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Vespa velutina predation impacts on *Apis mellifera* colony dynamics and reserves

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1. Introduction

Agroecosystems maintenance is dependent on the pollination services carried out by wind, water and animal transportation. Pollinators are essential for one third of global crop production and are necessary for fertilization of 60 to 90% of plant species (Kremen et al. 2007). Therefore, pollinators play key roles: an environmental role as they contribute to biodiversity preservation and an economical role as they enable a part of food production and. For instance, the colza and sunflower yields are increased by 35% to 40% when the bees' abundance (wild and domesticated) is multiplied by 100 (Perrot et al. 2018, 2019). The European honeybee, *Apis mellifera,* feeds on pollen (protein source for larvae) and nectar (carbohydrate source for adults) from fruit trees and bushes, nut trees, pastures, aromatic plants and wild plants. The honeybees are considered an indicator of environment quality (Nunes-Silva et al. 2019). The state of health of honeybee populations can indicate pollen sources of quality and/or in quantity, providing an appropriate habitat clear of insecticides with few pathogens and appropriate climatic conditions for the honeybee. Nonetheless, monoculture and urbanization expansions are currently problematic because of the reduction of biodiverse areas. In fact, these phenomena have a negative impact on the food quantity and quality for honeybees. On top of the loss of resources, the honeybee is exposed to a loss and a fragmentation of habitats (Naug 2009), climate change and pesticides (Goulson et al. 2015). In addition to the environmental stressors, the honeybee faces biotic challenges: viruses, bacteria, fungi, parasites and predators (Goulson et al. 2015). The sum of these biotic and abiotic challenges results in a large winter colony loss, around 12% between 2015 and 2016 (Steinhauer et al. 2016) and to decline of beekeepers, around 25%, between 1985 and 2005 in Europe (Potts et al. 2010).

Among honeybee's predators, *Vespa velutina,* was accidently introduced by boat transportation, into Europe in 2004, near Agen in France, from Eastern China and is now invasively spreading through Western Europe (Rortais et al. 2010). The hornet's spread is fast, it is estimated to 78 km per year in Western France (Robinet et al., 2017). Currently, 16 years after its introduction, the hornet is present in Spain (Goldarazena et al. 2015), Portugal (Grosso-Silva and Maia 2012), Italy (Porporato et al. 2014), United Kingdom (Keeling et al. 2017), Belgium (Garigliany et al. 2017), Netherlands and Germany (Witt 2015). The invasion of *Vespa velutina* in Europe received significant media attention (Monceau et al. 2014) and people are commonly afraid of the Asian hornet. It can be seen as a threat because of its aggressiveness and its nest location in urban

areas. *Vespa velutina* was classified an "invasive alien species" in 2012 in France (MAAF 2012) and since 2016 it is included in a European plan of invasion control (European Commission 2016). Then, different types of pest management have been tested such as nest destruction, trapping individuals with food baits or insecticides, using pheromones and implementing biological control. The problem of these methods is that they are not selective and impact the native biodiversity (Monceau et al. 2014). Another problem is that the traps are commonly used at the wrong moment, during the predation period. Finally, the nests are difficult to spot during the season as they are well hidden and often high in trees. As of today, not any efficient method to control the Asian hornet's expansion in Europe has been developed (Poidatz 2017). The introduced species feeds on various insects (honeybees, common wasps, hover flies, blow flies and house flies) (Villemant et al. 2011), then it shows ecological impacts as it presents a risk for native insects and an impact on the pollination services (Monceau et al. 2014). The hornet has also an economic impact as it disturbs beekeeper's activity, especially in an urban environment, where the hornet's population density is high (Monceau et al. 2017). Hence, the hornet's invasion shows general agroecological impacts. Consequently, Arc Atlantic POSitiVE, a European project, was launched from May 2019 to continue until May 2022, to conserve pollination services in Atlantic regions (Portugal, Spain, France, Ireland and United Kingdom) and to control the population of *Vespa velutina* (Atlantic Positive, 2020).

The hornet is a threat to honeybees starting from summer (Monceau et al. 2014). Before that, the hornet foundress comes to the end of wintering in February and builds by itself a primary nest. The first workers emerge during the end of the month of April. In June, the predation on honeybees begins and the predation peak starts mid-July, to feed the hornet's larvae until November or December when the hornet's population decreases. Characteristically, the Asian hornet hovers in front of the colony to catch foragers coming back loaded with resources (Monceau et al. 2013a). The honeybee foragers can have 40% body extra mass (Feuerbacher et al. 2003) which can be a handicap to easily avoid the predator (Monceau et al. 2013a). *Apis mellifera*'s lack of efficient anti-predator behaviour against the Asian hornet makes them vulnerable, honeybees are highly threatened by the predator's spread.

Indeed, compared to *Apis cerana*, a bee's species which has co-evolved in Asia with *Vespa velutina*, the Asian honeybee has developed a behaviour to defend the colony against the hornet predation (Ken 2005, Tan et al 2007 and 2012). A large number of bees (*Apis cerana*) are involved in the heat balling behaviour; the bees group into a ball

around the hornet to raise the temperature and to asphyxiate it until the hornet dies. The western honeybees, *Apis mellifera* are not able to defend as efficiently as *Apis cerana* against the newly introduced hornet in Europe, nevertheless a specific defensive behaviour has been observed from the Western honeybees. As it is difficult for the bees to sting the hornet's hard cuticle, a usually inactive pool of honeybees is recruited on the flying board, to form a 'bee carpet' (Nouvian et al. 2016), in order to scare the predator. The 'bee carpet' can engulf the hovering hornet if it comes too close to the colony, the hornet is then trapped and the 'bee carpet' asphyxiates the hornet until it dies (Arca et al. 2014). Nonetheless the 'bee carpet' strategy observed from *Apis mellifera* in Europe is not only associated with defence; it is also observed under high temperature, when the colony needs to lower the inner beehive temperature. The 'bee carpet' is in part composed of honeybee's guards. Guarding is one of the roles a honeybee can have during its life time. In fact, one of the characteristics of honeybees is to present polyethism (Calderone 1998). The honeybee workers play different roles during their life depending on their age and on the colony needs. Only a small part of the honeybee colony becomes guard (15%, Moore et al. 1987) to protect the colony entrance from intruders. The guards are easily recognizable by the specific behaviour they exhibit by raising their front legs in order to identify their nest mate. However, the defensive behaviour in honeybees is complex, it varies among the colonies and the defensive behaviour could be in part influenced by their genes (Hunt et al. 2007) and then be heritable (Breed et al. 2004). For beekeeping activities in France, honeybees have been selected years after years to result for gentleness from the honeybees towards humans. However, one may consider that this genetical selection is probably deleterious to the colony defensive behaviour against predators.

The present study aims at investigating the defence behaviours of two different genetic lines of European honeybees commonly bred in France: Buckfast and Caucasian colonies. For these two genetic lineages, we studied if the general dynamics of the honeybee colony was impacted, depending on the predation pressure. We also studied the pollen foraging dynamics to know if the hornet impacts the pollination services. Finally, as a consequence of the dynamics observed, we looked at the honeybee colony reserves to evaluate the genetic line influence or the predation impact on the production and on the beekeeping activity. Overall, our study aims to solve a current lack of information about the *Apis mellifera*'s capability to defend against the Asian hornet in natural conditions and to assess if there is any direct impact on the colony dynamics and indirect

impact on the reserves on the long-term. The study's intention is to help in the choice of apiary's environment and/or of the origin of the bees the beekeeper will breed. Our initial hypothesis, is that the presence of Asian hornet negatively impacts the activity of the foragers and indirectly impacts the pollen and honey reserves. Further, the lack of food leads to the restriction of brood rearing and ultimately to the colony collapse.

2. Methodology

2.1. Experimental methods

The present study was conducted at INRA Bordeaux in collaboration with La Rochelle Université CEBC (Centre d'Etudes Biologiques de Chizé) and ADANA (Association de Développement de l'Apiculture en Nouvelle Aquitaine). The results are based on field work during the *Vespa velutina* predation season, from June to the end of October 2019. The study was conducted with two of the most common *Apis mellifera* genetic lines in European beekeeping (Table 1): Buckfast and Caucasian, both selected for their honey production and their easiness to handle (Feltin 2013). Buckfast colonies were selected for the study because of their gentleness and Caucasian colonies because they are known to be less gentle than Buckfast. Buckfast colonies are generally more populous, produce more honey and start the foraging season earlier, than Caucasian colonies. They also show differences in the amount of reserves they need for the winter, the Caucasian colonies being originally from Eastern Europe have better capacities to face cold temperatures. The Buckfast colonies are limited to forage only flowers with shallow corolla as their bees have a slightly shorter tongue compared to the Caucasian strain. Bees' genetic background from each colony is planned to be analysed and validated. The observed swarms were from 2019, thus the colonies had never faced the Asian hornet before.

