the in-and-out activity of the bees but keeps hornets away from this entrance (Turchi and Derijard 2018). This physical control method does not aim to prevent the hornet's predation action per se, but to reduce the stress provided by the presence of hornets hovering in the vicinity of the beehive entrance (the so-called FP syndrome that can lead to a complete stop of the foraging activity) and therefore to help bees maintain their foraging activity even in the presence of the predator. Indeed, the FP has been established as the main risk factor for colony mortality related to the Asian hornet (Requier et al. 2019). The overarching objective of this study was to assess the effectiveness of the beehive muzzle as a control method of the Asian hornet's impacts on honey bees. For that, we monitored the predator–prey interactions at 22 pairs of honey bee colonies, consisting in one muzzle-equipped colony and one control colony without muzzle, throughout the invaded French territory. We first measured the hornet-related HF and FP based on field observations of the flight activity of bees and the predation activity of the Asian hornets at the beehives' entrance. We then estimated the mortality probability of the colonies using the BEEHAVE model (Becher et al. 2014)—a mechanistic model predicting honey bee colony dynamics which has been evaluated and supported by the European Food Safety Authority (EFSA 2015). Finally, we statistically tested whether the hornet-related HF, FP, and colony survival probability differed between muzzle-equipped and control colonies.

Materials and methods

Study sites and experimental design

The experiment was performed in 22 sites under Asian hornet pressure in France between 2013 and 2016 (Fig. 1a, more details in Supporting Information ESM 2). All sites had confirmed the presence of Asian hornets from at least 4 years before the start of the experiment (Rome and Villemant 2018). The sites consisted in established apiaries of 2–10 honey bee colonies (*Apis mellifera*) which were managed by local beekeepers. In each site, two colonies that were set at least 5 m apart or at both ends of the apiary were selected, totalising 44 colonies. The two colonies were selected based on similar colony structure (population size and amount of honey reserve) and hive type (Dadant hives with same colour). Two weeks before the first observation (the time span for bee colony adaptation to the muzzle), one colony was equipped with a beehive muzzle (Fig. 1b) and the second one was used as a control colony (without muzzle). All the observations were done on the same equipped–control pairs of colonies on each site and over time, without any colony change or replacement. The beehive muzzle consisted of a metal wire mesh of 6 mm square shape fixed using a plywood backing around the flight board of the beehive; it allows for a protection distance of 25 cm in front of the hive as honey bees can crawl through the mesh but not the hornets (Fig. 1b; see ESM 3 for the detailed design of the physical control tool).

Field observations of the prey–predator interaction

Visual observations were performed at the beehive entrance of the equipped–control pairs of colonies to quantify the foraging paralysis (FP, we observed in the field the foraging activity—the number of returning bees—to then calculate FP) and the homing failure (HF) associated with the hornet predation behaviour. A total of 388 observations were performed from July to December (194 observations on the 22 muzzle-equipped colonies and 194 on the 22 control colonies; ESM 2). Each observation consisted in 17 min of visual observation from a distance of 3–5 m of the beehive entrances. The first 15 min were dedicated to record the maximum number of hornets hovering simultaneously in front of the beehive (being careful to not double count the same individual) and the number of successful predations, i.e. the catch of a honeybee forager by a hornet. The two last minutes were dedicated to evaluate the flight activity of the bee colonies by counting the number of returning bees (more details in ESM 2). These observation durations (i.e. 15 and 2 min) were preliminary determined as the best trade-off between achievable sampling effort (not too time-consuming) and tractable accuracy for quantifying the numbers of hornets and honey bees, both at low or high densities. In particular, we performed an a priori statistical power analysis

