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**Original article**

# Preliminary report of honeybee physiological changes pre- and post-hybrid lavender season in high and low weight gain colonies

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**Abstract –** Pollinators foraging on monocultures like hybrid lavender are suspected to suffer physiological stress. We used biomarkers to assess changes in honeybee physiology pre- and post-hybrid lavender season, in high and low weight gain colonies. We observed post-season increased levels of enzymes involved in antioxidant defenses: catalase, superoxide dismutase, and glutathione peroxidase; increased levels of acetylcholinesterase, a biomarker of neural transmission; and decreased levels of alkaline phosphatase associated with the loss of midgut homeostasis. Compared to low weight gain colonies, high weight gain colonies showed lower levels of almost all analyzed biomarkers. We discuss the environmental and beekeeping factors likely underlying observed changes for which cause and effect remain to be established.

**honeybee / hybrid lavender / monoculture / physiology / stress**

## 1. INTRODUCTION

One of the primary causes of worldwide pollinator decline is known to be agricultural activities that alter natural landscapes (Potts et al. 2010; Goulson et al. 2015). Until now, only a few studies have addressed pollinator health by investigating, at the landscape level, the physiological integrity of organisms (Alaux et al. 2017; Simone-Finstrom et al. 2016; Vanbergen and the Insect Pollinators Initiative 2013). Yet, physiological responses represent a valuable source of information about an animal's adaptation to a variety of conditions (Le Maho 2002). Therefore, it seems crucial to improve our knowledge on pollinator health through the study of the physiological condition of a common pollinator, the Western honeybee (*Apis mellifera* L.), inhabiting monoculture environments.

Monocultures are common foraging landscapes in migratory honeybee management (Nicholls and Altieri 2012), though dramatic consequences of intensive farming have been observed for bees, via habitat degradation and fragmentation, including the loss of plant diversity and increased agrochemical pressure (Potts et al. 2010). Similarly, monocultures of lavender have continuously expanded since the 1950s in southeastern France, replacing formerly wild lavender

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fields where beekeeping was traditionally practiced (Candaele and Chaisse 2015). At present, 80% of lavender fields for essential oil production are composed of *Lavandula intermedia* clones, i.e., hybrids of *Lavandula angustifolia x Lavandula latifolia* commonly named "lavandin" (ADAPI 2012a). Hybrid lavender monocultures are known to produce abundant nectar but protein-deficient pollen (Barbier 1963). This pollen is usually absent from hybrid lavender honeys, suggesting that honeybees do not harvest it (unpubl.data). Therefore, it is suspected that colonies foraging in hybrid lavender landscapes suffer from nutritional stress, especially when secondary flora is scarce and/or has a low nutritional value for honeybees (Barbier 1963; ADAPI 2012b; Somerville and Nicols 2016). Physiological changes in honeybees after nutritional stress could be assessed through alkaline phosphatase (AP) activity, a biomarker of intestinal homeostasis and health condition (Lallès 2014). In mammals, AP is associated with multiple intestinal functions and low levels are related to malnutrition (Lallès 2014). In insects, AP shows strong activity in the midgut where it is involved, at least, with the absorption of metabolites and transport mechanisms (Eguchi 1995). In honeybees, low levels of AP were observed after infection with an intestinal parasite that damages the midgut epithelium and captures host ATP (Dussaubat et al. 2012).

Monoculture environments might also weaken honeybee health by inducing oxidative stress (Simone-Finstrom et al. 2016), which occurs when the balance between the production of reactive oxygen species (ROS) and antioxidant defenses is disrupted (Beaulieu and Costantini 2014). In healthy periods, ROS are normal byproducts of aerobic metabolism and other biological functions such as immune cell activity and detoxification, but an overproduction of ROS may induce oxidative damage to biological tissues (Beaulieu and Costantini 2014). Oxidative stress can be triggered in honeybees by multiple factors as varied as nutrition, exposure to xenobiotics, diseases, physical exertion, and aging (Balfour et al. 2013; Dussaubat et al. 2016; Kairo et al. 2016; Lipinzki and Zóltowska 2005; Margotta et al. 2018; Simone-Finstrom et al. 2016). Migratory beekeeping can also trigger oxidative stress, especially when migration occurs during immature stages, with long-term effects in adults (Simone-Finstrom et al. 2016). Honeybee oxidative stress has previously been assessed through the activity of three major antioxidant enzymes: catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GP) (Beaulieu and Costantini 2014; Dussaubat et al. 2016).

