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Effect of agroecological measures on honey bee colony development and survival in the Swiss context

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“Effect of agroecological measures on honey bee colony development and survival in the Swiss context”

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Abstract

Agroecological measures refer to all techniques that permit agricultural practices to be more respectful to the environment and biodiversity. Agroecological measures such as delayed mowing or providing floral resources in fields for pollinators to reduce the impact on the entomofauna can be implemented. In western Switzerland, farmers have implemented agroecological measures in meadows to be tested in the field of the “Agriculture & Pollinisateurs” project to verify their effectiveness on honey bee pollinator health. The measures tested concern specifically relinquishing the non-use of conditioner while mowing, implementing floral resource strips, and delayed mowing without conditioner in meadows. For this, volunteer beekeepers provided access to 300 honey bee colonies in order to determine how these agroecological measures influence their development and their winter survival.

The general objective of this thesis is to analyze the influence of this set of agroecological measures in meadows on (i) colony development in summer, (ii) winter mortality, and (iii) to determine the landscape types under which these agroecological measures are effective in supplying resources to honey bees. To measure the colony development, the ColEval method was developed, tested, and implemented. Before being able to evaluate the effect of these agroecological measures on honey bee colonies, it was important to consider the effect of the ectoparasitic mite *Varroa destructor*, which negative impact on colonies can mitigate the beneficial effects of agroecological measures on colony development. Therefore, we also verified if the treatment recommendations against *V. destructor* were followed by beekeepers and measured potential deviation in compliance and their consequences for the colonies.

The results obtained show that compliance with recommended *V. destructor* treatment regimens decreases infestation rates by this parasite and improves the survival of honey bee colonies over winter. After communicating the apparent link between low compliance and poor colony survival at the end of the first year to the beekeepers, we observed better compliance and increased colony survival in the second year. Following these observations on the treatments and therefore on the improvement of the sanitary conditions in the monitored apiaries, we described and statistically measured the varroa factor in order to be able to determine the effect on colony size in summer and autumn of the no-conditioner use, the implementation of floral resource strips and the delayed mowing combined with the use of no-conditioner while mowing temporary meadows. These measures had a positive effects on colony size likely contributed to the increased overwintering success. Moreover, our data suggest that in summer, areas where more than 76 hectares of agroecological measures were implemented, provided additional floral resources (compared areas with low agroecological density) for honey bee colonies in the field crops landscapes.

Our results show that selected agroecological measures in agricultural landscapes can benefit colonies provided that treatments against *V. destructor* are under control to ensure honey bee colony health.
The originality and interest of this study lies in its spatial scale covering a large part of western Switzerland, in the long-term follow-up of the colonies and in the fact that an interdisciplinary team of scientists worked in close collaboration with beekeepers, farmers and political decision-makers.

Keywords: agricultural landscapes, agroecological measures, agri-environmental schemes, *Apis mellifera*, colony size, honey bee, meadow management, winter colony losses, beekeeping management, compliance, pest control, *Varroa destructor*, field surveys

Résumé

Les mesures agroécologiques désignent l'ensemble des techniques qui permettent aux pratiques agricoles d'être plus respectueuses de l'environnement et de la biodiversité. Celles-ci peuvent être mises en œuvre dans les champs, comme le retardement de la fauche ou la mise à disposition de ressources florales dans les champs pour les pollinisateurs afin de réduire l'impact sur l'entomofaune. En Suisse romande, des agriculteurs ont mis en place des mesures agroécologiques dans des prairies qui seront testées sur le terrain dans le cadre du projet "Agriculture et pollinisateurs" afin de vérifier leur efficacité sur la santé des abeilles domestiques. Les mesures testées concernent notamment le renoncement à l'utilisation d'un éclateur lors de la fauche, la mise en place de bandes de ressources florales et la fauche retardée sans conditionneur dans les prairies. Pour cela, des apiculteurs volontaires ont donné accès à 300 colonies d'abeilles domestiques afin de déterminer comment ces mesures agroécologiques influencent leur développement et leur survie hivernale.

L'objectif général de cette thèse est d'analyser l'influence de cet ensemble de mesures agroécologiques dans les prairies sur (i) le développement des colonies en été, (ii) la mortalité hivernale, et (iii) de déterminer les types de paysages dans lesquels ces mesures agroécologiques sont efficaces pour fournir des ressources aux abeilles domestiques. Pour mesurer le développement des colonies, la méthode ColEval a été développée, testée et mise en œuvre. Avant de pouvoir évaluer l'effet de ces mesures agroécologiques sur les colonies d'abeilles domestiques, il était important de considérer l'effet de l'acarien ectoparasite *Varroa destructor*, dont l'impact négatif sur les colonies peut atténuer les effets bénéfiques des mesures agroécologiques sur le développement des colonies. Par conséquent, nous avons également vérifié si les recommandations de traitement contre *V. destructor* étaient suivies par les apiculteurs et mesuré les écarts potentiels de conformité et leurs conséquences sur les colonies.

Les résultats obtenus montrent que le respect des régimes de traitement recommandés contre *V. destructor* diminue les taux d'infestation par ce parasite et améliore la survie des colonies d'abeilles mellifères pendant l'hiver. Après avoir communiqué aux apiculteurs le lien apparent entre une faible conformité et une faible survie des colonies à la fin de la première année, nous avons observé une meilleure conformité et une augmentation de la survie des colonies la deuxième année. Suite à ces observations sur les traitements et donc sur l'amélioration des conditions sanitaires dans les ruchers suivis, nous avons décrit et mesuré statistiquement le facteur varroa afin de pouvoir mesurer un effet positif sur la taille des colonies en été et en automne de la non utilisation de l'éclateur, de la mise en place de bandes fleuries et du retard de fauche combiné à la non utilisation de l'éclateur lors de la fauche des prairies temporaires. Ces effets positifs sur la taille des colonies ont probablement contribué à l'augmentation du succès de l'hivernage. En outre, nos données suggèrent qu'en été, les zones où plus de 76 hectares de mesures agroécologiques ont été mises en œuvre, ont fourni des ressources florales supplémentaires (par rapport aux zones à faible densité agroécologique) pour les colonies d'abeilles domestiques dans les paysages de grandes cultures.

Nos résultats montrent que des mesures agroécologiques sélectionnées dans les paysages agricoles peuvent être bénéfiques pour les colonies, à condition que les traitements contre *V. destructor* soient maîtrisés pour assurer la santé des colonies d'abeilles domestiques. L'originalité et l'intérêt de cette étude résident dans son échelle spatiale couvrant une grande partie de la Suisse occidentale, dans le suivi à long terme des colonies et dans le fait qu'une équipe interdisciplinaire de scientifiques a travaillé en étroite collaboration avec des apiculteurs, des agriculteurs et des décideurs politiques.

Mots clés : paysages agricoles, mesures agroécologiques, programmes agroenvironnementaux, *Apis mellifera*, taille des colonies, abeille domestique, gestion des prairies, pertes de colonies en hiver, gestion apicole, conformité, lutte contre les parasites, *Varroa destructor*, enquêtes sur le terrain.

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« Si no escalas las montañas jamás podrás disfrutar el paisaje » - Pablo Neruda

Preface

This thesis manuscript has been organized in the "by articles" format, comprising a general introduction, four chapters in the form of scientific articles, three of which are published in peer-reviewed journals and a general discussion.
An "Appendices" section will follow, including all the supplementary materials of each chapter; and a "Thesis-related publications and communications" section which includes additional articles produced during this thesis and a list of conference participation.

Contents

Chapter 1
Introduction

1 Introduction

1.1 Context and issues of agriculture and pollinators

The common honeybee, *Apis mellifera*, is the most important managed insect pollinator in agriculture for many crops and thus contributes to increase the yield of human food production (Garibaldi et al., 2017). For honeybee colonies to be healthy and thus be able to deliver their pollination services, they need to feed on diverse nectar and pollen sources (Di Pasquale et al., 2013). However, many studies highlight poor floral resources as a cause of honeybee colony losses in agricultural agroecosystems (Odoux et al., 2014; Requier et al., 2015).