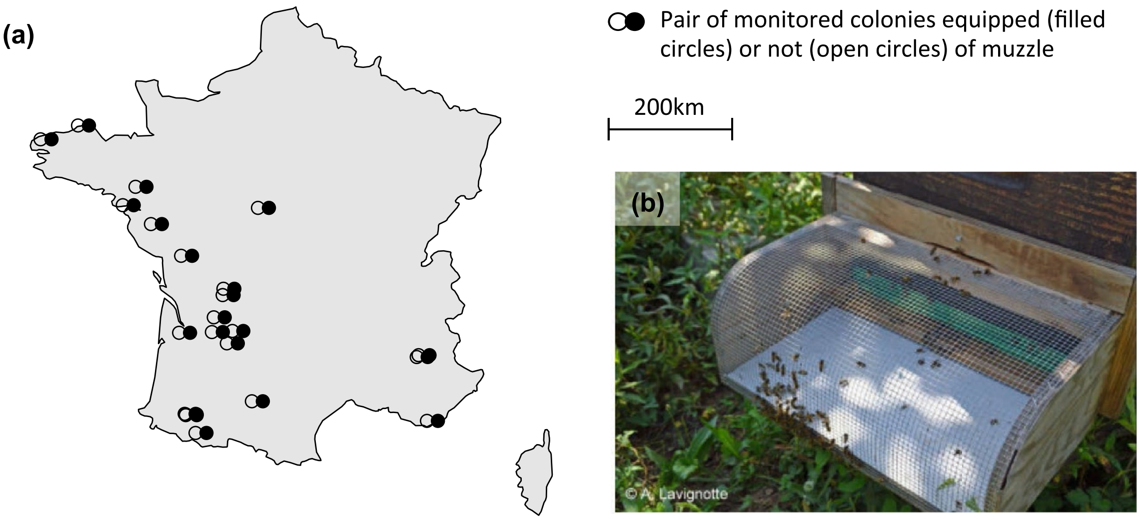

Fig. 1 Location of the study area in France with **a** 22 pairs of monitored honey bee colonies. **b** Beehive muzzle consists in a 6×6 mm mesh device placed at the entrance of the beehive that allows the in-and-out activity of the bees but block hornets out of this entrance

for assessing the ability of the study design to detect a potentially significant effect between treatments with a one-way analysis of variance (Sokal and Rohlf 1994). Given the observed coefficients of variation of honey bee (70.9%) and hornet (87.3%) visual counts, the power analysis revealed that the actual sample size (194 observations per treatment) would allow for the detection of effect sizes as small as 20% and 25% of the mean between treatments, respectively, with a satisfactory statistical power ($1-\beta=0.8$).

Those observations were performed randomly during the daily flight activity of bees and hornets (ranged from 9.30 am to 6.30 pm). We systematically performed the two observations (i.e. equipped colony and control colony) within the same hour. The FP was estimated as the relative flight activity of bee colonies compared to their maximum of flight activity (see statistical analysis). The HF was estimated for a 15-min slot as the ratio between number of bees caught (i.e. number of successful predations) and the total number of returning bees (previously multiplied by a factor of 7.5 for time match given that the flight activity of the bee colonies was observed on 2 min).

Modelling the muzzle effect on the hornet-stressed colony dynamics

We used the mechanistic BEEHAVE model (Becher et al. 2014) to assess the survival probability of honey bee colonies stressed by the Asian hornet predation. We performed 200 simulations to predict the daily colony growth of a bee colony population from the beginning of January to the end of May of the following year. This time period was chosen to include a complete winter season. The model was calibrated following Becher et al.'s (2014) initial colony settings, for which four key colony parameters were modified to increase stochasticity in the predictions and to improve representativeness of real field-condition variability (Requier et al. 2019). This stochasticity improvement includes random variations of the four key parameters: (1) the maximal egg-laying rate of the queen, (2) the initial adult population size, (3) the initial *Varroa destructor* infestation, and (4) the prevalence of virus-infected mites. We also enabled usual beekeeping management practices using the ad-hoc options, including *Varroa* treatments and honey harvests. To implement the Asian hornet predation impacts into the model, we followed Requier et al.'s (2019) procedure consisting in modifying the two parameters "forager mortality" and "maximal foraging distance allowed for the colony", from the day 240 (August 28) to the day 310 (November 6) (ESM 4). These parameters mimic the Asian hornet-related disturbances of HF and FP, respectively.

We distinguished two sets of 100 simulations to reproduce the variation of effect magnitude of the experiment (i.e. equipped or without beehive muzzle) given the empirical HF and FP assessments (see *results*). The two simulation sets differed in the covariate calibration values of the colony flight activity and the forager mortality probability. The first set of 100 simulations was parameterized as muzzle-equipped colony, for which we gradually decreased the maximal daily foraging distance allowed for the colony flight activity from the value of 2900 km per day (i.e. 84% of the default value of 3450 km per day) down to 2000 km (i.e. 76% of the default value), and we increased the forager mortality probability from the default value of 1.00e−05 to 1.35e−05 per second. The second set of 100 simulations was parameterized as control colony, for which we followed the same procedure decreasing the maximal daily foraging distance allowed for the colony flight activity from the default value down to the minimal theoretical value that does not lead to a complete stop of foraging (about 1.7 km), and we increased the forager mortality probability from the default value of 1.00e−05 to 1.35e−05 per second. Each of these simulations reproduced a different level of hornet predation pressure, ranging from null (no hornet predating at the beehive entrance) to high (20 hornets simultaneously predating at the beehive entrance).

Simulations were further summarized based on their predicted survival. Colony mortality was defined by the following two thresholds (Becher et al. 2014): (1) simulations that predict a population size smaller than 4000 adult bees during winter and (2) simulations that predict a total depletion of honey stock during winter. The survival probability was averaged per number of hornets simulated (i.e. from 0 to 20) and per set of "control" versus "muzzle" simulations (i.e. 100 simulations per set), totalising five replications per hornet level and simulated set. We then expressed the survival probability as the difference (Δ) between control and muzzle sets to show the potential effect of this control method on colony survival.