In honeybees, the neurotoxicity induced by exposure to insecticides has been estimated through acetylcholinesterase activity (AChE), an enzyme involved in the precise control of neurotransmission by hydrolyzing the neurotransmitter acetylcholine in the cholinergic synapses (Badiou-Bénéteau et al. 2012). Previous toxicological studies reported increased levels of AChE in surviving honeybees after exposure to deltamethrin (pyrethroid) (Badiou et al. 2008) and in immature stages, but not in adult honeybees, after exposure to thiamethoxam (neonicotinoid) (Badiou-Bénéteau et al. 2012; Tavares et al. 2017). Conversely, exposure to organophosphates and carbamates or other pollutants, like nanoparticles, causes a decrease in AChE activity as observed in humans (Lionetto et al. 2013) and in honeybee drones due to fipronil (phenylpyrazole) (Kairo et al. 2017).

Here, we investigated the modulation of these five biological markers in the honeybee to assess changes in intestinal functions (AP), antioxidant defenses (CAT, SOD, and GP), and neural transmission (AChE). Such physiological changes were suspected in honeybee colonies transferred to hybrid lavender monocultures because nutritional stress may occur, along with pressure from parasites and agrochemical exposure. The physiological status of the colonies was expected to reflect their production performance measured as final colony weight gain.

## 2. MATERIALS AND METHODS

This study was carried out in a zone producing lavender essential oils located in southeastern France. It was part of APIMODEL, a long-term field survey program set up to monitor honeybee colonies in a context of real-world professional beekeeping practices (Kretzschmar et al. 2016).

*Apiary description*Migratory apiaries were placed next to hybrid lavender fields (*Lavandula angustifolia x Lavandula latifolia* ) in the summer of 2013 and 2014. Data from both years were pooled resulting in 12 apiaries in total (see "Statistical analysis" for further information). Apiaries belonged to 7 professional beekeepers: 1 beekeeper with 2 apiaries in 2013; 2 beekeepers, each with 2 apiaries, in 2013; and one apiary in 2014; and 4 beekeepers, each with one apiary, in 2014. Apiary sites were located in southeastern France in the regions of "Provence-Alpes-Côtes d'Azur" (PACA) and "Auvergne Rhône-Alpes," which is a Mediterranean area characterized by 3 lavender production zones: (i) the Drôme valley at ± 300 m above sea level (MASL), where lavender fields are mainly surrounded by vineyards, (ii) the Valensole plateau at ± 500 MASL, where lavender fields are mainly surrounded by cereal production, and (iii) the Albion plateau up to the mountain of Lure at ± 800 MASL, which is a mostly semi-natural landscape. The 12 apiaries were distributed over 10 different sites: 4 sites in the Drôme valley, 3 sites in the Valensole plateau, and 3 sites in the Albion plateau/mountain of Lure; two apiaries were placed at the same site for both years, at the Albion plateau/mountain of Lure and at the Valensole plateau. GPS coordinates and a map of apiary sites are given in Online Resources 1(a-b).

Apiary management during the lavender nectar flow was similar for all apiaries and consisted mainly of adding honey supers to hives (i.e., new boxes with combs for honeybees to store nectar). During that period, there were no swarms nor *Varroa destructor* treatments and supplemental feeding was not necessary. Most hives were Dadant type but with some exceptions of Langstroth hives; all hives were equipped with queen excluders located above the first deep box, from the beginning until the end of the trial. Information on beekeeping management prior to the lavender season was not provided by beekeepers, and thus, we assumed that the apiaries had different backgrounds.

*Colony weight gain and honeybee samples*Beekeepers installed around 48 colonies per apiary, from which 20 colonies in 2013 and 24 colonies in 2014 were chosen to perform weight gain measures. Only those with no signs of disease and a productive queen were chosen. Biomarker analysis was done on honeybee samples from the 5 colonies exhibiting the highest final weight gains (HIGH) and the 5 colonies exhibiting the lowest final weight gains (LOW), to account for opposing colony production performances. Honeybee samples for biomarker analysis were collected at two time points: pre-season (PRE) at the beginning of nectar secretion in mid-June corresponding to the boreal summer, and post-season (POST) before plants were harvested for oil extraction in mid-August.