Table 1. Comparison of Buckfast and Caucasian bee colonies characteristics, adapted from (Feltin 2013)

	Buckfast (or Adam brother)	Caucasian *Apis mellifera caucasia*
Agressivity	-	+
Honey production	++	+
Colony population	++	+
Tongue	6,3mm	7mm
Flower foraging	Shallow corolla	Deep corolla (acacia)
Winter stock needs	+++	+
Easiness to handle	+++	++
Hybrid	Yes	No
Origin	Created for beekeeper's interest	Eastern Europe

The colonies were settled in two different apiaries, both located in South-West France, near Bordeaux, 18 kilometres apart. One apiary was at INRA in Villenave d'Ornon (Ferrade) (44°47'15.2"N0°34'35.1"W) in an urban environment surrounded by vineyards. The second apiary was located at Forêt-Bois INRA in Cestas-Pierroton (44°44'47.3"N 0°46'02.9"W) close to a forest of resinous trees. On both sites, no pesticide is used at a landscape scale, as this is forbidden. The only pesticides applied are on the colony, against honeybee parasites. Nevertheless, we could not control the potential chemical applications on a local scale, for instance in nearby private gardens.

Ferrade apiary was composed of six Dadant beehives with Buckfast colonies. Pierroton apiary was composed of thirty Dadant beehives with Caucasian colonies in one half, intercalated with Buckfast colonies in the other half. In both apiaries, the hives were 10 meters apart to avoid undue spatial interaction between the colonies and avoid one potential experimental confounding factor.

Measurements were performed every other week for each apiary and in total each colony was tested and evaluated during 10 weeks. The 'session' term was used to facilitate the assessment of the colony's evolution. One session was equivalent to one week of the experiment at Pierroton and one week of the experiment at Ferrade. The first session was in June and the last in October. To collect the samples' data that could be representative of the population, the colonies' evaluation order was randomly drawn before every session. Then, between each session, the colonies were not tested in an identical order, nor at the same time of the day. This experimental design avoided the measurement of the biological bias but measured the impact of the predation pressure or the impact of the genetic line.

The comparison between the two environments (Ferrade and Pierroton) was made only with Buckfast colonies (6 and 15 colonies, respectively). The comparison between the two genetic lineages (Buckfast and Caucasian) was made only at Pierroton apiary (15 and 15 colonies, respectively).

2.2. Data collection

2.2.1 Predation pressure measure: hornets count

The hornet predation pressure was evaluated by counting the number of hornets present around each colony on each session before each experiment. The count was made by

two persons, two to three meters away from the colony to have a global view of the predation pressure and the experimenters were placed on each side of the colony. The total number of hornets was agreed between the two persons to make sure each hornet was counted once. The hornets were counted as a scan sampling, every minute and three times per colony. The three counts of hornets per colony were highly repeatable (Spearman's rank correlation test, n=360, ρ=0.75, P<0.001) were significantly correlated between them. Based on this result, only the second count was used for the statistical analyses, because at this count, the disturbance on the colony from the experimenters was the lowest.

2.2.2. Behaviour and dynamics measure: video recording

The behaviour associated to defence, the global colony dynamics and the pollen foraging dynamics were evaluated with video records by a camera (Sony RX100 IV, 16 fps) settled in front of the beehive entrance. Recording of colony activity took place once every other week for each colony. A total of 360 videos was recorded (36 colonies x 10 sessions). Each video was recorded during four minutes. The video analysis was done *a posteriori* by two persons. The first person analysed a part of the first four sessions and the second person, after having been trained by the first person, analysed the remaining videos. The analysis was done using VLC media player in slow motion, image after image, if needed. In total only two minutes out of four minutes of recording of activity per colony and per session were analysed for two time frames: from 30" to 1'30 and from 2'30 to 3'30, to avoid the experimenter's perturbance on the colony (Figure 1).
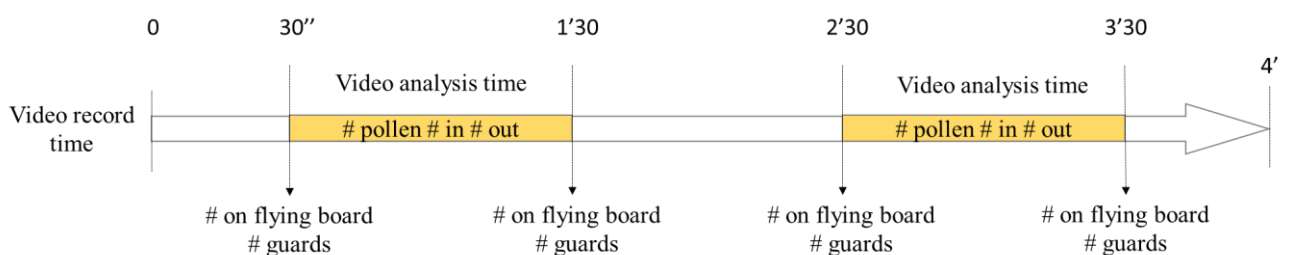
Figure 1. Video analysis diagram. The four minutes video is analysed from 30 seconds to 1 minute 30 and from 2 minutes 30 to 3 minutes 30. # = number of counted bees. Arrow = counting at a T time of bees on the flying board and bees guarding the colony. Yellow color = continuous counting during one minute of bees coming back with or without pollen and bees exiting the colony

The colony behaviour associated to defence was evaluated by counting the number of bees present on the flying board and the number of bees exhibiting a guarding behaviour on the starting and on the ending image of each time frame (T1= 30'', T2=1'30, T3=2'30 and T4=3'30). The video was watched five seconds before and after each T time (T1, T2,

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T3 and T4) in order to identify the moving guards on the flying board. The guards were recognized by their specific behaviour of raising their front legs towards a coming nest-mate or a predator, their jerky movements and their way of walking back and forth in front of the colony entrance. The four counts of bees on flying board (Spearman's rank correlation test, n=343, ρ= 0.96, P<0.001) and the four counts of guards (Spearman's rank correlation test, n=343, ρ= 0.80, P<0.001) were each significantly correlated between them. Based on this result, only the second count was used for the statistical analyses, because at this count, the disturbance from the experimenter was the lowest and the count gave an estimation of the colony activity at the middle of the video record.

The global colony dynamics was evaluated by continuously counting the number of foragers because they are the first Asian hornet's target. The analysis of the foragers' dynamics was preliminary to then analyse the predation pressure impact on the reserves. The number of bees coming back to the colony with and without pollen and the number of bees exiting, during two minutes per colony were counted using a traffic counter. A bee was counted as a bee coming back to the colony, only if it was coming from outside of the video screen, the ones who were already on the flying board and entered the colony were excluded from the counting. In fact, the bees that could possibly go back and forth from the flying board to the colony were ignored, because they are not foragers. The number of foraging bees coming back with pollen on the back legs were counted. Concerning the exits, all bees flying out, outside of the video screen, were counted as foragers.

To assess the predation pressure during the video record, the presence or absence of hornets was also noted. This variable was not taken into consideration for the statistical analysis, because the video frame limited the global vision of the predation pressure on the colony. The hornet count per colony executed on the field before the video record was used for the statistical analysis, as it was an accurate measure of the predation pressure. To assure a correct counting during the video analysis, when an entire session was analysed, two videos were randomly selected to be double checked for Pierroton apiary and one video was picked for Ferrade apiary. Then for one colony, the first count was compared to the mean of the double checks with a Chi square test. The first count and the mean were statistically similar. Then only the first count was used for the statistical analyses, because a discrete variable was more appropriate for a counting variable analysis rather than a continuous variable.

2.2.3. Reserve measure: ColEval

The reserves of bee colonies were assessed by using the tool 'ColonyEvaluation' (ColEval) by visually estimating the proportions of honey storage (including nectar storage) and pollen storage (Maisonnasse et al. 2016 and Hernandez et al. 2020). The evaluation was performed once every other week on each colony by a duo of researchers, one who assessed the proportions on each frame from the colony and one who took notes. The observed proportions were then converted in dm², knowing that a total Dadant frame surface is equal to 11.34 dm².

2.3. Environmental descriptors

2.3.1. Environmental composition: QGIS

The apiaries' environments were described to show the proportion of urban and green areas and the vegetation types in green areas. The honeybees forage on an average radius of three kilometres from the hive, then the environments were described in circle diagram of three kilometres around each apiary. The QGIS (version 2.18.17) software, a geographical information system, was used for the environmental analysis, using the data from CORINE Land Cover (CLC) 2012.