Statistical analysis

All statistical analyses were performed using the R Project for Statistical Computing version 3.3.3 (R Development Core Team 2018).

Muzzle effect on the hornet-related FP and HF

To evaluate the benefits of the muzzle, we first analysed whether this biodiversity-friendly control method affects the hornet-related FP. For that, we fitted a generalized linear mixed-effects model (GLMM) with Poisson error

Table 1 Summary of the GLMM models performed to test the effects of the Asian hornet and its interaction with beehive on bee foraging paralysis and homing failure

	Estimate	SE	*Z* value	*P* value
Foraging paralysis (model 1)				
Muzzle	− 0.177	0.021	− 8.310	< 0.001
Hornet	− 0.043	0.005	− 11.537	< 0.001
Muzzle × Hornet	0.047	0.006	7.964	< 0.001
Homing failure (model 2)				
Muzzle	− 0.115	0.148	0.778	0.436
Bee activity	− 0.147	0.009	− 15.930	< 0.001
Muzzle × Bee activity	0.001	0.013	0.108	0.914

structure (*glmer* function in the *lme4* R-package) to test the correlative links between the number of returning bees and (1) maximum number of hornets hovering in front of the beehive in the same time as a quantitative predictor, (2) the presence of muzzle as a categorical predictor (two levels: yes or no), and (3) interaction between the maximum number of hornets and the presence of muzzle (model 1 in Table 1). The date and site of observations were specified as random variables in a nested design (date within site) to account for the spatio-temporal non-independency of the repeated measurements. The FP was then calculated and expressed as the per cent flight activity of bees relative to its maximal value (the higher number of returning bees predicted by the model). We also evaluated the effectiveness of the muzzle at reducing hornet-related HF. To do so, we fitted a binomial GLMM with a logit-link function to test the correlative link between the proportion of HF and (1) the number of returning bees as a quantitative predictor, (2) the presence of muzzle as a categorical predictor, and (3) the interaction between the number of returning bees and the presence of muzzle (model 2 in Table 1). Likewise, date and site of observations were specified as random variables in a nested design (date within site) to account for the spatio-temporal non-independency of the repeated measurements. The model residuals were extracted and inspected against fitted values (residuals vs. fitted plot and normal Q–Q plot) to ensure the residual normality and the homoscedasticity assumptions were fulfilled.

Muzzle effect on the survival probability of hornet-stressed colonies

We analysed the potential benefits of this control method on the survival probability of hornet-stressed colonies. For that, we fitted a linear model (LM) to test the correlation between the control–muzzle difference (Δ) in survival probability and the number of simulated hornets. Model residuals were also extracted and inspected against fitted values to check the suitability of the statistics.

Results

Muzzle effect on the hornet-related FP and HF

The visual observations led to a range of 0–20 hornets hovering at beehive entrances independently of the presence of beehive muzzle that negatively impacted the flight activity of the bee colonies (Table 1). The presence of the muzzle reduced the flight activity of the bee colonies, but in the meanwhile interacted positively with the hornet loads (Table 1). Over this positive interaction, the muzzle mitigated the FP of the colony related to the presence of hornets hovering at beehive entrances (Fig. 2a). Thus, the foraging activity in the presence of hornets ranged from 84 to 76% of its baseline value (i.e. 16–24% of FP, respectively; Fig. 2a) in muzzle-equipped colonies, instead of ranging from 100 to 35% in control colonies (i.e. 0–65% of FP, respectively; with prediction 95% CI reaching up to 76% of FP). The muzzle led to the reduction up to 41% in FP. The hornet-related HF was significantly dependent on the flight activity of the bee colony but not on the muzzles nor on the interaction between both factors (Table 1). Thus, the HF is maximal under the condition of very low flight activity of the bee colony, and quickly decreases with the increase in the flight activity of the bee colony, independently of the presence of beehive muzzle (Fig. 2b).

Muzzle effect on the survival probability of hornet-stressed colonies

A prior validation step excluded three simulations that collapsed before the implementation date of the hornet impacts. Among the 197 remaining simulations, no collapse occurred during the impacting period of the hornet (i.e. from August 28 to November 6, ESM 4). Instead, collapse events only occurred during and after the subsequent winter, from January 13 to May 1. The survival rate of the muzzle-simulated colonies was higher (55%) than the survival rate of control-simulated colonies (35%). Interestingly, the survival probability of hornet-stressed colonies significantly increased in muzzle-equipped colonies compared to control colonies ($n = 197$, $F = 14.38$, $R^2 = 0.43$, $P = 0.001$). Although the survival probability of hornet-stressed colonies equipped with muzzles was marginally lower than control colonies in a context of low amounts of hornets simulated (i.e. less than five hornets hovering at the beehive entrance), theoretical simulations