Honeybee colonies were weighed PRE and POST to obtain their final weight gain. For PRE and POST weight measures, the main body and suppers of each colony were weighed separately using electronic scales.

As part of the APIMODEL program, two other experimental handlings were performed on the colonies in parallel with our trial. (i) After PRE weight measuring (see above) and until the end of the trial, colonies were weighed every 2 days (data not shown). The weighing method consisted of an electronic scale adapted to a mechanical pallet lifter (see Online Resources 1(c-d) for scale pictures). Each colony was placed over an individual pallet, and the pallet with its colony was lifted for 30 s. This enabled us to weigh each colony on-site without displacing or opening it and with no other intervention except when smoking was necessary. (ii) Colonies were evaluated with the ColEVaL method, a quantitative description of adult honeybees and brood surface (Hernandez et al. 2020) (data not shown). The weighing method and ColEval were designed to exert minimum stress on honeybee colonies. Honeybee sampling for biomarker analysis was carried out before weighing and ColEval to avoid honeybee stress.

For physiological marker analysis, honeybees were collected in plastic bags. To minimize honeybee stress and post-sampling variation of biomarkers, honeybees were immediately anesthetized with $CO_2$ and frozen at − 20 °C in portable freezers. At the laboratory, samples were stored at − 20 °C until analysis.

To obtain a consistent representation of the worker honeybee population, sampling was carried out from the surface of combs containing eggs, young larvae, and sealed brood where the distribution of successive age classes, according to van der Steen et al. (2012), is similar between combs (41% of one-, 23% of two-, 17% of three-, 11% of four-, and 8% of 5-week-old honeybees).

*Biomarkers*The enzymatic activity was spectrophotometrically measured in the midgut for AP (AP-midgut), in the midgut and head for CAT (CAT-midgut and CAT-head) and SOD (SOD-head and SOD-midgut), and in the head for GP (GP-head) and AChE (AChE-head). Enzyme assays were carried out on 30 worker honeybees per colony, pooled by 3 heads or midguts, to obtain 10 pools (sub-samples). Enzyme extraction was modified from Badiou-Bénéteau et al. (2012). Briefly, bee tissue was homogenized at 4 °C with a TissueLyser (Qiagen) (5 × 10 s at 30 Hz) in phosphate buffer pH 7.4 (10 mM NaCl, 1% (w/v), Triton X-100, and 40 mM sodium phosphate, containing 2 mg/mL of antipain, leupeptin, and pepstatin A; 25 U/mL of aprotinin, and 0.1 mg/mL of trypsin inhibitor, as protease inhibitors), to make a 10% (w/v) extract. The homogenates were then centrifuged at 15,000×*g* for 20 min at 4 °C and resulting supernatants were used immediately for analysis of enzyme activities. All enzyme assays were performed in triplicate and results were expressed as milli-units of absorbance/minute per mass of tissue (mUA/min/mg of tissue).

*Catalase* (CAT-head and CAT-midgut) was measured according to a slightly modified procedure of Beers and Sizer (1952), in a medium containing 30 mM $H_2O_2$ and 100 mM sodium phosphate at pH 7.0. The reaction was monitored through the decrease in absorbance at 240 nm due to the consumption of $H_2O_2$ at 25 °C.

*Superoxide dismutase* (SOD-head and SOD-midgut) activity was indirectly measured at 25 °C as the rate of reduction of nitroblue tetrazolium when the superoxide anion radical was generated during the oxidation of xanthine by xanthine oxidase, as described by Boldyrev et al. (2001). The reaction mixture contained 50 mM sodium carbonate, 0.1 mM EDTA, 0.1 mM xanthine, 0.0833 U/mL xanthine oxidase, 0.025 mM nitroblue tetrazolium, and 50 mM sodium-potassium phosphate pH 7.8. The enzyme activity was measured at 560 nm.

*Glutathione peroxidase* (GP-head) activity was monitored at 340 nm and 25 °C in a reaction mixture containing 50 mM Na/K phosphate buffer pH 7.4, containing 1 mM EDTA, 0.16 mM NADPH, 0.85 mM reduced glutathione, 0.25 U/mL glutathione reductase, and 0.2 mM tert-butyl hydroperoxide, modified from Boldyrev et al. (2001).