The evolution of agricultural production systems during the second half of the twentieth century brought important changes to the development of the rural territory, mainly through agricultural intensification, fragmenting and alteration of natural ecosystems (Bernard et al., 2006).

Livestock areas have also undergone profound changes as a result of the decline of natural grasslands with composite flora, their early mowing in spring, the expansion of grasslands dominated by grasses and few melliferous plants (as Fabaceae), and the frequent increase in animal load per hectare (Decourtye et al., 2005). The repercussions of these changes in practices can be observed in plant communities whose pollinator populations are closely dependent of nectar and pollen resources (Jauzein, 2001). Indeed, one of the leading causes of the decline in pollinators would be the loss of semi-natural habitats and the decrease in the quality and quantity of floral resources in the landscape (Tscharntke et al., 2005).

In agricultural landscapes, after the flowering period of entomophilous species (oilseed rape, sunflower, faba bean) a dearth period occurs, leading to a decrease in floral resources for honey bee colonies (Requier et al., 2015; Wintermantel et al., 2019).

To respond to short periods of food shortage well known to beekeepers, corrective methods are commonly used in beekeeping practices, such as transhumance or feeding colonies during times of shortage. Transhumance is a way for beekeepers to move their colonies to new floral resources available in the environment (Le Conte and Navajas, 2008). However, when necessary, beekeepers sometimes resort to artificial feeding, which does not provide the same nutritional quality to honey bee colonies as natural sources of pollen and nectar (Pedersen and Omholt, 1993). The good development of the colonies is important in order to allow it to function and to ensure the food supply necessary for its health and stimulate brood rearing.

For the same purpose, agroecological measures can be implemented to increase the food supply in the environment to promote the development of honey bee colonies

(Decourtye et al., 2010; Odoux et al., 2014; Requier et al., 2015; Alaux et al., 2017; Wintermantel et al., 2019). Such measures consist in implementing diversified floral resources, using melliferous plants as floral strips and in preserving the flora of the interstitial landscape elements (Decourtye et al., 2010). Therefore, agricultural landscape and their associated management practices through the implementation of agroecological measures, could provide a benefit to honey bee colonies.

To understand the losses and the weakening of the honeybee colonies in Europe (Potts et al., 2010), pilot apiaries have been set up in Europe to monitor the development of colonies in their agroecosystems (Genersch et al., 2010; Odoux et al., 2014; Kuchling et al., 2018; Kretzschmar and Frontero, 2017; Kretzschmar and Maisonnasse, 2022). Indeed, there is no simple explanations for the increase of colony winter losses in European countries, but several factors seem to be linked to winter colony mortality. However, the colony strength and the presence of biotic agents, such as parasitic mites, and pathogens, have been clearly identified to be linked to winter colony losses (Smith et al., 2013b; Steinhauer et al., 2018). Many studies have shown that the biotic agent *Varroa destructor* is a key factor in high colony losses during wintering (Genersch et al., 2010; Hernandez et al., 2022).

Therefore, it is essential to consider this loss factor in field surveys to disentangle the weight of other factors, such as beekeeping practices and environmental stresses. In fact, studies show that beekeeping practices (especially through the varroa treatment implementation) influence the colonies mortality, as does the agricultural environment of apiaries (Giacobino et al., 2017) . Therefore, beekeeping management must be considered to understand honeybee health and dynamics during field surveys. These monitoring studies have allowed for a better understanding of bee-landscape relationships over the long term and have identified important factors to be taken into account in order to understand the health and dynamics of honey bees. These studies with pilot apiaries were conducted over several years to ensure robustness in the evaluation of the seasonal and interannual dynamic parameters of honey bee colonies in specific agricultural systems such as oilseed rape (Odoux et al., 2014), sunflower or lavander crops (Kretzschmar and Frontero, 2017; Kretzschmar and Maisonnasse, 2022). These studies provided valuable and robust datasets for testing specific honey bee factors, such as the influence of landscape design, agricultural inputs, and human interventions, or to show the landscape valuation of floral resources for honey bees in agroecosystems in order to foster agricultural policy initiatives for the well-being of honey bees (Decourtye et al., 2010). Nonetheless, past studies have not been able to assess and quantify the direct effect of specific agroecological measures under real field conditions that will improve honey bee colony development.

The project “Agriculture and pollinators” financed by the Swiss Federal Office for

Agriculture (OFAG); proposed a set of agroecological measures that could be implemented by farmers in meadows, in order to identify those that effectively increase floral resources for the benefit of honey bee health (Sutter et al., 2019).

The main measures implemented in the "Agriculture and pollinators" project were designed to encouraged farmers to act on floral resources availability and on the use of agricultural equipment and techniques preserving pollinators in meadows. The measures tested concerned specifically the relinquishing of the use of a conditioner while mowing, implementing floral resource strips, and delayed mowing in meadows. Also, some measures can also be combined, such as the combination of mowing without conditioner use and leaving an unmown floral strip and the combination of mowing without conditioner and delaying mowing (Chapter 4).

The conditioner hastens the drying of grass after mowing, thereby improving hay quality, but is harmful to bees and other insects visiting the flowers during mowing (Fluri and Frick, 2002; Humbert et al., 2009). These studies on conditioners observed dead insects in the field during mowing operations, but the impact on bee colonies development has not been documented.

Furthermore, the beneficial effects of delayed mowing and flower strips have only been demonstrated under experimental conditions for pollinators (Buri et al., 2014; Buhk et al., 2018) but the combinations of these measures with the conditioner use have not been documented. This project evaluated these measures under field conditions to verify their effectiveness on honey bee pollinator health. For this, volunteer beekeepers provided access to 300 honey bee colonies in the framework of this project in order to determine how these agroecological measures influence their development and their winter survival (See below the description of the scientific monitoring of this project 1.2).

This inter-cantonal project, with the participation of the cantons of Vaud, Jura, and Bern, has made it possible to set up a large monitoring system under real field conditions in an extensive and landscape varied territory for the enhancement of floral resources for the benefit of pollinators.

1.2 Description of the scientific monitoring on a territorial scale

A four-year colony monitoring study was conducted in 30 apiaries located in the cantons of Jura, Bern, and Vaud in Switzerland (See Figure 11). Beekeepers‘ associations in the cantons relayed calls for participation in the study to beekeepers who were eligible. All beekeepers between the ages of 18 and 70 were eligible to participate in the study (after 30 participants had been recruited for the study, enrollment was halted). These volunteer beekeepers practiced local beekeeping according to Swiss national recommendations of beekeeping practices varroa management [SSA ApiService - Apicultural Health Service (Apiservice, 2021)]. In this study, 300 colonies (*Apis mellifera*) were kept in Dadant or Swiss Bürki beehive systems. Four times per year, all apiaries were monitored (in April, June, end of July/beginning of August, and October). At each visit, we estimated the colony size i.e., the number of adult workers bees and the number of capped brood cells according to a method developed for this study described below (described in chapter 2). These variables enable us to evaluate the colony development during all beekeeping season. To determine the influence of *V. destructor* infestation rates on colony mortality, adult honey bee workers (mean = 300, SD = 50) were sampled on brood frames at the beginning of April, June, August and October to determine their mite infestation rates respectively (described in chapter 3) To determine the influence of the beekeeping practices on honey bee colony health and development, the beekeepers were asked to record their beekeeping tasks as the implementation of varroa treatments, queens age, or honey harvest thought a mobile application (ApiNotes©). Colony mortality was recorded beginning of April, after the overwintering period for all years of the study. In case of colony losses, beekeepers replaced the dead colonies with nuclei prepared in the spring of the previous season, and new colony identity numbers were attributed to them. The surrounding landscape were documented for all years and will be described in the chapter 4 and in chapter 5. Honey bee colony weight was also measured using automatic scales (Youbee©) installed on five hives per apiary. Measurements are taken every ten minutes and are sent to the Youbee© platform every four hours. From these scales, we extracted weight data from spring and summer using segmented linear regression to analyze the honey bee colony productivity in their different landscape environment (See chapter 5). The local weather through cumulative precipitations was also documented for all years and will be described in the chapter 5.