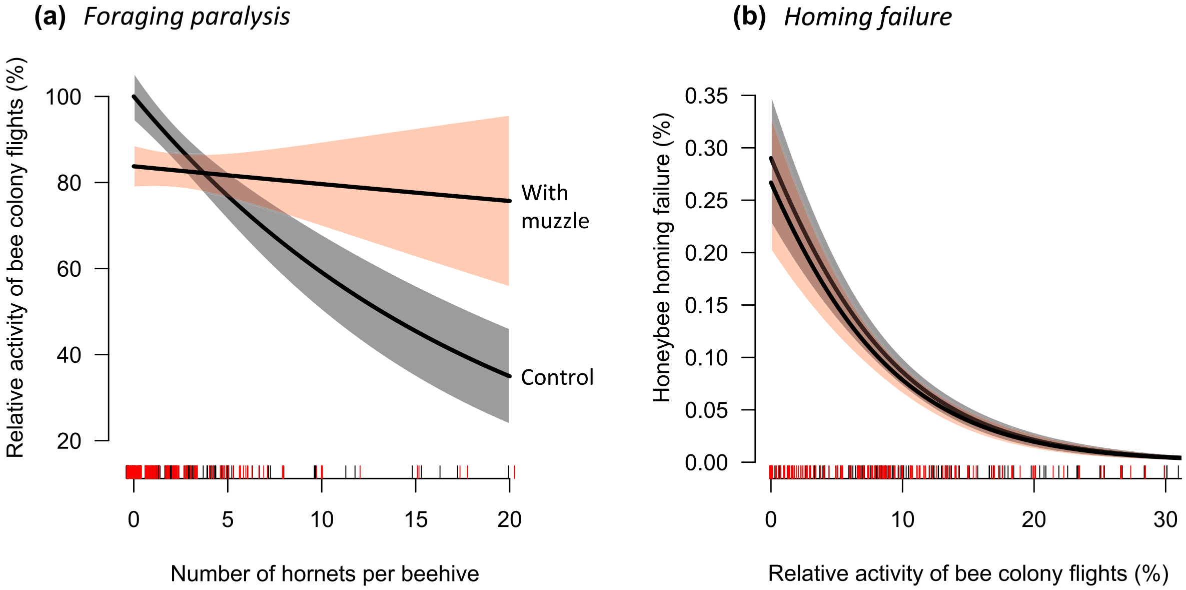

Fig. 2 Effect of the beehive muzzle on the hornet-related impacts of bee foraging paralysis (FP) and homing failure (HF). **a** The number of Asian hornets hovering in front of beehives triggers FP, with a sharp decrease in honey bee flight activity in control colonies mitigated by the presence of beehive muzzle. **b** As flight activity decreases, hornets increase their bee capture success, increasing the risk of HF due to hornet predation, independently of the presence of beehive muzzle. Thick lines show the model predictions with shaded areas indicating the 95% CI

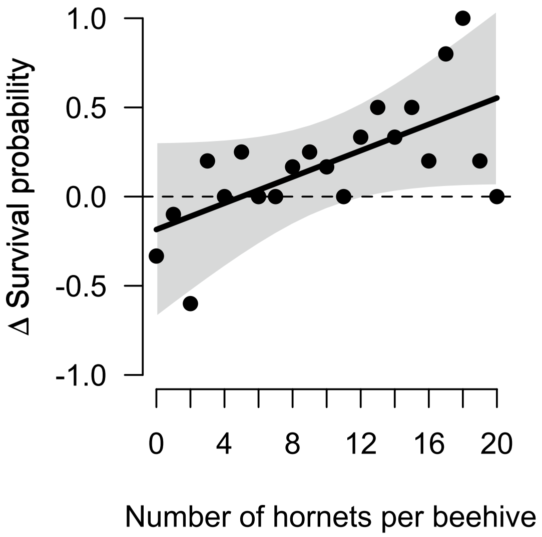

Fig. 3 Effect of supplementing beehive muzzle on the survival probability of hornet-stressed bee colonies. The presence of beehive muzzle increased the survival probability of simulated hornet-stressed colonies in a context of high amounts of hornets hovering at the beehive entrance (i.e. more than five hornets). The Δ value consists in the difference of survival rate between control and muzzle colonies. The horizontal dotted line shows the no benefit of the beehive muzzle on the survival probability. Thick lines show the model predictions with shaded areas indicating the 95% CI

predict that the muzzle increases the survival probability of hornet-stressed colonies up to 51% in a context of high amounts of hornets (i.e. more than five hornets) (Fig. 3).