*Acetylcholinesterase* (AChE-head) activity was measured at 412 nm and 25 °C in a medium containing 0.3 mM acetylthiocholine iodide (AcSCh.I), 1.5 mM 5,5′-dithiobis(2-nitrobezoic acid) (DTNB), and 100 mM sodium phosphate pH 7.0 (Belzunces et al. 1988).

*Alkaline phosphatase* (AP-midgut) activity was assayed at 25 °C in a medium containing 20 μM of $MgCl_2$, 2 mM of *p* -nitrophenyl phosphate as a substrate, and 100 mM Tris-HCl pH 8.5 (Bounias et al. 1996). The reaction was measured at 410 nm.

### 2.1. Statistical analysis

We used R version 3.6.0 (2019-04-26), RStudio version 1.2.1335, and package *lme4* (Bates et al. 2015) to perform a linear mixed-effect analysis of the relationship between "enzyme activity," "sampling time point," and colony "final weight gain." As fixed effects, we entered into the model "sampling time point" (PRE and POST) and "final weight gain" (HIGH and LOW) without an interaction term, which was previously verified as statistically insignificant for all biomarkers. Enzyme activity PRE/POST was paired data because honeybee samples were taken from the same colonies on both dates; this type of data is taken into account by linear mixed models. As random effects, we had intercepts and slopes for "apiary" considering the "colonies" in an apiary. Apiaries from both years 2013 and 2014 were pooled (a total of 12 apiaries) given that apiaries were not actually repetitions from 1 year to another because (i) sites were all different with the exception of two and (ii) beekeepers rebuild apiaries with their best colonies just before lavender

season. Thus, the variability from year, site, and production zone was represented in the term "apiary." This structure of the random effect enables characterizing the variation between apiaries due to individual differences, such as secondary flora, climate, sites, production zones, year, previous beekeeping practices, and genetic background. The better-fit model was chosen based on AIC and ANOVA comparisons among alternative models.

Homoscedasticity and normality were checked to confirm the use of a linear Gaussian mixed model (Winter 2013). *p*-values were obtained using package *lmerTest* (Kuznetsova et al. 2017). We checked for influential data points using package *influence.ME* (Nieuwenhuis et al. 2012); no data exclusion was necessary.

## 3. RESULTS

The linear mixed-effect model found significant effects of "sampling time point" (PRE/POST) for almost all biomarkers: CAT-head, CAT-midgut, SOD-head, GP-head, AChE-head, and AP-midgut, with the exception of SOD-midgut (Figure 1). Only the AP-midgut model showed a negative estimate for "sampling time point," because of the decrease of enzyme activity levels from PRE to POST; all other "sampling time point" estimates were positive as enzyme activity levels increased from PRE to POST (Table I). The linear mixed-effect model also found significant effects of "final weight gain" for all biomarkers except GP-head (Figure 2); all estimates were negative and thus associated with lower levels of enzyme activities in HIGH compared to LOW colonies (Table I). See Online Resources 1(e) for distribution of colony weight gain (kg) in HIGH and LOW classes. Previously tested interaction terms between "sampling time point" and "final weight gain" were not statistically significant for all biomarkers.

## 4. DISCUSSION/CONCLUSION

Post-season honeybee colonies compared to pre-season colonies showed increased levels of biomarkers involved in antioxidant defenses and neural transmission, with decreased levels of the biomarker involved in