1.3 PhD aims

The main objective of this thesis is to analyze the influence of a set of agroecological measures in meadows on (i) honey bee colony development in summer and (ii) winter mortality; and (iii) to determine the landscape types under which these agroecological measures are effective in supplying resources to honey bees.

More in details, the agroecological measures proposed, were expected to increase brood production in summer by 10% and limit winter colony losses to a maximum of 10% in the apiaries (Sutter et al., 2019). Therefore, developing a method for quantifying the various components of colony size, namely the quantity of brood and adult bees, was essential for measuring the colony development in their different environments. Accordingly, the ColEval method presented in this thesis enables the evaluation of colony development to observe if agroecological measures benefit honey bee populations. This method is an effective tool for field surveys, allowing a large number of colonies to be assessed and comparable data to be obtained to understand environmental stresses (such as habitat loss and floral resources), or to evaluate the effect of beekeeping practices (Hernandez et al., 2020, 2022; Kretzschmar and Maisonnasse, 2022). Indeed, in this study, beekeepers are required to manage their colonies according to recommended beekeeping practices (Apiservice, 2021), especially in regard to the implementation of treatments against *V. destructor*. In fact, this ectoparasite is known for having a strong negative impact on colony development and mortality (Genersch et al., 2010; Traynor et al., 2020) and one of the direct effects on this parasite are treatments applied by the beekeepers. In this study, we thus considered important to ensure that the beekeepers indeed respected the treatment recommendations against the *V. destructor* in order to understand the weight of this factor involved in honey bee colony health and development before evaluating the effect of agroecological measures implemented in meadows.

This monitoring allowed us to collect a consequent set of empirical data on the development of colonies during the season (from April to October, and during four consecutive years – 2018 to 2021), on their sanitary conditions (*V. destructor* infestation rates), on the implemented mite treatments as well as on the census of honey bee winter losses. In addition, we gathered data on landscape composition and structure with the implementation of the agroecological measures described in this study. Combining these datasets allowed us to address the main objective of this thesis by identifying viable agroecological measures on meadows, specifically on temporary meadows, that can be implemented to promote honey bee colony health in the agricultural landscape. The following chapters illustrate in detail all the methods and results obtained.

1.4 Chapters organisation

Chapter 2 - The chapter entitled “ColEval: Honeybee COLony Structure EVALuation for Field Surveys”, is a scientific article published in Insects in 2020 (https://doi.org/10.3390/insects11010041). This article presents a rapid and accurate estimating method for the evaluation and comparison of the structure of numerous honeybee colonies, allowing to conduct large honey bee colony surveys. This method is an essential tool for the description of the evolution of honeybee colony sizes in the framework of our monitoring project to relate colony development to agroecological measures in the surrounding landscape.

Chapter 3 - The chapter entitled “Compliance with recommended *Varroa destructor* treatment regimens improves the survival of honey bee colonies over winter”, is a scientific article published in Research in Veterinary Science in 2022 (https://doi.org/10.1016/j.rvsc.2021.12.025). This article highlights the influence of treatment regimens implemented by beekeepers against *V. destructor* on honey bee colony winter losses. We have demonstrated in this study that the compliance of *V. destructor* treatments is an essential condition to limit honey bee winter mortality. In large field surveys as our study, it is important to have a control of varroa infestation rates and a correct implementation of the treatment regimens by the beekeepers for efficacy, because *V. destructor* is known to have a strong effect on honey bee colony mortality and could override or annihilate the beneficial effects of agroecological measures implemented in meadows by farmers on colony development.

Chapter 4 - The chapter entitled “Agroecological measures in meadows promote honey bee colony development and winter survival”, is a scientific article accepted in Ecosphere in October 2022. This article highlights the influence of three agroecological measure implemented in temporary meadows on honey bee colony development and winter survival.

Chapter 5 - The chapter entitled “Agroecological measures in meadows provide additional supply of floral resources in crop-dominated landscapes”, reports an as yet unpublished analysis and providing more evidence that supplying resources in summer to honey bee in sufficient amount contributes to the survival of the honey bee colonies. It describes the amount of floral resources through areas of agroecological measures that are needed to make a difference in landscape types and the associated climate.

Chapter 2
ColEval: Honeybee COLony Structure EVALuation for Field Surveys

Hernandez, J., Maisonnasse, A., Cousin, M., Beri, C., Le Quintrec, C., Bouetard, A., ... & Kretzschmar, A. (2020). ColEval: Honeybee colony structure EVALuation for field surveys. *Insects*, 11(1), 41. https://doi.org/10.3390/insects11010041

2 ColEval: Honeybee COLony Structure EVALuation for Field Surveys

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Abstract

Methods for the evaluation and comparison of the structure of numerous honeybee colonies are needed for the development of applied and fundamental field research, as well as to evaluate how the structure and activity of honeybee colonies evolve over time. ColEval complements existing methods, as it uses an online reference image bank for (human) learning and training purposes. ColEval is based on the evaluation of the surface area percentage occupied by different components of a honeybee colony: adult worker bees, open and capped brood, honey, nectar, and pollen. This method is an essential tool for the description of the evolution in the size of honeybee colonies. The procedure makes allowances for tendencies between different observers and uses them to calculate accurate measurements of honeybee colony evaluation. ColEval thus allows for a posteriori comparison of under- or over-evaluation made by different observers working on the same project; it is thus possible to eliminate observer bias in the measurements and to conduct large surveys.

Keywords: *honeybees; colony structure; field experiment; human estimations of colony hive*

2.1 Introduction

In order to provide the practical knowledge that will help beekeepers to maintain colonies; secure honey productivity; minimize damage from bio-stressors (*V. destructor* (Rosenkranz et al., 2010; Nazzi and Le Conte, 2016), virus, *Nosema spp.*, . . .); and apprehend environmental constraints such as habitat loss, flower paucity, and pesticide exposure, honeybee researchers need precise and efficient tools that deliver comparable data (Meixner et al., 2010). In the current context of worldwide concerns about honeybee decline (Ellis et al., 2010; Neumann and Carreck, 2010; Potts et al., 2010; Evans et al., 2009; Pettis and Delaplane, 2010), field research at an apiary scale requires effective and efficient methods for the evaluation of the population structure of colonies (Dietemann et al., 2013a; Delaplane et al., 2013). The key components of the colony structure are the number of adult worker bees and the number of capped and open brood cells, which together indicate the future size of the colony. Food stores necessary for the colony (honey, nectar, and pollen stored in combs) are crucial to ensure survival during autumn and winter periods when foraging is not possible. Thus, the presence of such stores is a good indicator of colony vitality and resilience.

Several methods already exist to evaluate the different components of honeybee colonies occupying hives (Marchetti, 1985; Imdorf et al., 1987; Imdorf and Gerig, 2001), using an assessment of colony components on combs with a partition of the observed space into a small number of quadrats of equal area. If the number of quadrats is small (between 6 and 9, for example), the measure of several colonies is better fitted by a multinomial variable. The higher the number of quadrats, the closer the counting is from the continuous variable, which is more suitable for downstream statistical analysis and modelling (Delaplane et al., 2013; Pirk et al., 2013). Other methods use combs photography to allow for reliable visual assessments, but quality photographs are not easy to obtain under field conditions (variable weather, honeybee behavior, and so on). Even if the counting can be done by image analysis in silico, the result of automatic image analysis is hampered by limitations in image definition (low quality of contrast that could lead to an error of counting evaluation) (Cornelissen et al., 2009; WSC, 2014).