Discussion

The invasive Asian hornet *Vespa velutina* threatens honey bees in Western Europe by inducing bee foragers' homing failures (HF) and foraging paralysis (FP) of colonies that reduce colony survival probability (Monceau et al. 2013; Requier et al. 2019). Here, we tested whether the use of beehive muzzles, a low-cost and biodiversity-friendly control method, can mitigate these Asian hornet impacts on honey bees. We found that the beehive muzzle did not affect the hornet-related homing failure, but drastically reduced the foraging paralysis. Beehive muzzle therefore helps honey bee colonies maintain their foraging activity even in the presence of the predator. Once modelled, these muzzle-based effects increased substantially the survival probability of hornet-stressed colonies in the context of high abundances of Asian hornets. These results suggest that supplementing beehive muzzle can mitigate the detrimental effect of the Asian hornet on European honey bees.

The Western honey bee *Apis mellifera* is an important pollinator of many crops (Garibaldi et al. 2017) and wild plants

(Huang et al. 2018), and further produces honey and other beekeeping products. Large-scale monitoring has revealed an ongoing decline of honey bee populations in Europe (e.g. van der Zee et al. 2012; Gray et al. 2019), the USA (e.g. Kulhanek et al. 2017) and in several other regions across the world (e.g. Latin America: Requier et al. 2018; China: Liu et al. 2016; South Africa: Pirk et al. 2014). This decline is depicted by high honey bee colony mortality rates, especially during winter season in Europe (Neumann and Carreck 2010; Gray et al. 2019). The decline is likely to be related to interactions among multiple combined stressors (reviewed in Potts et al. 2010, 2016; Goulson et al. 2015; Steinhauer et al. 2018). For instance, the agricultural disturbance of floral resource diversity, availability, and composition, resulting in the scarcity of particular flowering species and/or monotonous offers of specific crop flowers, negatively affects managed honey bee colony dynamics and survival (e.g. Requier et al. 2017). Pesticides, e.g. neonicotinoids as well as pyrethroids and fungicides, were also highlighted to severely affect the behaviour and fitness of honey bees (e.g. Prado et al. 2019). Additionally identified critical causes are diseases, pathogens, and parasites (reviewed in Potts et al. 2010, 2016; Goulson et al. 2015; Steinhauer et al. 2018). Recently, the Asian hornet has been added to this list of multiple stressors likely involved in the mortality of honey bee colonies in Western Europe (Leza et al. 2019; Requier et al. 2019).

Here, we showed that the beehive muzzle, a physical control method, reduces the stress related to the Asian hornet that could lead to a positive effect on the survival of hornet-stressed colonies. By reducing the stress of the bee colony facing hovering hornets, this mesh placed around the beehive's flight board allows bee workers to continue foraging. Although several studies have highlighted the need to control for the invasive Asian hornet's impact on honey bees, none of the proposed control methods have tested their efficiency per se. Conversely, it is well established that the current control methods are not well adapted and lead to important environmental impacts (ESM 1). For instance, locating and destroying nests or trapping hornets in mass cannot sustainably reduce the populations of any social invasive wasp (Beggs et al. 2011). Moreover, in the absence of specific baits mass trapping remains a threat to numerous non-target species of the local entomofauna (Rome et al. 2011; Rojas-Nossa et al. 2018, Rodríguez-Flores et al. 2019). Beehive muzzle represents for beekeepers an effective low-cost technique to reduce Asian hornet impact, while promoting economic saving given the estimated cost less of 15 € per homemade beehive muzzle (including material and construction time) instead of a cost up to 100 € for a hornet-related dead colony replacement. The beehive muzzles have to be installed at the start of the predation period (typically in mid of August in France; Monceau et al. 2013; Requier et al. 2019) and removed before the spring. Although our results using a 6 mm mesh device (see ESM 3) would lead to a potential negative effect on the foraging activity of honey bees in the absence of Asian hornets, we recommend testing a larger mesh device (e.g. 8–10 mm) that would facilitate the in-and-out activity of bees without reducing the protection against the predator. This tool can also be useful to control other predators that impact colonies particularly during the winter season, especially large insects and small mammals (Simone-Finstrom et al. 2017). However, even using beehive muzzle, beekeepers may consider to provide supplemental feeding with sugar syrup during pre-wintering or wintering periods to enhance the survival probability of hornet-stressed colonies though caution in supplemental feeding methods is required to avoid any disturbance in bee colony thermoregulation during the critical period of overwintering (Requier et al. 2019).