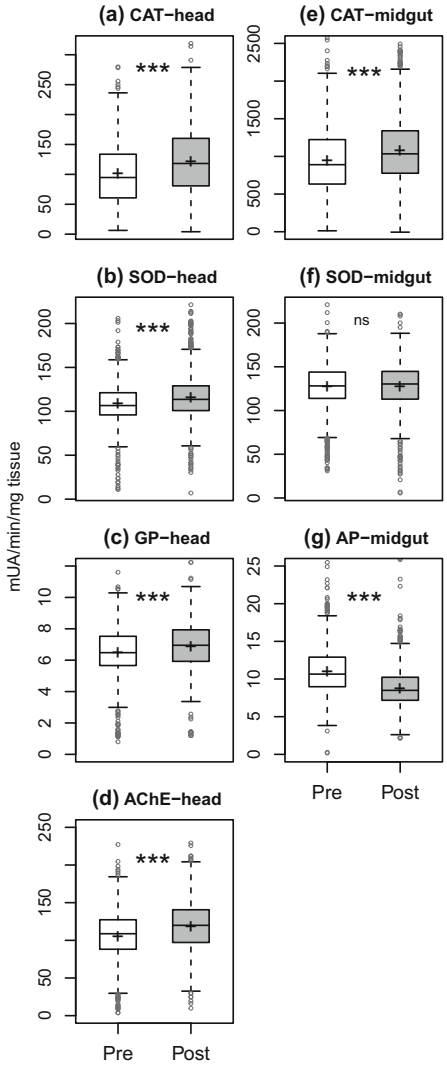



**Figure 1.** Honeybee enzyme activities PRE- and POST-hybrid lavender foraging season are shown for (**a**) catalase in the head, (**b**) superoxide dismutase in the head, (**c**) glutathione peroxidase in the head, (**d**) acetylcholinesterase in the head, (**e**) catalase in the midgut, (**f**) superoxide dismutase in the midgut, and (**g**) phosphatase alkaline in the midgut. Boxplots show the 1st and 3rd interquartile range with line denoting median and plus sign denoting mean; whiskers encompass 90% of 120 colonies beyond which outliers are represented by circles. Scale limit for the *y*-axis was settled to better visualize **c**, **d**, **e**, and **g** graphics. For each biomarker, statistical significance between PRE and POST is indicated at the top of the graphic by "*" when < .05, "**" when < .01, "***" when < .001, and "ns" when no significant differences were detected by the linear mixed model.

**Table I..** Statistical details of coefficients from the seven biomarkers' linear mixed models. The estimate, standard error (SE), degree of freedom (Df), *t*-value, and corresponding *p*-value are shown for each biomarker. All of the biomarker models include as fixed effects "time point" (PRE/POST) and "final weight gain" (HIGH/LOW), and as random intercept and slope "apiary" considering "colonies." The estimated coefficient for "time point" is the calculated slope using POST vs. PRE as the reference category, and the estimated coefficient for "final weight gain" is the slope using HIGH vs. LOW as the reference category.

| Dependent variable | Categorical fixed effect | Estimate | SE | Df | Statistics *t*-value | *p*-value |
|---|---|---|---|---|---|---|
| CAT-head | Time point: post | 18.234 | 1.480 | 2049.08 | 12.323 | <2e−16*** |
| | Final weight gain: high | −8.688 | 1.585 | 1318.70 | −5.483 | 5.0le−08*** |
| CAT-midgut | Time point: post | 140.66 | 14.19 | 2098.90 | 9.431 | <2e−16*** |
| | Final weight gain: high | −57.22 | 16.35 | 2067.87 | −3.501 | 0.000474*** |
| SOD-head | Time point: post | 7.1646 | 0.8523 | 2296.70 | 8.406 | <2e−16*** |
| | Final weight gain: high | −3.8266 | 0.9302 | 2086.45 | −4.114 | 4.05e−05*** |
| SOD-midgut | Time point: post | −0.7456 | 0.7419 | 2335.14 | −1.005 | 0.31503 |
| | Final weight gain: high | −2.3197 | 0.8153 | 2327.02 | −2.845 | 0.00448** |
| GP-head | Time point: post | 0.40845 | 0.05874 | 2121.42 | 6.954 | 4.72e−12*** |
| | Final weight gain: high | −0.10117 | 0.06328 | 1722.74 | −1.599 | 0.11 |
| AP-midgut | Time point: post | −2.2552 | 0.1343 | 2194.90 | −16.786 | <2e−16*** |
| | Final weight gain: high | −0.6136 | 0.1460 | 1830.49 | −4.202 | 2.77e−05*** |
| AChE-head | Time point: post | 12.502 | 1.254 | 2317.4 | 9.969 | <2e−16*** |
| | Final weight gain: high | −2.980 | 1.337 | 1355.82 | −2.228 | 0.026* |

midgut homeostasis (Figure 1). Regardless of the time point (PRE and POST combined), HIGH weight gain colonies showed lower levels for most biomarkers (Figure 2) suggesting a healthier physiological condition than LOW weight gain colonies, with the exception of the biomarker for midgut homeostasis.