Improvements in honeycomb image analysis are underway (Yoshiyama et al., 2011; Sparavigna, 2016; Jeker et al., 2012; Colin et al., 2018; Alburaki et al., 2015, 2017); the use of these techniques is impaired by the time needed to take and analyze photographs. These methods do not yet offer a suitable way to describe a large number of colonies per day or, consequently, to record the high variability attached to hives and apiaries. The structure of honeybee colonies has been shown to be highly variable in large surveys at apiary scale, or even during one particular honey flow at colony scale (Genersch et al., 2010; Kretzschmar et al., 2016). For this reason, it is important to consider the number and the variability of colonies observed, because it has a crucial bearing on the statistical validity of most experiments. For example, cohorts of a minimum of 25 hives per group compared per replicate are needed when attempting to validate the efficiency of *V. destructor* treatments, or when providing specific instructions for the use of new products or methods against mites (Kretzschmar, unpublished data). The development of image analysis methods has not yet been used in large apiary surveys, which is most likely related to the time cost associated with these techniques.

A high number of colonies observed repeatedly over time, with minimal disturbance to the study objects, is also required by recent approaches addressing the modelling of colony structure dynamics (Becher et al., 2013), which, again, require a rapid method of colony size estimation. A large team is needed to observe multiple colonies, and it is thus often necessary to involve people with varying levels of expertise (beekeepers, engineers, technical staff, and researchers) in the research projects to evaluate the colonies. Individual variations in assessments between observers must thus be taken into account in order to deliver standardized and comparable measures. The common bias encountered in social sciences; that is, inexperienced participants, false counting, observer expertise evolution (Gonsamo and D'Odorico, 2014; Fitzpatrick et al., 2009), should be addressed by developing learning, training and evaluation support for all team members, regardless of their level of expertise. It is for these reasons that we chose to improve an easy to handle field technique, supported by evaluation tools and fitting the statistical and modelling needs in honeybee epidemiological research (Kretzschmar et al., 2016; Alaux et al., 2018; Dubois et al., 2018; Kretzschmar and Frontero, 2017; Rollin and Garibaldi, 2019; Monchanin et al., 2019; Sutter et al., 2019). In the long-term surveys of honeybee weight performance under lavender or sunflower honey flow (Kretzschmar et al., 2016; Kretzschmar and Frontero, 2017), 200–600 hives are surveyed at the beginning and at the end of honey flow (circa 2000 to 6000 frames evaluation). The development of a large and long-term survey is the first step in honeybee epidemiologic studies. ColEval is devoted to being used for this development. Image analysis would provide reference counts of open or capped brood cells, but it fails in giving accurate counts of bees. ColEval is presented here as a convenient trade-off between accuracy and efficiency in describing, under field conditions, the components of a hive at a given time.

In order to account for the aforementioned constrains, an efficient and precise description method with human learning and comprehensive training is needed to (i) describe a sufficient number of apiaries for field experiments and (ii) measure the statistical variability between observers when experiment scale and schedule requires division of labor between sev-

eral persons. The method presented in this paper offers a solution to the above-mentioned constraints, specifically addressing the aims of being able to describe a large number of honeybee colonies while maintaining statistical validity. To ensure the highest quality of data when quantitatively evaluating the five components of colony structure (number of adult workers, number of open and capped brood cells, surface area of honey, nectar, and pollen stores) and enable assessment of a large number of colonies per day, we created a set of three learning and training tools: (i) a learning, training, and self-evaluation tool; (ii) a specific field application tool to improve the quality of the counting; and (iii) a method to compare and adjust results from different observers. The impact of performing repeated ColEval measurements on honeybee colonies was also tested to validate this method.
Nevertheless, the authors make clear that ColEval is a method of evaluation that provides a training tool for minimizing the variability of the measures at individual level, knowing this method includes the inherent variability of human quantification.

2.2 Materials and Methods

2.2.1 Description of the ColEval Method

The ColEval method consists of the description of the entire colony; both faces of each comb of the brood box and suppers are screened, along with their walls, to visually estimate the quantity of adult workers. The observer analyzes every comb in the hive. Once the evaluation of a comb is complete, it is placed in a different box to prevent workers changing combs, as this would bias the assessment of the next combs. Once all combs have been screened, the observer evaluates the number of adult bees on the walls of the inspected hive.

Each component (adult workers, capped and open brood, honey, nectar, and pollen) is evaluated through the proportion of the surface it covers on each comb side and wall (the latter for adult workers only). This proportion is converted into a percentage of the total comb surface and is then transformed (see below subsection 2.2.3 and Table 1) into the following:
- Number for worker bees;
- Number for capped and open brood cells;
- Area (dm^2) for cells filled with honey/nectar or pollen.

For practical reasons, the percentages are rounded to the nearest 5 (counting by steps of 5 percentage points). When several layers of worker bees cover a comb, the estimation could be higher than 100%.

2.2.2 Learning and Training

- Image bank and reference counts

Table 1: Estimated values of the coefficient to transform the percentage of comb coverage into number of brood cells and adult workers or into area of honey/nectar and pollen. Values given for 1%.

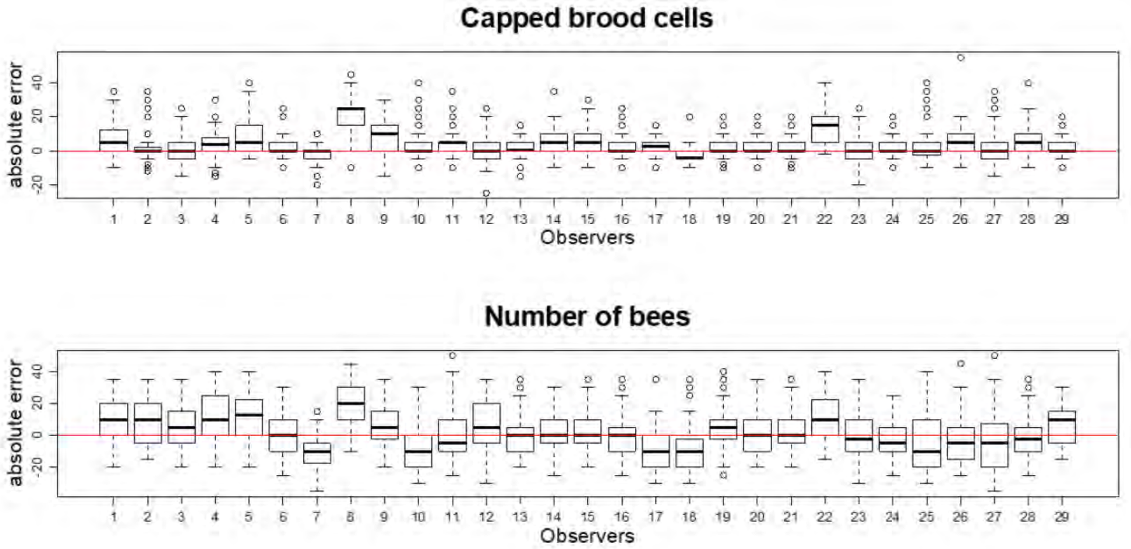
Hive Type	Number of Honeybees	Number of Capped Cells	Number of Open Cells	Area of Pollen (dm^2)	Area of Honey/Nectar (dm^2)
Dadant (hive)	14	40	40	0.1134	0.1134
Langstroth (hive)*	11	30.2	30.2	0.0903	0.0903
Dadant (supper)	7	20	20	0.057	0.057

* For Langstroth hive, the suppers are generally either another Langstroth hives or Dadant suppers.