2.3.2. Climatic conditions

The average temperature per day (in degrees Celsius) and the average humidity per day (in percent) were measured by weather stations at each INRAE site (Ferrade and Pierroton). The weather conditions data was collected for each experimental session from INRAE Climatik application (INRAE 2019). The temperature and the session, as well as, the humidity and the session were highly correlated (Spearman's rank correlation test, $n=20$, $\rho=-0.74$, $P<0.001$ and $n=20$, $\rho=0.66$, $P<0.01$, respectively). Based on this result, all the weather variables were excluded from the statistical analyses.

2.4. Statistical analyses

The distribution of each variable was tested with the *fitdistrplus* package (Delignette-Muller and Dutang 2015). The variations of the number of hornets per colony fitted a negative binomial family. The variations of the number of bees involved in colony defence and the variation of dynamics fitted a binomial family. The variations of the reserves in the colony fitted a gausian family. According to the variable distributions, the Generalized

Linear Mixed Effects model (GLMM) was selected to analyse the data, using the *lme4* package (Bates et al. 2015). In order to test the difference between the two genetic lineages, the session, the number of hornets per colony and the apiary were used as the fixed variables. In order to test the difference between the two apiaries, the session, the number of hornets per colony and the apiary identity were used as the fixed variables. The colony identity was considered as the random effect variable to avoid pseudo-replication that could lead to misinterpretation of the results, the interest of the analysis was the overall impact observed on the genetic line or on the apiary not the impact observed on each colony. The marginal R^2, the variance explained by only the fixed effects and the conditional R^2, the variance explained by the fixed effects and the random effects (Vonesh et al. 1996) were checked for the selected models using *MuMIn* package (Barton 2019). The conditional R^2 was higher than the marginal R^2, then, the use of the colony identity as a random effect was justified because it had an impact in the selected models (Cameron et al. 1997). The model with the lowest Akaike's Information Criterion (AIC) was selected (Burnham and Anderson 2004). When two models differed by less than 2 points, the most parsimonious, with fewer parameters was selected. The collinearity between independent variables was checked using Variance Inflation Factors (VIF), using the *car* package (Fox et al. 2019). As recommended by Zuur et al. (2010), the parameter over 3 was cut-off and the next model was selected. When it was needed, an overdispersion parameter was added to the analysis as a random effect. The fit of the model residuals was checked via the *DHARMa* package (Hartig 2020). All statistics were run using the statistical software R version 3.6.2 (R Core Team 2019) implemented by the *lsmean* package (Lenth 2016) to calculate the estimate of the models.

3. Results

3.1. Environmental composition

The three km foraging radius is represented as diagrams in circles as honeybees forage up to three km away from their colony on average. Ferrade apiary is in the middle of an urban area. It is essentially surrounded by buildings (81%) and roads (5%) but not exclusively, as there is a small proportion of green area which represents annual crops (7%), public park (4%), mixed forest (2%) and vineyards (1%) (Figure 2 and Appendix 1).

Pierroton apiary is principally surrounded by forest (73%), as it is located in INRA Cestas Forest. The vegetation composition is mainly 'coniferous forest' (45%) and

'transitional woodland-shrubs' (26%). There is a large occupation by agriculture too (22%). Nonetheless, there are also 149 hectares of urban occupation (5%).
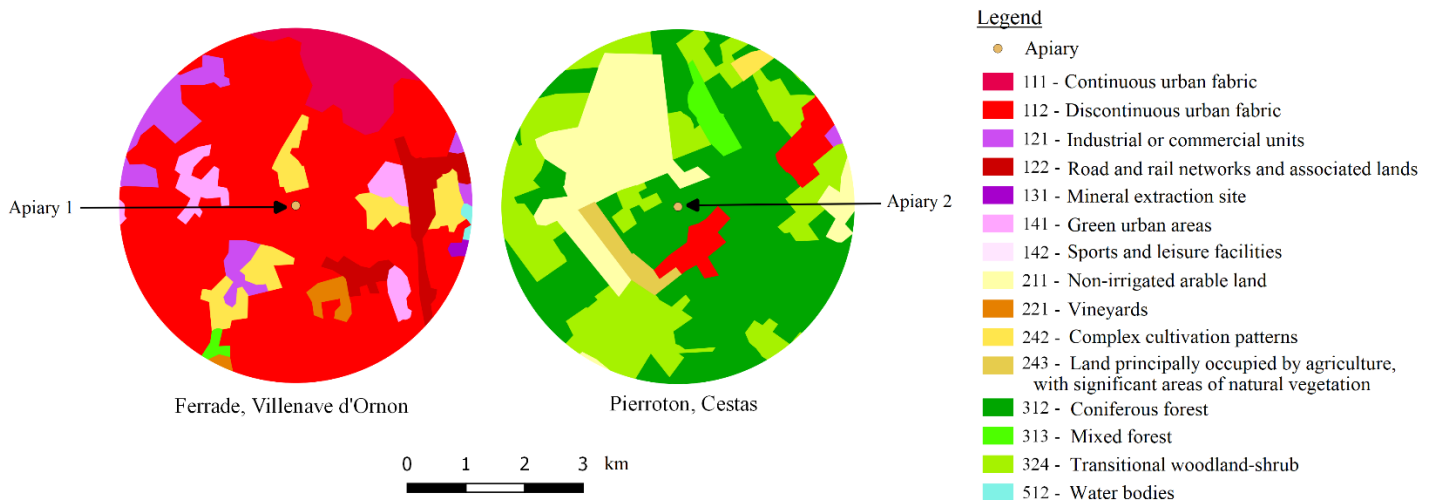
Figure 2. Apiaries' environment diagram in a radius of three kilometers: Ferrade (left) and Pierroton (right). Source: CORINE Land Cover 2012.

3.2. Predation pressure

The hornet predation pressure began in mid-July (third session) at Ferrade apiary and it began in mid-August (fifth session), at Pierroton apiary (Figure 3). The predation pressure increased through the season at Ferrade apiary. The variability of the number of hornets was explained by the effect of the session and the apiary in the selected model (GLMM, n=360, Table 2). The predation pressure was higher at Ferrade apiary (mean predicted values with [95% confidence interval]: 3.88 hornets [1.66-6.10]) than at Pierroton apiary (0.12 hornet [0.05-0.18]).
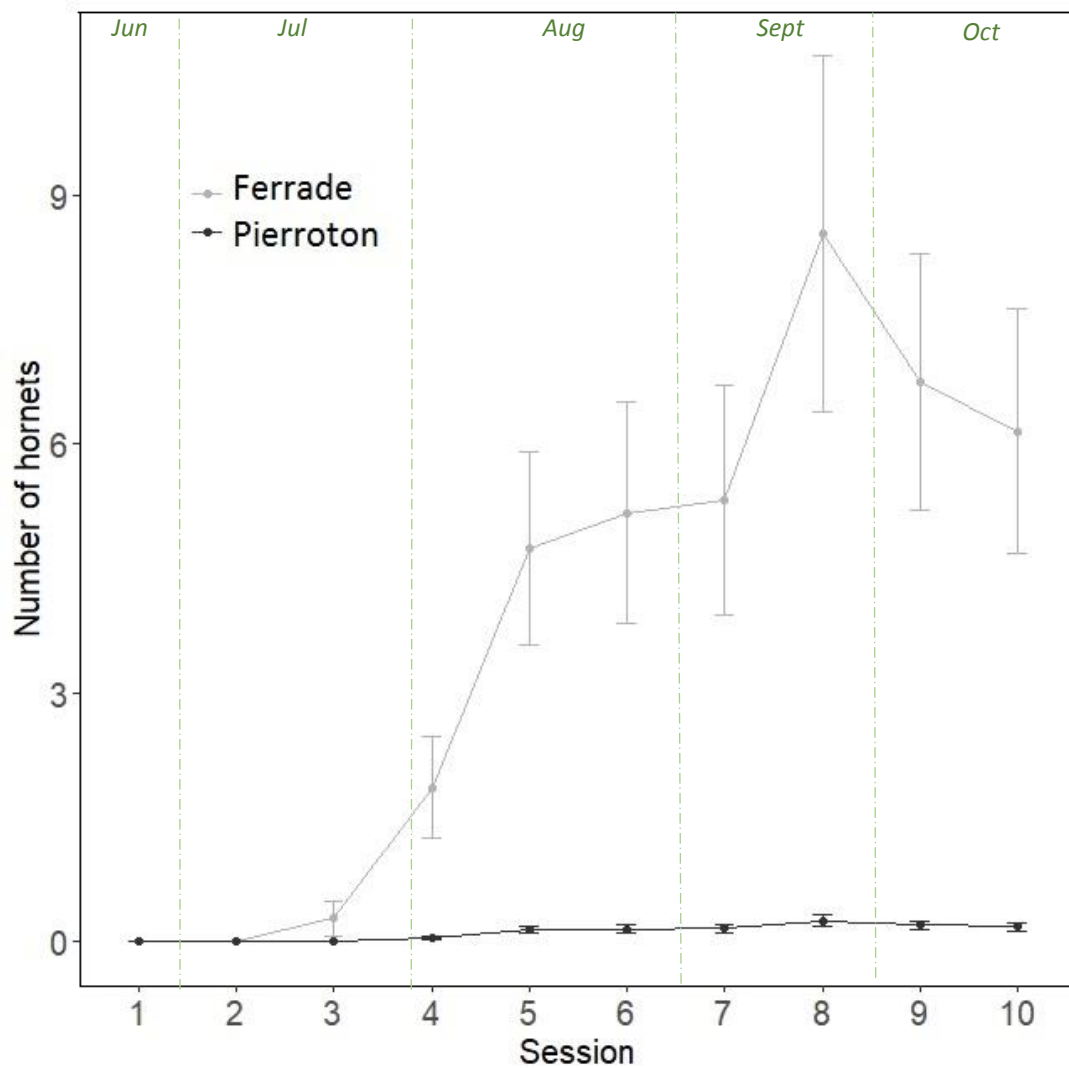
Figure 3. Seasonal evolution of the average number of hornets per honeybee colony according to the apiary with the standard deviation.