Biological invasions, in combination with other anthropogenic drivers (Brook et al. 2008), are responsible for many socio-economic and ecological impacts worldwide, including massive economic costs (Bradshaw et al. 2016), health-related issues (Mazza et al. 2014; Schindler et al. 2015), and ecological damages such as biodiversity loss and species extinctions (Bellard et al. 2017). Here, we showed that the beehive muzzle, a physical control method, can help to increase the survival probability of hornet-stressed colonies without using environmental-cost biocides or expensive material. This control method could therefore be recommended to beekeepers as an effective low-cost technique to protect their beehives. The current study is the first formal assessment of hive muzzles as a honey bee protection device against the Asian hornet. A part of our findings is based on theoretical modelling assessments, which remain to be consolidated by field data. Given the urgency of the situation, with the rapidly expanding Asian hornet threat and the flourishing of initiatives to control its economic impact, we encourage scientists and beekeeping institutes to gather large-scale standardized field data on the muzzle efficiency. Additional colony survival data are needed to further validate the method in a wider range of environmental contexts. Importantly, any colony survival monitoring should cover the whole winter period and next spring arousal so that the potentially delayed effects (Requier et al. 2017) will be properly detected.

Author contributions

FR and QR conceived the idea and designed methodology; QR collected the data; FR analysed the data and ran the simulations; FR wrote the first version of the manuscript; FR, QR, CV, and MH contributed critically to the drafts and gave final approval for publication.

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Compliance with ethical standards

Conflict of interest No conflict of interest was reported by the authors.

Human and animals rights All applicable international, national and/or, institutional guidelines for the care and use of animals were followed.

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A biodiversity-friendly method to mitigate the invasive Asian hornet's impact on European honey bees

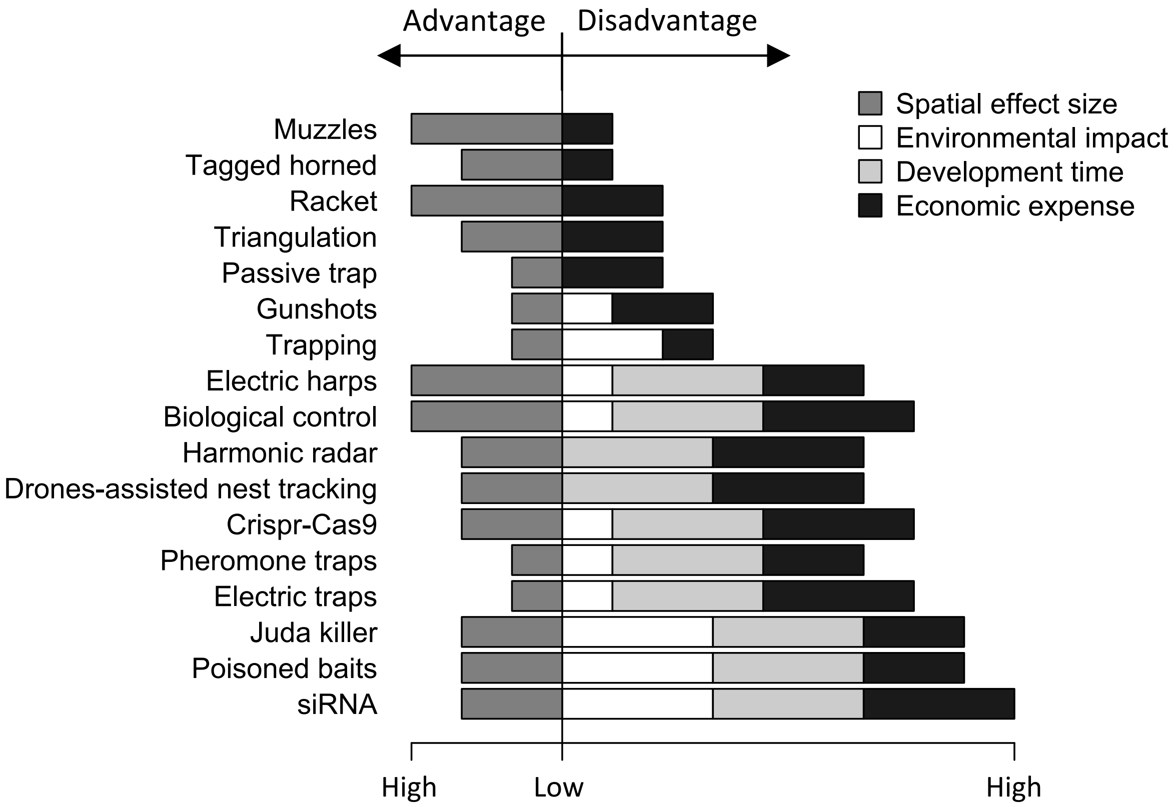
Requier F, Rome Q, Villemant C, Henry M

Journal of Pest Science

ELECTRONIC SUPPLEMENTARY MATERIAL

Content **pj**

ESM 1. Ranking of the technical cost – benefit ratio among existing biological and physical control methods of the Asian hornet *Vespa velutina*. The estimates are quantitative-derived from the review of Turchi and Derijard (2018), with a respective attribution of the values 0, 1, 2 and 3 to the categories “none”, “low”, “medium” and “high”.