An image bank was created with photos (n = 300) of comb sides covered with various numbers of adult worker bees and photos (n = 600) of comb sides from which adult workers have been removed to make the capped brood cells visible (Hernandez, 2013). Reference images for the open brood cells are not provided here, because they are particularly hard to photograph, even under laboratory conditions (43h for 16 photos of high quality in special light chamber; P. Le-Bivic, INRA, personal communication), and are difficult to see on photos taken in apiary conditions owing to light conditions (Imdorf et al., 1987; Jeker et al., 2012) (brood comb picture in Appendix A.1.3). Adult workers and capped brood have been manually counted on these photos with the help of ImageJ software to provide a reference count (Abràmoff et al., 2004). Several formats of hives are used by beekeepers. The main formats in Europe and the United States are Dadant and Langstroth. To cope with these differences, combs of the latter two formats are included in the picture bank and a rule of transformation of percentages to counts is provided (Table 1). The image bank (photos of combs or virtual images), learning tools, and instructions for use are available via the permalink: http://w3.avignon.inra.fr/lavandes/biosp/colevalENG.html .

- Learning training and processes

During the learning and training sessions, several series of 20 photos drawn at random from the image bank are displayed on a computer screen to simulate a whole hive. Evaluation of either the number of adult bees or capped cells is typed in by the observer, recorded by the R software (R, 2018), and then compared to the reference counts. At the end of a counting series (20 images), several descriptors are calculated, as follows:
the average absolute error for each counting: $\frac{\sum_1^n |evaluation - reference|}{n}$, calculated from a series of n counts (see Figure 1);
the effective error of the total series: $\sum_1^n evaluation - \sum_1^n reference$, for a series of n counts;


Capped brood cells

Number of bees

Figure 1: Absolute error of 29 learning observers for capped brood (upper) and worker bees (lower). Each observer has evaluated two or three series (20 images each) of both capped brood and honeybee images.

the effective relative error for the total series: $\frac{\sum_1^n (evaluation - reference)}{\sum_1^n reference}$.
Finally, for the regression coefficient α of the counting series (see Figure 2), the regression follows the line equation $Evaluation = \alpha \,.\, Reference$ ($Intercept = 0$), where α depicts the tendency of the observer to under or over-evaluate and provides the coefficient of correction when the counting of several observers is to be compared. The reference counting of each observer is then $Reference_{observer\ i} = Evaluation_{observer\ i} / \alpha_{observer\ i}$.
Along successive series, the observer can thus evaluate her/his ability to describe the comb components based on observation of her/his own errors. For each photo, a reference count is provided. This enables the observer to see why their estimation was wrong; especially, the observer can check if the error depends on the quantity of adult bees.

- Additional corrective procedure for adult honeybee workers

We hypothesized that the assumption that honeybees formed a unique layer on the comb was in most cases not met in field realistic settings. In an attempt to better estimate the number of adult worker bees on a comb, we compared the estimates obtained with the ColEval method described above with an estimate obtained by weighing the workers. Five observers performed the assessments of 150 combs simultaneously. We considered that the weight of one individual equals 0.140 g (Lehziel, 2011). From this comparison, a coefficient of under- or over-evaluation, as well as the average coefficient, was estimated for each observer (Figure 3).

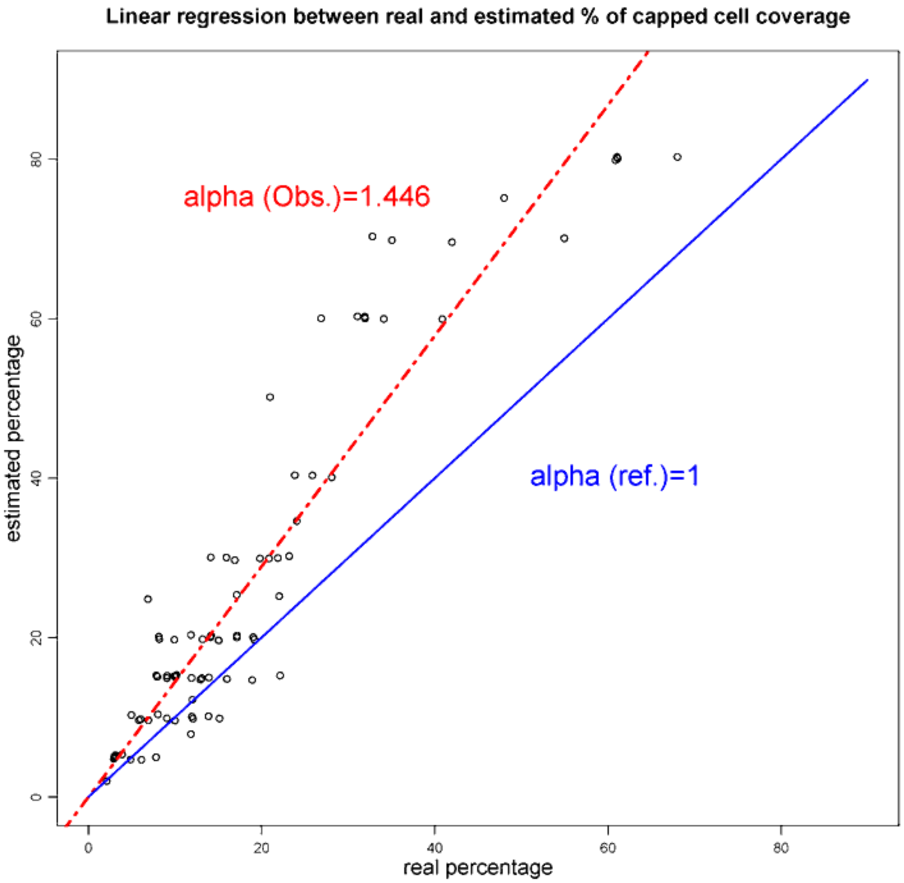


Figure 2: Linear regression between real and estimated percentage of capped brood coverage for two series of 20 images showing the tendency for one observer to deviate from the real counts: red dotdashed line shows the observer evaluation; blue solid line is the reference line ($\alpha = 1$). The coefficient $\alpha = 1.446$ give the tendency of over-evaluation of this observer.