Table 2. GLMM of the impact of session and apiary on the number of hornets. The results are presented by the estimated coefficient of the effect variable influence on the response variable, with the standard error and the 95% confidence interval of the selected model.

Response variable	Effect variable	Class	Estimate	Standard error	95% Low CI	95% Up CI
Number of hornets	(Intercept)		-19.1	35.08	-87.86	49.66
	Session	2	1.77	209.02	-407.91	411.45
		3	17.83	35.09	-50.94	86.6
		4	19.72	35.08	-49.04	88.48
		5	20.65	35.08	-48.11	89.41
		6	20.74	35.08	-48.02	89.5
		7	20.77	35.08	-47.99	89.53
		8	21.24	35.08	-47.52	90
		9	21.01	35.08	-47.75	89.77
		10	20.91	35.08	-47.85	89.67
	Apiary	Pierroton	-3.5	0.22	-3.92	-3.07

### 3.3. Impact of Asian hornet according to the genetic line

Buckfast and Caucasian colonies were both at Pierroton apiary, under a low predation pressure.

3.3.1. Defensive behaviour

The number of bees involved in the colony defence increased until mid-August (fifth session). The proportion of guards varied from 2.77% [0.84-8.70] in mid-June (first session) to 19.79% [15.64-24.71] in early September (seventh session). The variability of the number of bees involved in the colony defence was explained only by a season effect, in the selected model and the genetic lines had no influence (GLMM, n=286).

3.3.2. Colony dynamics

The proportion of the number of bees coming back was the highest in early July(second session) for Buckfast colonies and for Caucasian colonies (49.60% [41.91-57.31] and 58.10% [50.45,65.39], respectively) and was the lowest in late September (eight session) (27.01% [12.21-36.54] and 34.27% [25.30-44.52], respectively) (Figure 4). The variability of the colony dynamics was explained by a season effect and a genetic line effect, in the selected model (GLMM, n=292, Table 3). Buckfast colonies showed an average lower proportion of bees coming back (41.92% [37.23-46.61]), compared to Caucasian colonies (50.28% [45.34-55.22]) over the season.
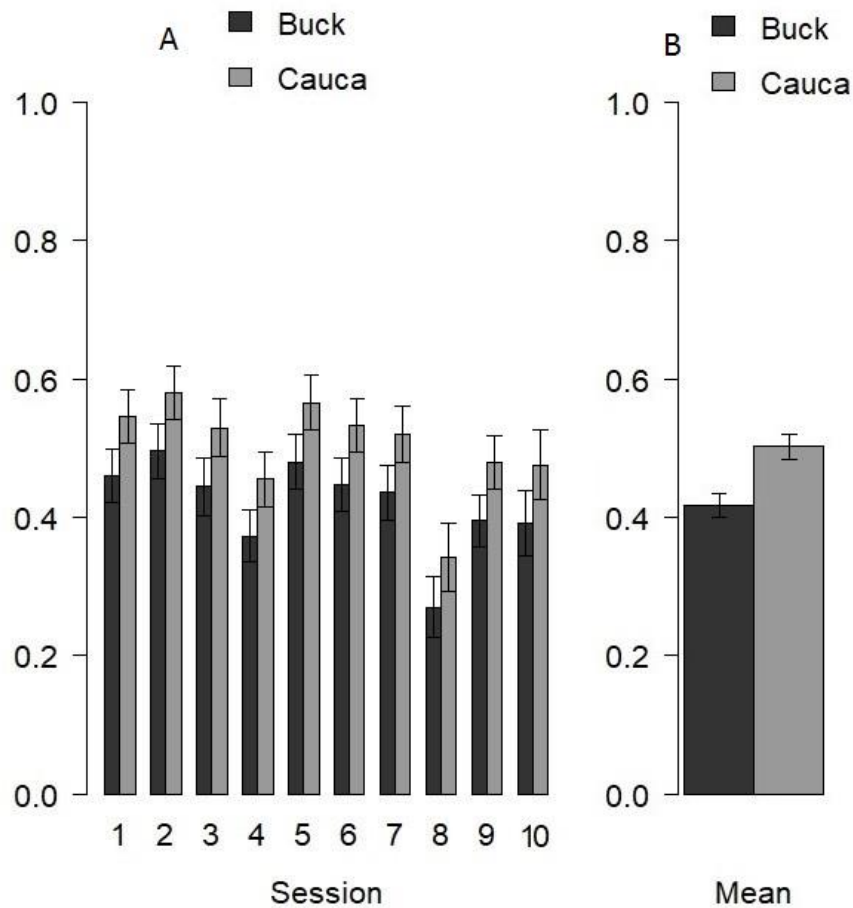
Figure 4. Genetic line effect on the general colony dynamics, proportion of bees coming back to the colony out of bees flying out. Seasonal evolution (A), and average dynamics per genetic line (B), Buckfast colonies (dark) and Caucasian colonies(light) with the 95% confidence interval.

The proportion of the number of bees coming back with pollen was the highest in mid-June (first session) (35.81% [35.74-35.88]) and was the lowest in late October (last session) (8.47% [8.44-8.50]). The variability of the collected resource was explained only by a season effect as the genetic line had not influence, in the selected model (GLMM, n=292).