Post-season results agree with previous studies on migratory colonies foraging in monoculture landscapes of poor nutritional value, where colonies showed low nutritional stores (vitellogenin, insulin-like peptide 1, lipids generally), high levels of humoral immunity (Smart et al. 2016), and high oxidative stress (lipid peroxidation) (Simone-Finstrom et al. 2016).

Based on scientific literature, we speculate that key factors common to hybrid lavender monocultures triggering post-season increased levels of oxidative stress biomarkers, as we observed (Figure 1), may be related to nutritional stress because of both the poor protein content of hybrid lavender pollen and insufficient quality and/or quantity of secondary pollen (Barbier 1963; Simone-Finstrom et al. 2016), defense responses against parasites (Lipinzki and Zóltowska 2005; Dussaubat et al. 2012), sublethal exposure to pesticides (Dussaubat et al. 2016; Kairo et al. 2016), and colony migration (Simone-Finstrom et al. 2016). Another factor, not yet demonstrated in honeybees, is that lavender essential oils upon contact exposure may elicit antioxidant responses (Hancianu et al. 2013). Colony handling may also stress honeybees; however, standard beekeeping and experimental procedures carried out during the season (see Materials and Methods) were conceived to minimize any long-term effect on honeybee physiology.

Colonies with HIGH weight gain showed lower levels of oxidative-stress biomarkers compared to LOW colonies (Figure 2). Interestingly, Kretzschmar et al. (2016) observed that colonies with strong weight gain had lower initial parasitic infestations of *V. destructor* and had a younger population by the second half of the season, compared to colonies that accumulated little weight. Both factors, *V. destructor*

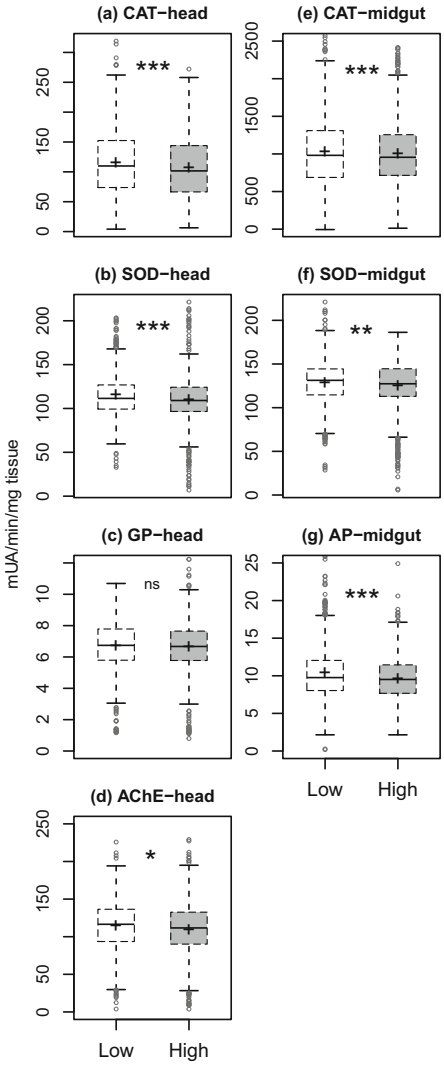



**Figure 2.** Honeybee enzyme activities of LOW and HIGH weight-gain colonies placed at hybrid lavender monocultures are shown for (**a**) catalase in the head, (**b**) superoxide dismutase in the head, (**c**) glutathione peroxidase in the head, (**d**) acetylcholinesterase in the head, (**e**) catalase in the midgut, (**f**) superoxide dismutase in the midgut, and (**g**) phosphatase alkaline in the midgut. Boxplots show the 1st and 3rd interquartile range with line denoting median and plus sign denoting mean; whiskers encompass 90% of 120 colonies beyond which outliers are represented by circles. Scale limit for the *y*-axis was settled to better visualize **c**, **d**, **e**, and **g** graphics. For each biomarker, statistical significance between LOW and HIGH is indicated at the top of the graphic by "*" when < .05, "**" when < .01, "***" when < .001 and "ns" when no significant differences were detected by the linear mixed model.

(Lipinzki and Zóltowska 2005) and aging (Margotta et al. 2018), are known to trigger oxidative stress in honeybees. Biomarkers CAT and SOD in the head are not sensitive to aging (Margotta et al. 2018). Therefore, in our study, low levels of oxidative stress biomarkers in HIGH colonies might be more likely related to a low parasitic charge than to a younger population.