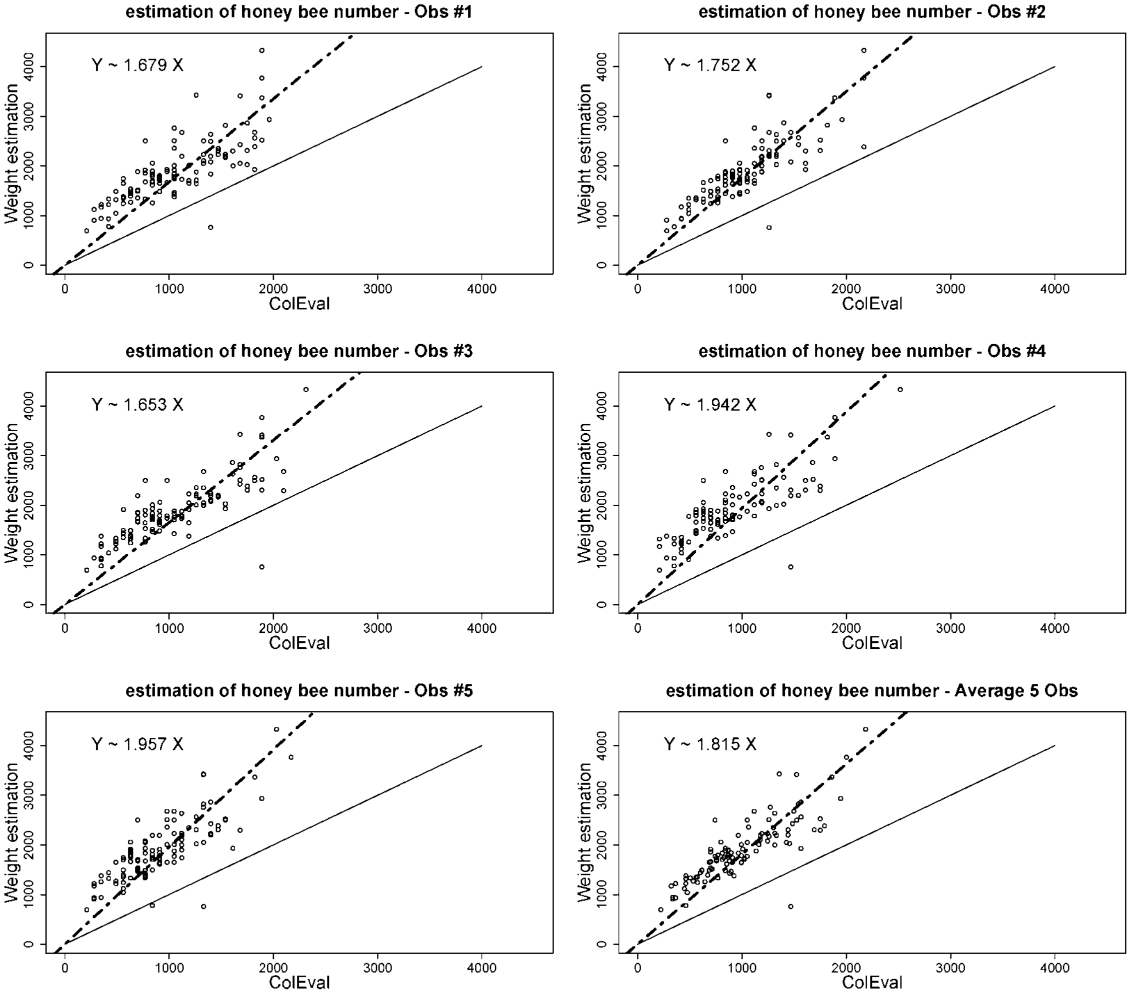


Figure 3: Linear regression between ColEval evaluation and weight-based evaluation of number of adult honeybee workers (dot dashed line). The equation $Y = \alpha X$ gives the tendency of over-evaluation; solid line is the line with $\alpha = 1$. X-axis: bee number evaluated by ColEval; Y-axis: bee number evaluated by weighing.

- Correction between observers

Three observers performed ColEval evaluations (Figure 4). Each of them described one-third of the same colonies in each apiary to determine inter-individual errors. The evaluation of each observer is corrected, for each component, taking the mean of all the counts as the reference and applying a correction coefficient ($= \frac{mean(observer)}{mean(total)}$) to each observer (Figure 4).
New observers are expected to train with an experienced observer. Thus far, all observers in the various programs in which ColEval is used started with the initial group who established the method (namely, Julie Hernandez, Alban Maisonnasse, and Andre Kretzschmar). Observers are expected to engage in regular training and to maintain their level of accuracy. Nevertheless, it is clear that specific trends (either over or under estimation) are attached to each observer. As it is always possible to account for these trends, the main point is to check that the trend is constant.

2.2.3 Data Collection Support Spreadsheet

Field spreadsheets used to document the coverage percentage of each of the five components on each side of each comb of the hive are provided in Appendices A.1.1, A.1.2). They are transcribed on formatted spreadsheets (format type .xlsx, .csv, .ods, or others), which transform percentage evaluation into numbers or surface areas, with the coefficient transformation values given in Table 1 (see above in section 2.3.1 Data transformation).
Calculation of the theoretical number of cells and adult workers on comb.
The number of cells covering 100% of a comb side was measured on 30 combs. For the evaluation of the number of adult honeybee workers covering 100% of a comb side, the body surface area of 130 individuals was measured with image analysis (ImageJ—(Abràmoff et al., 2004)). Hypothesizing that honeybees formed a unique continuous layer, the maximum number of honeybees needed to cover 1% of a comb side was estimated (See Table 1). This maximum number fits nicely with the number obtain by Imdorf et al. (Imdorf and Gerig, 2001) and Burgett and Burikam (Burgett and Burikam, 1985).

2.2.4 Impact on Colony

Two groups of 50 colonies were surveyed with ColEval at one-month and one-week intervals, respectively, during a four-week period. The first group was thus surveyed twice, whereas the second was surveyed five times.

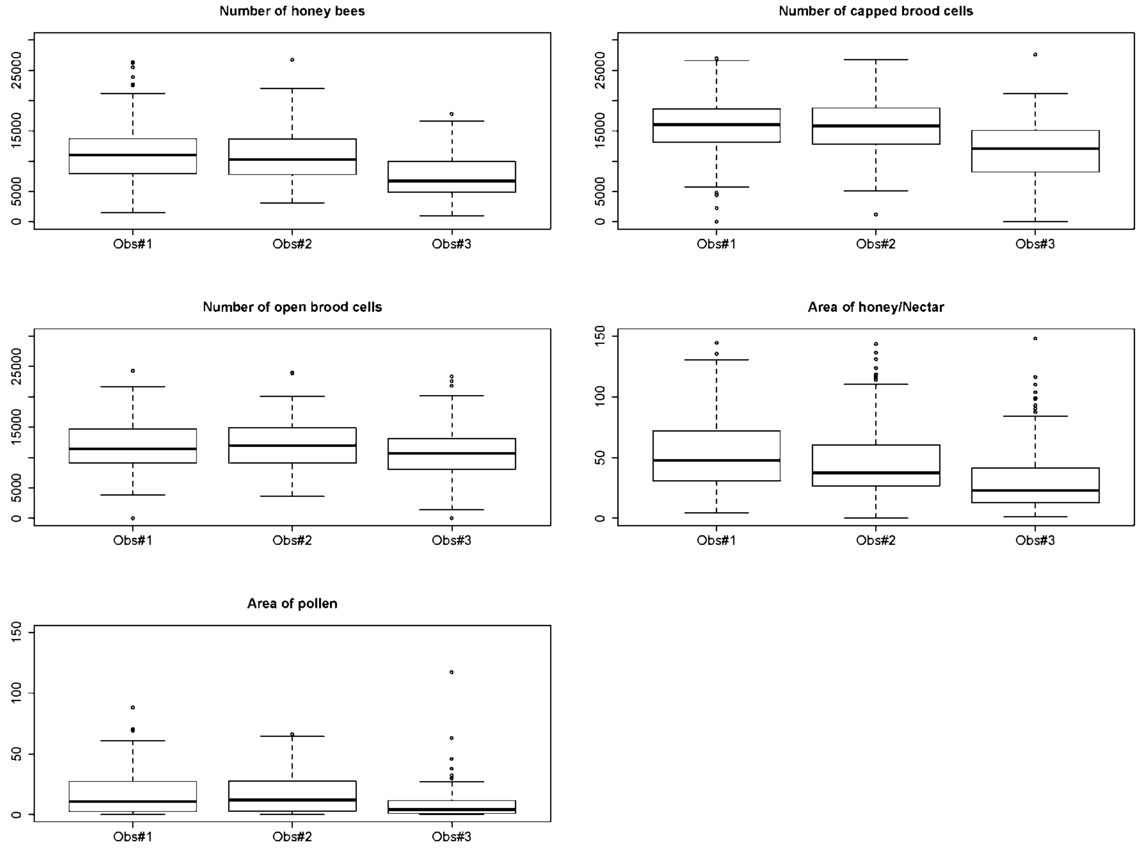


Figure 4: Comparison of the distribution of the evaluation of the five ColEval components by three observers.

2.3 Results

2.3.1 Data Transformation

To transform the percentage evaluation into numbers or surface areas, we calculated the coefficient transformation values given in Table 1. Each value given in the table is the number of items (adult workers or cells) or the comb area (dm^2) corresponding to 1% of the total area (=area inside the wood comb) of a comb side of a given hive format.
The estimated percentage of the theorical number of cells covering the comb is transformed into number of cells (40 cells for Dadant hive type, 30.2 cells for Langstroth hive type, and 20 cells for Dadant supper represented 1% of the comb — Table 1). The estimated percentage of honeybees covering the comb is transformed into the number of worker bees (14 honeybees for Dadant hive type, 11 honeybees for Langstroth hive type, and 7 honeybees for Dadant supper represented 1% of covered comb — Table 1). One hundred percent coverage gives the maximum number of bees in one continuous layer on a comb.