3.3.3. Colony reserves

The honey surface in the colony was the highest in mid-August (fifth session) for Buckfast colonies and in late October (last session) for Caucasian colonies (Figure 5). The variability of the honey surface included the effect of the session interacting with the effect of the genetic line, in the selected model (GLMM, n=300, Table 3). Caucasian colonies showed a lower average honey surface in the colony over the season (83.00 dm² [68.67-97.33]), compared to Buckfast colonies (93,69 dm² [72.12-115.27]).
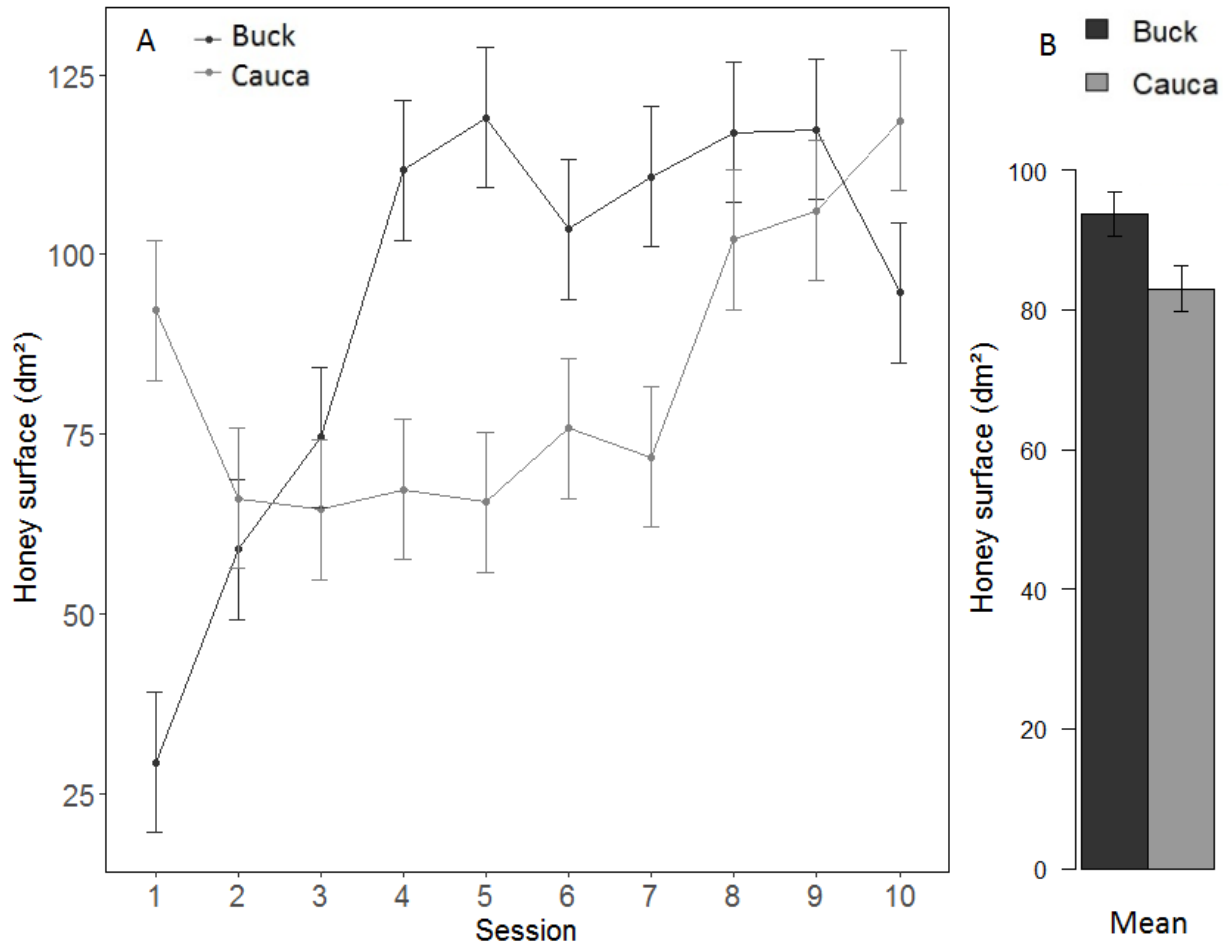
Figure 5. Genetic line effect on honey surface reserves per colony (dm²). Seasonal evolution (A) and average honey surface per genetic line (B), Buckfast colonies (dark) and Caucasian colonies (light), with the 95% confidence interval.

The pollen surface in the colony was the highest in late July (fourth session) for Buckfast colonies and in early October (ninth session) for Caucasian colonies (Figure 6). The variability of the pollen surface was explained by the same variables as the honey, in the selected model (GLMM, n=300, Table 3). Buckfast colonies showed a lower average pollen surface in the colony over the season (23.94 dm² [20.52-27.36]), compared to Caucasian colonies (27.01 dm² [22.41-31.60]).
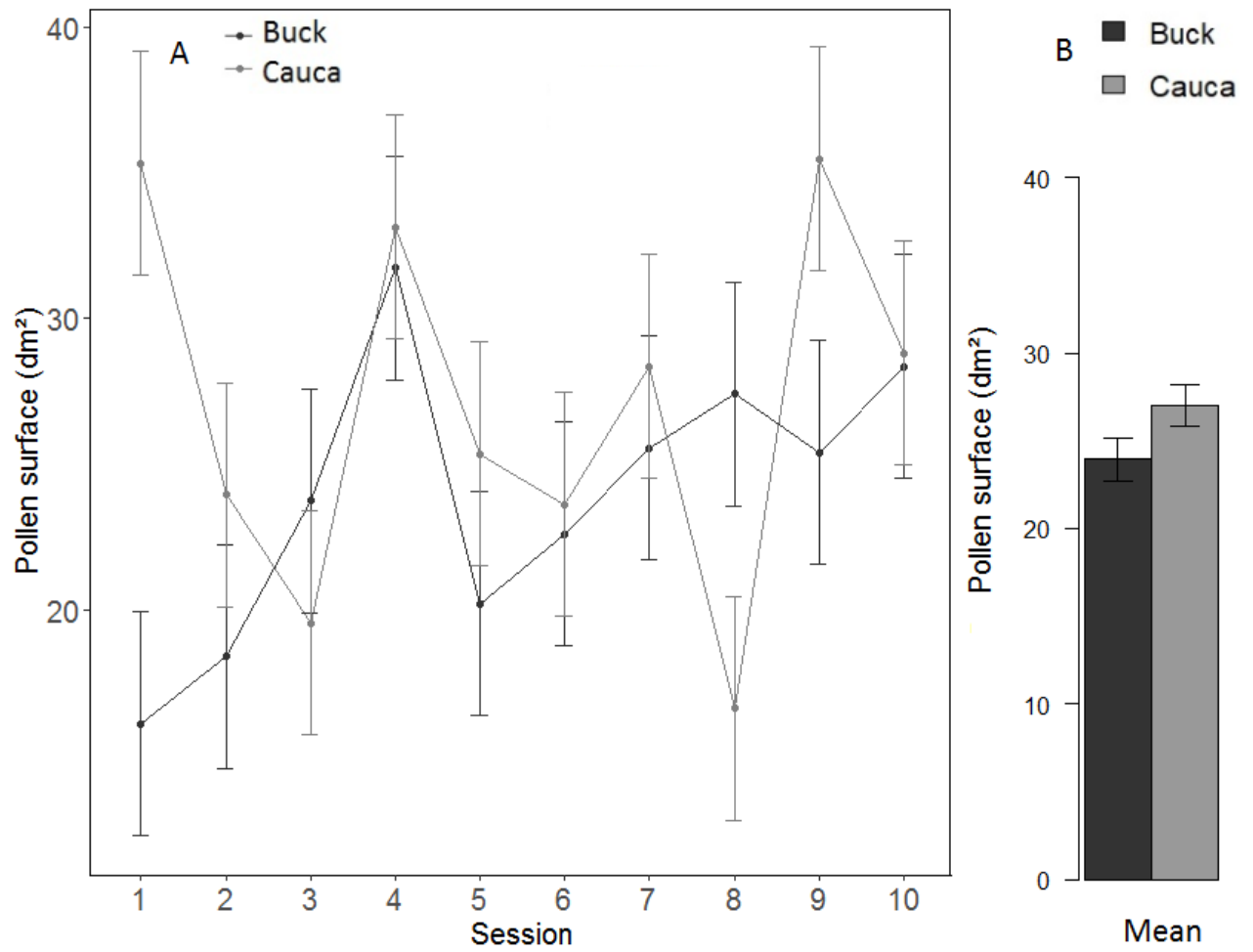
Figure 6. Genetic line effect on pollen surface reserves per colony (dm²). Seasonal evolution (A) and average of pollen surface per genetic line (B), Buckfast colonies (dark) and Caucasian colonies (light) with the 95% confidence interval.

Table 3. GLMM of the impact of session and genetic on the general dynamics and on the honey surface per colony, at Pierroton. The results are presented by the estimated coefficient of the effect variable influence on the response variable, with the standard error and the 95% confidence interval of the selected model.

| Response variable	Effect variable	Class	Estimate	Standard error	95% Low CI	95% Up CI
In/out ratio	(Intercept)		-0.16	0.16	-0.47	0.15
at Pierroton	Session	2	0.15	0.21	-0.27	0.56
		3	-0.06	0.22	-0.49	0.36
		4	-0.36	0.21	-0.77	0.06
		5	0.08	0.21	-0.33	0.5
		6	-0.05	0.21	-0.46	0.36
		7	-0.1	0.22	-0.52	0.32
		8	-0.83	0.26	-1.35	-0.32
		9	-0.26	0.21	-0.68	0.15
		10	-0.28	0.25	-0.76	0.2
	Genetic	Caucasian	0.34	0.1	0.14	0.54
Honey surface	(Intercept)		29.27	9.76	10.15	48.39
at Pierroton	Session	2	29.67	13.73	2.76	56.57
		3	45.27	13.73	18.36	72.17
		4	82.47	13.73	55.56	109.37
		5	89.73	13.73	62.83	116.64
		6	74.27	13.73	47.36	101.17
		7	81.60	13.73	54.69	108.51
		8	87.67	13.73	60.76	114.57
		9	88.13	13.73	61.23	115.04
		10	65.47	13.73	38.56	92.37
	Genetic	Caucasian	63.00	19.41	35.96	90.04
	Session*Genetic	2*Caucasian	-55.93	19.41	-93.98	-17.88
		3*Caucasian	-73.00	19.41	-111.05	-34.95
		4*Caucasian	-107.47	19.41	-145.52	-69.42
		5*Caucasian	-116.47	19.41	-154.52	-78.42
		6*Caucasian	-90.73	19.41	-128.78	-52.68
		7*Caucasian	-102.07	19.41	-140.12	-64.02
		8*Caucasian	-77.87	19.41	-115.92	-39.82
		9*Caucasian	-74.27	19.41	-112.32	-36.22
		10*Caucasian	-39.13	19.41	-77.18	-1.08

3.4. Impact of Asian hornet on Buckfast

Buckfast colonies were in two environments, one with a low hornet predation pressure (Pierroton) and one with a high predation pressure (Ferrade).