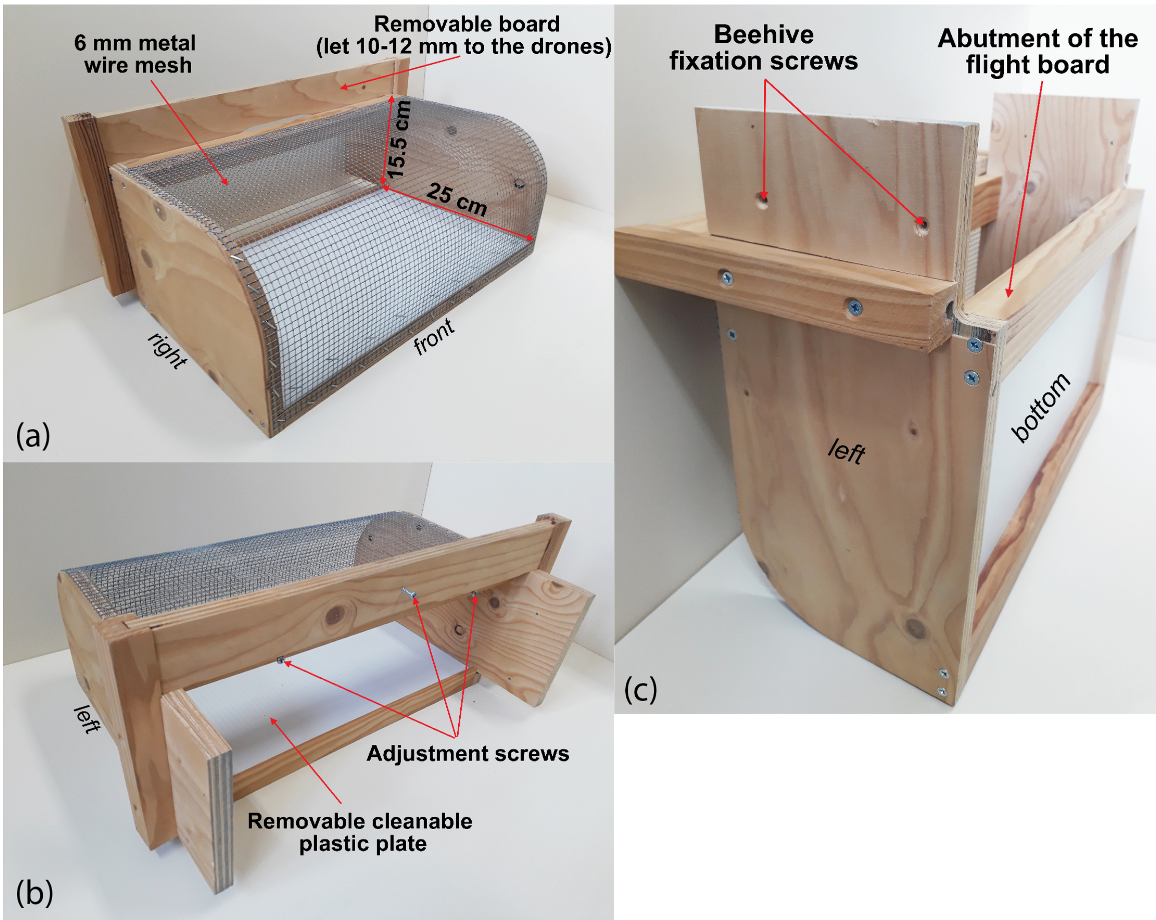


ESM 2. Details on the visual observation dataset.