2.3.2 Method Validation

- Learning process

The average absolute error for each counting and the regression coefficient α of the counting series (see above in section 2.2.2 Learning training and processes) are presented in Figures 1 and 2.

- Additional corrective procedure for adult honeybee workers

As seen in Figure 3, a coefficient of under- or over-evaluation as well as the average coefficient was estimated for each observer. The estimation of honeybee numbers by ColEval should be multiplied by a coefficient of 1.8 to more accurately approach the real number of worker bees. Additionally, the distribution of the residual errors both for each observer and on average for the five observers is presented in Figure 5.

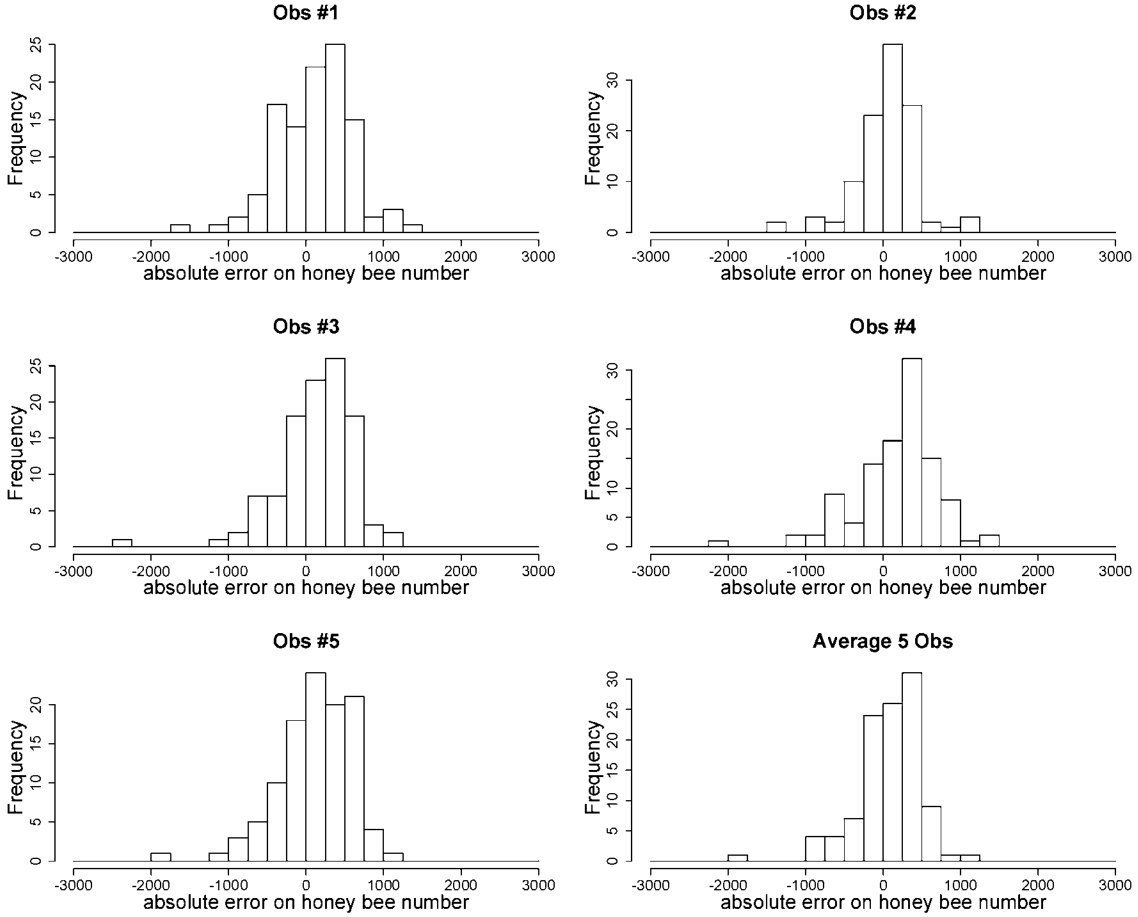


Figure 5: Histogram of residuals of the linear regression for the five observers and on average.

- Standardization between observers

Taking observer tendency α on field measurements into account improves the quality of the results. Figure 5 illustrates the distribution of the evaluation of the five ColEval components by three observers, and their respective learning score. This figure clearly shows that observer #3 under-evaluated the components compared with the two other observers. To level off the evaluations of observer #3, they were corrected using the mean values for each component evaluated by the two others as reference (see above in section 2.2.2. Correction between observers).

- Impact on colony

In our field experiments, no disturbance of honeybee colonies was noticed in terms of colony performance (weight gain) in short-term experiments. The average total weight of the colonies at the end of the survey was not different between the two groups (32.96 kg ± 4.6 and 33.5 kg ± 4.8, respectively).

2.4 Discussion

ColEval has already been applied to several large surveys (totaling approximately 15,000 colonies) within research programs of the research unit on honeybees at the National Institute of Agronomic Research (INRA) of Avignon (France), and many scientific publications used it in their monitoring design to characterize honeybee colony size (Kretzschmar et al., 2016; Alaux et al., 2018; Dubois et al., 2018; Kretzschmar and Frontero, 2017; Rollin and Garibaldi, 2019; Monchanin et al., 2019; Sutter et al., 2019). Application of the method on this scale has clearly demonstrated that ColEval is an easy method to learn and to use. Two people (one observer and one person in charge of taking notes) can assess the demographic and food store components of about 30 to 40 colonies per day. As ColEval observers increase their skills with practice over time and with regular training sessions, the correction of observer bias can be better taken into account and can be used to better analyze the results. In fact, some observers have more difficulty estimating when adult bees are high in number, whereas for others, it is the contrary. In the case of capped brood evaluation, some observers struggle to take into account the presence of mosaic brood (Kretschmar unpublished). Repeated training sessions thus improve the quality of observers' estimates. To address this bias correction, it is advisable to record the identity of the observer on the field sheet when several observers are working on the same project (see A.1.1, A.1.2 in Appendices).

In our opinion, ColEval improves the methods developed by Liebefeld (Delaplane et al., 2013; Imdorf et al., 2010) and the methods based on combs photography (WSC, 2014; Avni et al., 2015), because of the increased precision deriving from its learning method on a broad photographic basis, and because of the possibility to compensate for over- or underestimation for each observer. This also allows for assessments made by several observers participating in a project to be homogenized/standardized. Compared with earlier methods, ColEval proposes a single way to measure all the components of a colony with percentage evaluation. ColEval yields a precise description. As the coverage percentage of all the elements (excluding adult worker bees) amounts to 100%, the observers can take an intuitive partition of the whole area of the comb surface into its components (including voids). Doing this minimizes the total error, which is averaged when the evaluation of the different components are summed up, while the errors of each component are summed up if the components are evaluated independently.

The ColEval method has shown (see section 2.2.3) that the number given as reference for the number of worker bees on a comb surface by the Liebefeld method (Imdorf et al., 2010), or in methods described in the BEEBOOK (Delaplane et al., 2013), should be increased to improve the accuracy of the evaluation. The difference between these methods and ColEval could lie in the different behaviors of workers bees, which could be more intensely clustered together in our case. The differences in individual honeybee weight cannot be excluded either.

As the evaluation of the number of bees by weight is time demanding for field evaluation, it may be more efficient to evaluate a coefficient correction with a preliminary experiment, which can be repeated from time to time (as also mentioned in Imdorf et al. (Imdorf et al., 2010)). Then, instead of considering that 1400–1500 workers bees completely cover one Dadant comb face, it is more realistic to consider 2610 workers bees as the reference (1450 × 1.8 = 2610; see Figure 3). The same result is obtained by Burgett and Burikam (Burgett and Burikam, 1985).