3.4.1. Defensive behaviour

The number of bees involved in the colony defence increased until Mid-August (fifth session). The proportion of guards varied from 2.34% [0.86-6.14] in June (first session) to 17.73% [13.09-23.57] in early September (seventh session). The variability of the number of bees involved in the colony defence was explained only by a season effect as the apiary had no influence, in the selected model (GLMM, n=199).

3.4.2. Colony dynamics

The variability of the colony dynamics was explained by an apiary effect, in the selected model (GLMM, n=206, Table 4). The colonies at Pierroton showed an average lower proportion of bees coming back (42.90% [39.16-46.72]), compared to colonies at Ferrade (55.11% [47.03-62.94]) over the season (Figure 7).
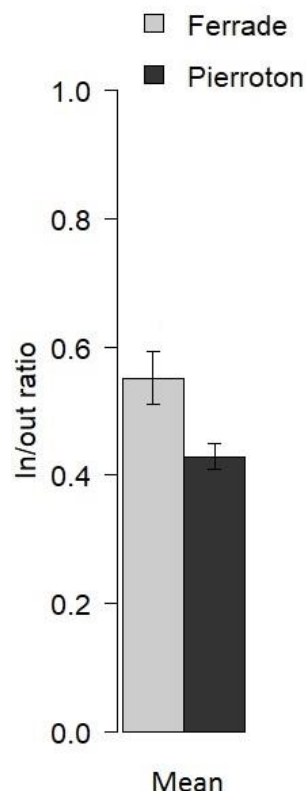

Figure 7. Effect of the environment on the general colony dynamics, average proportion of bees coming back to the colony out of bees flying out, at Ferrade (light) and Pierroton (dark), with the 95% confidence interval.

The proportion of the number of bees coming back with pollen was the highest in mid-August (fifth session) (39.37% [27.34-52.85]) and was the lowest in early September (seventh session) (5.99% [2.85-12.16]). The variability of the collected resource was only explained by a season effect as the apiary had not influence, in the selected model (GLMM, n=292).

3.4.3. Colony reserves

The honey surface in the colony was the highest in mid-July (third session) at Ferrade and in early October (ninth session) at Pierroton (Figure 8). The variability of the honey surface included the effect of the session interacting with the effect of the genetic line, in the selected model (GLMM, n=210, Table 4). Colonies at Ferrade showed a lower average honey surface in the colony over the season (64.70 dm² [46.10-83.30]), compared to colonies at Pierroton (93.69 dm² [72.12-115.27]).
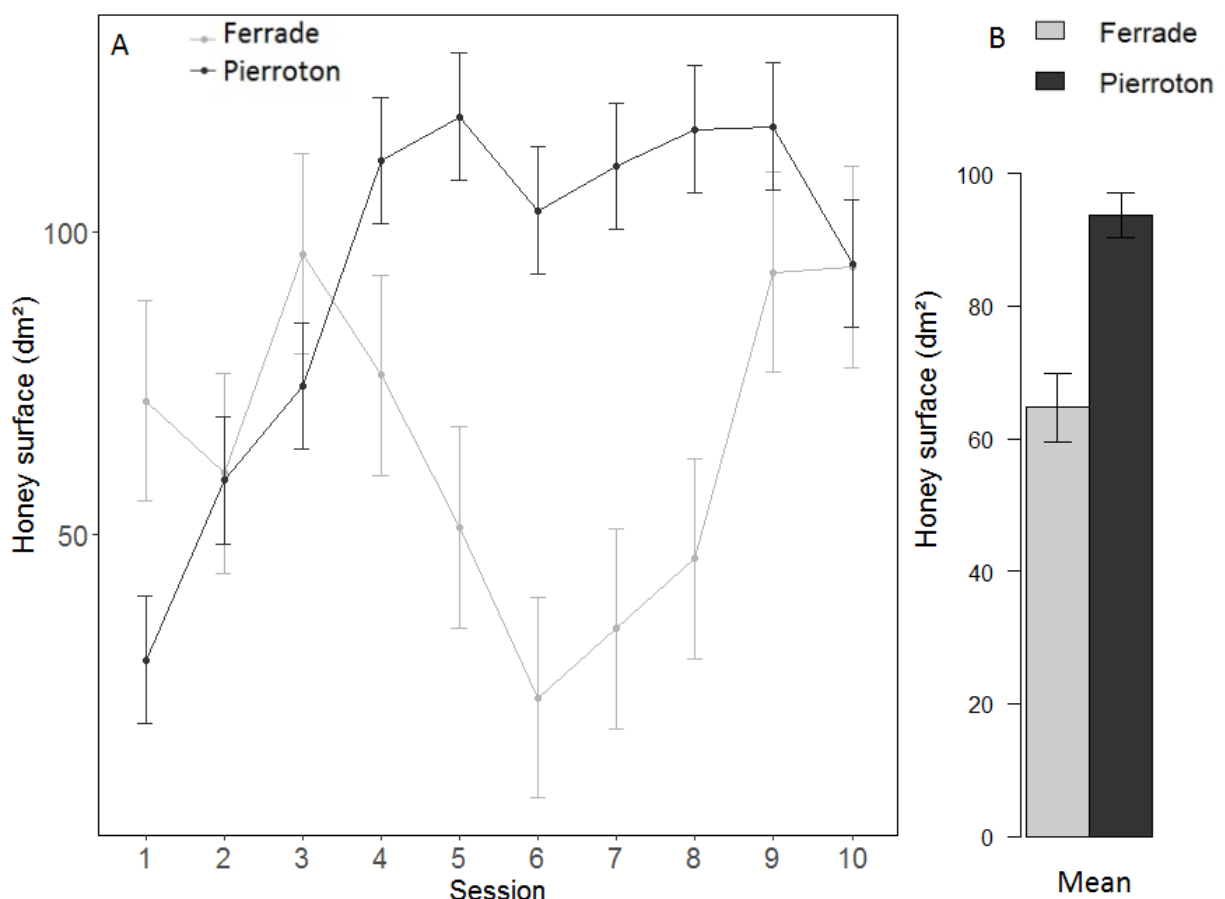
Figure 8. Environment effect on the honey surface reserves per colony (dm²). Seasonal evolution (A) and average of honey surface per environment (B), at Ferrade (light) and at Pierroton (dark), with the 95% confidence interval.