							Control				Muzzle			
Region	Apiary name	Latitude	Longitude	Year	First date obs (d-day)	Last date obs (d-day)	No. obs.	No. bees (min - max)	No. hornet (min - max)	No. predation (min - max)	No. obs.	No. bees (min - max)	No. hornet (min - max)	No. predation (min - max)
Bretagne	B1	48.5694	-4.3317	2016	222	315	9	1 - 305	0 - 11	0 - 5	9	5 - 300	0 - 6	0 - 3
Bretagne	B2	48.8331	-3.3042	2016	216	307	14	15 - 75	3 - 8	5 - 14	14	13 - 60	2 - 8	5 - 14
Nouvelle-Aquitaine	N16	45.5923	0.6644	2013	263	291	4	50 - 150	0 - 1	0 - 0	4	20 - 100	0 - 1	0 - 0
Nouvelle-Aquitaine	N32	43.2980	-0.3482	2014	242	305	14	3 - 86	1 - 20	1 - 22	14	0 - 130	1 - 20	0 - 28
Nouvelle-Aquitaine	N17	45.7131	0.6852	2013	225	309	11	0 - 26	0 - 2	0 - 2	11	0 - 11	0 - 2	0 - 1
Nouvelle-Aquitaine	N18	45.1672	0.4206	2013	251	300	8	11 - 80	0 - 0	0 - 0	8	16 - 100	0 - 0	0 - 0
Nouvelle-Aquitaine	N19	44.9063	0.9314	2013	256	303	7	34 - 90	0 - 2	0 - 4	7	21 - 80	0 - 1	0 - 1
Nouvelle-Aquitaine	N20	44.8718	-0.5304	2013	264	345	7	24 - 31	0 - 2	0 - 4	7	22 - 102	0 - 3	0 - 2
Nouvelle-Aquitaine	N34	44.8857	0.3687	2014	259	287	5	15 - 90	0 - 3	0 - 2	5	30 - 90	0 - 1	0 - 1
Nouvelle-Aquitaine	N35	44.6698	0.7792	2013	215	308	35	0 - 212	0 - 3	0 - 2	35	0 - 119	0 - 2	0 - 2
Nouvelle-Aquitaine	N46	43.3134	-0.3495	2014	251	306	4	0 - 1	3 - 10	0 - 1	4	0 - 0	2 - 18	0 - 0

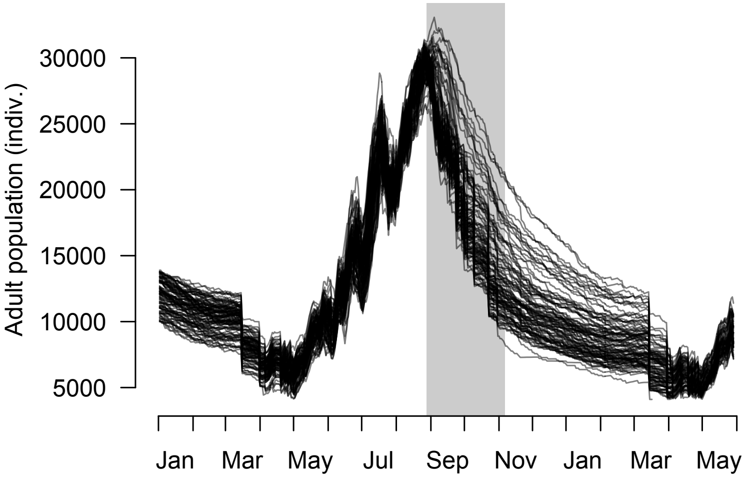
Occitanie	O1	43.3226	-0.3782	2016	91	360	17	0 - 42	0 - 3	0 - 3	17	0 - 38	0 - 4	0 - 4
Occitanie	O2	43.5629	1.4662	2013	286	286	1	20 - 20	2 - 2	2 - 2	1	1 - 1	1 - 1	0 - 0
PACA	Pr1	44.4089	5.9894	2016	222	300	5	15 - 46	0 - 1	0 - 1	5	18 - 41	0 - 2	0 - 2
PACA	Pr2	44.4437	6.0399	2016	222	300	5	12 - 46	0 - 3	0 - 4	5	7 - 40	0 - 2	0 - 4
PACA	Pr3	43.1886	6.2697	2016	222	277	5	7 - 24	0 - 0	0 - 0	5	15 - 26	0 - 0	0 - 0
Pays-de-la-Loire	Pa1	47.6610	-1.7261	2016	237	265	7	12 - 38	0 - 5	0 - 3	7	6 - 33	0 - 7	0 - 4
Pays-de-la-Loire	Pa2	46.9492	-1.3033	2016	260	301	6	15 - 48	0 - 5	0 - 2	6	11 - 45	1 - 6	0 - 3
Pays-de-la-Loire	Pa3	47.3098	-2.0764	2016	215	275	5	0 - 30	0 - 10	0 - 1	5	0 - 178	0 - 17	0 - 3
Pays-de-la-Loire	Pa4	47.2590	1.6333	2016	247	300	4	8 - 118	0 - 16	0 - 4	4	3 - 7	0 - 5	0 - 3

ESM 3. Detailed design of the beehive muzzle. (**a**) Latero-frontal view, (**b**) latero-back view, (**c**) lateral view.



ESM 4. Temporal pattern of the honey bee colony population size simulated with the BEEHAVE colony model parameterized with a range of hornet impact levels following its effect on (**a**) control colonies without muzzle vs. (**b**) muzzle-equipped colonies. The control simulations (**a**) show a typical pattern of foraging paralysis effect (i.e. a higher size of the population in comparison with the control simulations) during (in grey) and after the Asian hornet stress period. This is related to the stop of flight activity and therefore an increase of individual survivorship per bee. See Requier et al. (2019) for more details. The muzzle-equipped simulations (**b**) show the absence of such a foraging paralysis effect.

(a) *Control*



(b) *Muzzle*