The low levels of AP observed post-season support the hypothesis of increased nutritional stress in honeybees. Low AP levels have been associated with malnutrition in mammals and subsequent loss of midgut homeostasis (Lallès 2014) and with disturbed intestinal functions in honeybees (Eguchi 1995). This hypothesis agrees with the inhibition of the queen's egg laying observed in colonies foraging on hybrid lavender (Barbier 1963), a phenomenon that can be associated with nutritional stress (Schmickl and Crailsheim 2004).

We were surprised to observe lower levels of AP activity in HIGH weight gain colonies than LOW weight gain colonies (Figure 2). We speculate that nutritional stress might be stronger in HIGH colonies because they are more likely to be characterized by a younger population as previously mentioned (Kretzschmar et al. 2016) and therefore have greater nutritional needs than LOW colonies. Other markers of nutritional status like lipid content, fat body, or vitellogenin might be helpful to further pursue this question.

The observed higher AChE post-season levels indicate an increased activity of neural transmission. In terms of exposure to pesticides, such an increase in AChE activity may be observed after contamination by substances targeting the voltage-dependent sodium channel, like pyrethroid insecticides (Badiou et al. 2008). Exposure to environmental pesticides accumulated in the surrounding flora could explain this phenomenon, given that residues of agrochemicals were detected on secondary pollen from pollen traps at the same apiaries but from colonies different than those in our study (Kretzschmar et al. 2016). However, further data are needed to link the AChE response to the sublethal acute and chronic exposure to pesticides believed to be used on surrounding crops.

Lower AChE levels were observed on HIGH weight gain colonies compared to LOW weight gain colonies (Figure 2). Because it stands to reason that both HIGH and LOW colonies were equally exposed to environmental pollutants, if they were, such differences could hardly be explained by a differential exposure to agrochemicals. Interestingly, AChE activity has been demonstrated to be lower in forager than "nurse" honeybees, probably favoring cholinergic neurotransmission with a

subsequent increase in cognitive capacities needed to carry out foraging tasks (Shapira et al. 2001). This suggests a demographic-based physiological difference with behavioral consequences between HIGH and LOW weight gain colonies, to be further investigated.

In conclusion, the pre-/post-season physiological changes in honeybees reported here seem to agree with the increasing scientific evidence showing monocultures as stressful anthropogenic landscapes for pollinators (Alaux et al. 2017; St. Clair et al. 2020; Dolezal et al. 2019; Simone-Finstrom et al. 2016; Smart et al. 2016; reviews: Goulson et al. 2015; Nicholls and Altieri 2012; Potts et al. 2010; Vanbergen and the Insect Pollinators Initiative 2013). We speculate that nutritional and toxicological stress from the hybrid lavender environment, as well as parasitic load and colony migration, could be the main pressures underlying the observed physiological changes. The individual contributions of these factors to the observed physiological changes remain to be studied by comparing under controlled nutritional conditions and health status and by contrasting with sedentary beekeeping. Colony weight gain groups were clearly characterized by different physiological profiles, regardless of the sampling time point, suggesting a potential use of biomarkers as predictors of colony performance.

## SUPPLEMENTARY INFORMATION

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## AUTHOR CONTRIBUTIONS

AM, AK conceived study; CD, AM, AK, JLB designed survey; AM, AK, CD field work; CD laboratory; CD, AK analyzed data; CD writting. CD, AM, AK, JLB, LPB discussed results, revision

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## DATA AVAILABILITY

The dataset generated and analyzed during the current study is given in Online Resource 2.

## COMPLIANCE WITH ETHICAL STANDARDS

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated invertebrate species.

**Rapport préliminaire sur les changements physiologiques des abeilles avant et après la saison de la lavande hybride - une comparaison des colonies ayant une forte et une faible prise de poids**

**Abeille mellifère / lavande hybride / monoculture / physiologie / stress.**

**Ein vorläufiger Bericht über physiologische Veränderungen bei der Honigbiene vor und nach der Saison von Lavendelhybriden - ein Vergleich von Völkern mit starker und schwacher Gewichtszunahme.**

**Honigbiene / Hybridlavendel / Monokultur / Physiologie / Stress.**

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