The pollen surface in the colony was the highest in early July (second session) at Ferrade and in late August (sixth session) at Pierroton (Figure 9). The variability of the pollen surface was explained by the same variables as honey, in the selected model (GLMM, n=210). Colonies at Ferrade apiary showed a lower average pollen surface over the season (13.95 dm² [6.74-21.26]), compared to colonies at Pierroton apiary (23.94 dm² [20.52-27.36]).
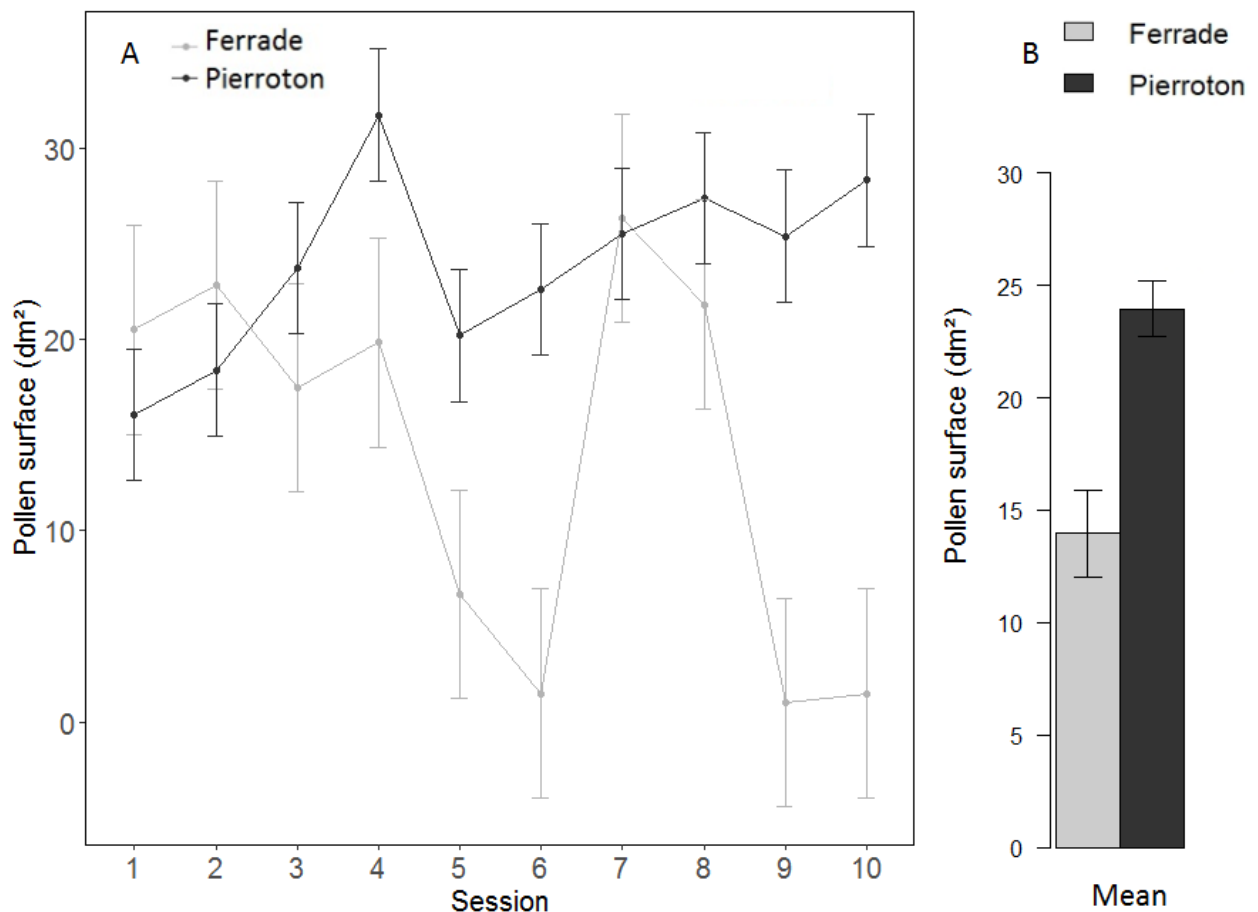
Figure 9. Environment effect on the pollen surface reserves per colony (dm²). Seasonal evolution (A) and average of pollen surface per environment (B), at Ferrade (light) and at Pierroton (dark), with the 95% confidence interval.

Table 4. GLMM of the impact of the session and the apiary on the general dynamics and on the honey surface per colony, for Buckfast. The results are presented by the estimated coefficient of the effect variable influence on the response variable, with the standard error and the 95% confidence interval of the selected model.

Response variable	Effect variable	Class	Estimate	Standard error	95% Low CI	95% Up CI
In/out ratio for Buckfast colonies	(Intercept)		0.21	0.17	-0.12	0.53
	Apiary	Pierroton	-0.49	0.18	-0.85	-0.13
Honey surface for Buckfast colonies	(Intercept)		72.00	16.55	39.56	104.44
	Session	2	-11.83	23.40	-57.70	34.04
		3	24.33	23.40	-21.54	70.20
		4	4.33	23.40	-41.54	50.20
		5	-20.83	23.40	-66.70	25.04
		6	-49.00	23.40	-94.87	-3.13
		7	-37.50	23.40	-83.37	8.37
		8	-26.00	23.40	-71.87	19.87
		9	21.33	23.40	-24.54	67.20
		10	22.17	23.40	-23.70	68.04
	Apiary	Pierroton	-42.73	19.58	-81.11	-4.35
	Session*Apiary	2*Pierroton	41.50	27.69	-12.78	95.78
		3*Pierroton	20.93	27.69	-33.34	75.21
		4*Pierroton	78.13	27.69	23.86	132.41
		5*Pierroton	110.57	27.69	56.29	164.84
		6*Pierroton	123.27	27.69	68.99	177.54
		7*Pierroton	119.10	27.69	64.82	173.38
		8*Pierroton	113.67	27.69	59.39	167.94
		9*Pierroton	66.80	27.69	12.52	121.08
		10*Pierroton	43.30	27.69	-10.98	97.58

4. Discussion

The aim of the study was to investigate about the impact of *Vespa velutina* on *Apis mellifera* colony dynamics and reserves, regarding genetic lineages and in natural conditions, to know if the predation impacts the pollination services and the beekeeper's activity. The hornet predation pressure was higher in the urban environment than in the forest area. In the apiary located in the forest area, the honeybee colony genetic lineage influenced the global dynamics and the colony reserves. Comparing the two apiaries of Buckfast colonies, the hornet predation pressure disturbed the honeybee colony general and foraging dynamics and impacted the colony reserves.

In Europe, *Vespa velutina* usually hunts honeybees from July to October (Monceau et al. 2014), when the hornet needs to feed its larvae with protein sources that are found in the muscles of the honeybee's thorax. As expected, during our study the hornets were present from July to October, at the moment when the honeybees need to storage honey resources before the winter (Winston, 1987). *Vespa velutina* commonly settles in urban environments, where the hornet can find large quantity of food and adapted nest locations (Monceau et al. 2017). In South Korea, where the Asian hornet is also an invasive species, it is largely spreading in the urban centre and parks rather than in forest areas (Choi et al. 2012). In our study, as expected, the predation pressure was high in the urban environment (Ferrade) and low in the forest environment (Pierroton). The observed difference of predation pressure on the colonies can be explained by the variance of the hornet's diet regarding its environment. The Asian hornet feeds on a variety of insects and prefers the honeybees (66%) to flies (17%), other hornets (7%) and wasps (6%) in an urban context (Villemant et al. 2011). In a forest area, *Vespa velutina* shows a balanced diet between honeybees (33%), flies (32%) and hornets (28%). Then the hornet predation pressure on honeybees might be diluted between different types of preys in a forest environment, where the system is richer in arthropods (Monceau et al. 2013b). On top of the predation dilution, the presence of hornet's nests was rare around Pierroton apiary, whereas 12 hornet's nests were found around Ferrade in a perimeter of a couple of kilometres. Then, the two honeybee genetic lineages: Buckfast and Caucasian were studied at Pierroton under an extremely low predation pressure.

Buckfast and Caucasian colonies are both selected for beekeeping activity, in part because of their easiness to handle for the beekeeper (Feltin 2013). Buckfast colonies,

being hybrid, are selected to be the gentlest honeybees, but there is no scientific data about behaviour difference between *Apis mellifera* genetic lineages. Then, in our study, we hypothesized that the Caucasians, would be more aggressive and show better abilities to defend against the hornet predator. Our results showed that the genetic lineage does not influence the proportion of bees on the flying board involved in guarding the colony. Only the seasonal effect influenced the evolution of the behaviour associated to defence. The results indicate that both genetic lineages showed a standard biological seasonal evolution of guarding behaviour, adapted to the colony's needs (Free 1954).

Knowing that the Asian hornet targets the honeybee foragers (Monceau et al. 2013a), we studied the colony global and pollen foraging dynamics. Buckfast and Caucasian colonies are also mainly selected for beekeeping activity because of their high productivity (Feltin 2013) and Buckfast are selected to be the most productive colonies. Nevertheless, not any scientific study has evaluated the difference between Buckfast and Caucasian colony dynamics, thou we hypothesized that as Buckfast are more productive they might be more active. In our study, Buckfast colonies showed an average of 10% lower general dynamics compared to Caucasian colonies. During the time windows of observation, Buckfast colonies exited more than they entered the colony, whereas Caucasians showed balanced dynamics. To understand the observed dynamics and to evaluate each genetic lineage ability of resource storage, we measured the accumulated reserves in the colonies. Buckfast usually storage more resources to survive through the winter than Caucasians which are adapted to cold winter conditions as they are from Eastern Europe (Honko et al. 2002, Cengiz et al. 2017). In our study, as expected, the Buckfast produced 12% more honey than Caucasian colonies on average. Then the results suggest that Buckfast are more efficient than Caucasians in collecting food resources, even if they fail in coming back to the colony, they are able to collect large resources. Our second hypothesis is that Buckfast shows a larger foraging range than Caucasians to forage flowers and then come back later in the day, which was not observed on the video records that lasted only 4 minutes. If Buckfast are able to forage further than Caucasians, then Buckfast can forage larger food resources that are not available to Caucasians. In the next study, we could look at the dynamics during the entire day thanks to a bee counter placed at the colony entrance to know if they really fail coming back to the colony or not. The study compared the colonies' lineages at the colony scale and in the future it would be interesting to look at the behaviour and dynamics of the genetic line at the apiary scale, under different levels of predation pressure.

Our study compared the Buckfast behaviour differences under two levels of hornet predation pressure, high in the urban environment (Ferrade) and low in the forest area (Pierroton). The honeybee defence behaviour response against *Vespa velutina* in Europe exists but it is currently not efficient enough to limit the hornet predation pressure (Monceau et al. 2018). The honeybee colony is able to recruit bees and guards on the flying board to form a 'bee-carpet' to engulf and asphyxiate the hornet (Arca et al. 2014). Surprisingly, in our study the proportions of bees involved in the defensive behaviour of the colony was similar between Ferrade and Pierroton apiaries whereas the two environments showed differences in hornet predation pressures. Our study suggests that the colonies don't adapt their defensive behaviour to the increasing predation pressure, which advocates a lack of defence ability against the hornet. Though, the highest proportion of guards was observed in September, probably because they adapted their behaviour to the colony needs to protect their reserves before the winter (Free 1954). Even though the colonies are not able to adequately respond to the predation pressure, their dynamics might be directly impacted by the hornet's presence.

Previous studies (Monceau et al. 2018, Requier et al. 2019) showed that the foraging activity is disturbed by the Asian hornet. There is a compromise between the number of bees involved in the colony defence and the number of bees in charge of foraging (Giray et al. 2000). In our study, we observed that the dynamics were 12% higher at Ferrade, where the predation pressure was high than at Pierroton, where the predation pressure was low. As expected, the observed difference shows that bees at Ferrade come back more than they exit. The study approves our hypothesis of a foraging paralysis, caused by the presence of hornets. Looking at the consequence of the lowered foraging activity, we observed the colony reserves. The colonies at Pierroton had an average of 45% of honey reserves and an average of 72% of pollen reserves higher than the colonies at Ferrade. Then the honeybee's colonies are less active and less productive under high hornet predation pressure than the colonies under low predation pressure. Our study shows that the presence of the hornet impacts the pollination services executed by *Apis mellifera* and that the beekeeping activity is disturbed by the hornet's presence. As the colonies under predation pressure forage less resources, we hypothesize that the colony populations are reduced, because they have less pollen reserves available for their larvae. We also hypothesize that as a consequence of lack of available food for the adult individuals and as a consequence of forage paralysis, the adult population proceeds

to cannibalism and eats the larvae to survive (Schmickl and Crailsheim 2001). In addition to the Asian hornet's direct impact on the honeybee colony, we suggest that the predation pressure has indirect impacts on the winter colony survival (Requier et al. 2019). The hornet presence, on the top of weakening the colonies by preventing the honeybees to forage, the hornets were observed plundering the honeybee reserves in the colony at the end of the season. We then hypothesized a large hornet impact on honeybees on the long term, on the winter colony survival. Effectively, though we observed a general winter colony loss (19%) lower than the national average (30%), we noticed a difference between the two apiaries. There was 33% of winter colony loss at Ferrade compared to 16% at Pierroton (if we consider Buckfast and Caucasian colonies). Evidently, it is not accurate to blame only the hornet predation pressure for the winter colony loss. Many parameters have to be considered as contributors to the colony loss: quality and quantity of food resources, pollution, climate change, pathogens and other predators (Goulson et al. 2015). Indeed, at Ferrade apiary, due to the urban environment, lower food reserves were noticed compared to Pierroton apiary as the Ferrade colonies had to be fed from July until the end of the season. Planting plants with melliferous flowers for the next season could be a solution to provide a sufficient food quantity and to avoid high competition between the pollinators. Moreover, for the next season the balanced sampling will be carefully respected in order to avoid an additional bias.

5. Conclusion

In a nutshell, the Asian hornet directly impacts the honeybee colonies during the summer and early autumn, in an urban environment. Our study shows that the common genetic lineages bred for beekeeping activities differ in their general colony dynamics. Overall, Buckfast colonies are more productive than Caucasian colonies under a low hornet predation pressure. Our results present the consequence of the Asian hornet high predation pressure on Buckfast colonies; the colony activity is reduced and consequently the honey and pollen reserves are reduced. Based on these results, we can presume that the beekeepers suffer from the high presence of the Asian hornet and that the bee's pollination services are negatively affected. However, it would be interesting to resume the present study to compare the response of other honeybee genetic lineages under different levels of hornet predation pressure. Moreover, further studies are needed on the long-term effects of the hornet predation on honeybee colonies. For instance, it would be interesting to study the colony's capability of learning from one season to the next season,

to know if the honeybees can show a better adaptability to the predation pressure when they have previously met the Asian hornet.

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7. Appendices

7.1. Appendix 1

Table 3. Land occupation three km around each apiary, in hectares (ha)and percent (%). Urban occupation (in brown) and vegetation (in green).

Land occupation	Ferrade (ha)	Pierroton (ha)	Total (ha)	Ferrade (%)	Pierroton (%)
Sports and leisure facilities	0.01		0.01	0.00%	
Green urban areas	118.42		118.42	4.19%	
Mineral extraction site	7.20		7.20	0.25%	
Transitional woodland-shrub		729.18	729.18		25.79%
Coniferous forest		1281.26	1281.25		45.32%
Mixed forest	12.61	57.54	70.16	0.45%	2.04%
Water bodies	6.96		6.96	0.25%	
Road and rail networks and associated lands	153.47		153.47	5.43%	
Land principally occupied by agriculture, with significant areas of natural vegetation		64.04	64.04		2.27%
Complex cultivation patterns	192.48	19.83	212.31	6.81%	0.70%
Non-irrigated arable land		525.46	525.46		18.59%
Continuous urban fabric	227.53		227.53	8.05%	
Discontinuous urban fabric	1904.18	143.82	2047.99	67.35%	5.09%
Vineyards	39.53		39.53	1.40%	
Industrial or commercial units	164.92	6.19	171.10	5.83%	0.22%
Total	**2827.32**	**2827.32**	**5654.63**	**100%**	**100%**

Author: Faustine Quiles   **Year: 2020**

To what extent are Apis mellifera colony dynamics and reserves impacted by Vespa velutina predation?

Résumé: (15 lines) (note: for non-speaking French students provide an abstract of 20-25 lines in English)

Les abeilles jouent des rôles environnementaux et économiques primordiaux. Ainsi, elles contribuent à la préservation de la biodiversité et à la production de nourriture par le biais de la pollinisation. Les butineuses *Apis mellifera* sont actuellement menacées par la prédation de *Vespa velutina*, une espèce invasive de frelon en Europe. Il est alors important de comprendre dans quelles mesures les abeilles et l'apiculture sont impactées, en fonction de la lignée génétique de la colonie et en fonction de la pression de prédation du frelon. Nous avons étudié pendant une saison, en conditions naturelles, le comportement des abeilles associé à la défense, les dynamiques générales et de butinage des colonies, à partir d'enregistrements vidéo. Nous avons également étudié la quantité des réserves par colonie, à partir de la méthode Colony Evaluation. Les résultats montrent que la lignée génétique de la colonie impacte son activité et production, sous une faible pression de prédation du frelon. L'étude prouve que les dynamiques des colonies Buckfast sont impactées par la présence du frelon et que les quantités de réserves disponibles dans la colonie sont négativement affectées. Etudier les effets de la prédation du frelon asiatique sur les colonies d'abeilles sur le long terme pourrait apporter une perspective intéressante.

Abstract: (15 lines)

The honeybees play key environmental and economical roles as they contribute to the biodiversity preservation and to food production, through pollination. Nevertheless, *Apis mellifera* foragers are currently threatened by *Vespa velutina* predation, an invasive hornet species, in Europe. Therefore, it is important to understand to what extent the honeybees and the beekeeping activity are impacted, depending on the colony genetic line and depending on the hornet predation pressure. We studied during one season, in natural conditions, the honeybee behaviour associated with defence and the general and foraging colony dynamics, based on video recording. We also studied the amount of reserves per colony based on Colony Evaluation method. The results show that the colony genetic lineage has an impact on the colony activity and productivity under a low hornet predation pressure. The study proves that Buckfast colony dynamics are impacted by the hornet presence and that the amount of reserves available in the colony are negatively affected. Studying the long-term effects of the Asian hornet predation on weakened honeybee colonies may provide an interesting perspective.

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