This correction is also useful because it provides a more realistic quantification owing to the overlapping of adult worker bees that can be better interpreted by beekeepers participating in an apiary-level assessment of colony strength and dynamics. Additionally, applying this correction coefficient on the evaluations of the number of adult worker bees on 600 colonies nested in 24 apiaries of a large survey (unpublished data) shows an average colony size, at the time of this experiment (colonies on lavender honey flow in June in South France), of 17,514 ± 8321 worker bees. This number is closer to data found in literature from "Winston, 1991; Burgett and Burikam 1985" (Burgett and Burikam, 1985; Winston, 1991) compared with the estimation before correction of 9730 ± 4572 adult workers per colony.

Compared with the previous methods of describing colonies based on the evaluations of one or several observers, ColEval provides user-friendly training and learning tools required for the candidates to "calibrate" themselves before describing colonies. ColEval addresses this by providing a large image bank (adult workers and capped brood cells) as a tool for observer self-training without requiring handling and weighing of colonies.

Another problem with some other observation methods is that the time required for assessments could be incompatible with fieldwork conditions (variable weather, adverse weather conditions, too cold condition for brood, robbing, bee's aggressiveness, and so on). In addition, these methods do not work for frequent and repeated description of the large number of colonies required by large surveys and would disturb the colonies for too long.

In our field experiments using the ColEval method, no disruption of honeybee colonies was noticed in terms of colony performance (weight gain) in short-term experiments. This result is compatible with those presented in the Liebefeld method with a three-week interval survey (Imdorf et al., 2010). It still has to be proven that performing ColEval on colonies at one- or two-week intervals does not affect long-term honeybee behavior, especially regarding reproduction.

As ColEval provides continuous variables, it facilitates the use of these variables in generalized linear models, mixed or not. It enables better evaluation of the variability of these components in order to more accurately evaluate the response of population structures to bio-stressors and environmental conditions.

Despite the improvements brought by ColEval, it should be noted that the evaluations obtained must be considered as approximations. The way to make these approximations as realistic as possible is to practice and to perform regular training sessions using the image bank and reference counting.

Supplementary Materials

The supplementary materials (in this thesis available at the Appendices A.1.1, A.1.2, A.1.3) are accessible in the online published version of this article (Hernandez et al., 2020) and at the following url: http://www.mdpi.com/2075-4450/11/1/41/s1; and the files are described as : Field sheet in .xlsx with in column the percentages to be described of each of the five components on each side of each comb of the hive: "SM1_ColEval_fieldsheet.xlsx". ColEval spreadsheet in .xlsx to convert percentages to numbers of adult worker bees, capped brood cells, area in dm^2 of honey/nectar and pollen: "SM2_ColEval_spreadsheet.xlsx". Image of brood comb (open and closed brood cells) in png: "SM3_broodComb.JPG".

Author Contributions

Conceptualization, J.H, A.M and A.K; Methodology, J.H, A.M and A.K.; Validation, all authors; Formal Analysis, J.H, A.M and A.K; Writing-Original Draft Preparation, J.H and A.K.; Writing-Review & Editing, J.H, A.A and A.K..; Supervision, A.A and A.K..; Funding Acquisition, A.A and A.K. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest

Chapter 3

Compliance with recommended *Varroa destructor* treatment regimens improves the survival of honey bee colonies over winter

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3 Compliance with recommended *Varroa destructor* treatment regimens improves the survival of honey bee colonies over winter

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Highlights

- We surveyed compliance of beekeepers to *Varroa destructor* mite control recommendations
- Honey bee colony infestation rate and mortality were measured to assess compliance impact
- Noncompliance led to higher mite infestation rates and colony mortality
- A colony had up to 25 times higher risks of dying when not treated as recommended
- Communicating the impact of deviations from recommendations improves compliance

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Abstract

The ectoparasitic mite *Varroa destructor* affects honey bee colony health and survival negatively, thus compelling beekeepers to treat their colonies every year. A broadly used mite control regimen is based on two organic molecules: formic and oxalic acids. To ensure optimal efficiency, several applications of these acids at pre-defined time points are recommended. These recommendations are mainly based on experiments conducted under controlled conditions. Studies evaluating the effectiveness under natural field conditions are lacking. We enrolled 30 beekeepers in a longitudinal study in three cantons in Switzerland and monitored the management and health of their colonies for two years. We assessed compliance with mite control recommendations and measured *V. destructor* infestation rates, indexes of colony productivity (brood size and honey harvest), and colony mortality in 300 colonies. We observed a 10-fold increased risk of colony dying when beekeepers deviated slightly from the recommended treatment regimen compared to compliant beekeepers (odds ratio: 11.9, 95% CI: 2.6–55.2, $p = 0.002$). The risk of colony death increased 25-fold in apiaries with substantial deviations from the recommendations (odds ratio: 50.4, 95% CI: 9.7–262.5, $p < 0.0001$). The deviations also led to levels of *V. destructor* infestation ahead of wintering, which was likely responsible for colony mortality. After communicating poor survival at the end of the first year to the beekeepers, we observed better compliance and colony survival in the second year. Our results highlight the positive impact of compliance with the recommended *V. destructor* treatment regimen on the health of honeybee colonies and the need to better communicate the consequences of deviating from the recommendations to improve compliance. Compliance also occasionally decreased, which hints at concept implementation constraints that could be identified and possibly addressed in detail with the help of social sciences to further promote honey bee health.

Keywords: *Apis mellifera, pest control, Varroa destructor, compliance, colony mortality, beekeeper management*

3.1 Introduction

During the last 15 years, increased colony mortality of the Western honeybee, *Apis mellifera*, an economically important insect, has fostered intense research on the factors affecting its health (Steinhauer et al., 2018; Smith et al., 2013a). The possible causes of colony losses identified include parasites and pathogens (Smith et al., 2013a; vanEngelsdorp and Meixner, 2010). In particular, the invasive ectoparasitic mite *Varroa destructor* is regarded as the main biotic threat to A. mellifera of European origin (Guichard et al., 2020b; Traynor et al., 2020). This mite functions as a vector of viruses (Berthoud et al., 2010; Le Conte et al., 2010), reducing the lifespan of adult honey bee workers (Dainat et al., 2012) and the ability of colonies to survive, especially over winter (Rosenkranz et al., 2010; Traynor et al., 2020). Without mite control, a colony is predicted to collapse within one to three years (Ritter, 1981; Korpela et al., 1992; Fries and Rosenkranz, 1996; Le Conte et al., 2007), compelling beekeepers to apply effective mite control yearly to maintain their stocks and productivity (Rosenkranz et al., 2010). The implementation of control measures is aimed at reducing the *V. destructor* infestation levels of the so-called “winter bees”, which are long-lived individuals who are thought essential to ensure colony survival over winter (Van Dooremalen et al., 2012). Several recent studies have shown that beekeepers can reduce winter colony mortality by applying varroacidal treatments (Oberreiter and Brodschneider, 2020; Jacques et al., 2017; Haber et al., 2019; Giacobino et al., 2015, 2016, 2017). However, colony losses remain excessive and fluctuate in an unpredictable manner (Charrière and Neumann, 2010; Oberreiter and Brodschneider, 2020; Brodschneider et al., 2018; Gray et al., 2020). This observation is attributable to factors other than *V. destructor* (Smith et al., 2013a) but can also be due to failures in mite control due to incorrect implementation, which is yet to be investigated systematically. Failure implementation is especially likely for the so-called “alternative control methods” because they rely on several applications of organic acids at particular times, which leaves a margin for deviations (Dietemann et al., 2012). Several applications are required to reach sufficient treatment efficacy, equivalent to that of the previously used products containing synthetic active compounds, such as pyrethroids (e.g., tau-fluvalinate and flumethrin) and phosphorothioates (e.g., coumaphos) (Rosenkranz et al., 2010). Their application at particular times during the beekeeping season is due to the dependency of efficacy on ambient factors, which affect the distribution of the active ingredients within the colony (Rosenkranz et al., 2010; Beyer et al., 2018). While this dependency is problematic to determine application time, it is an advantage because, when correctly used, these ingredients evaporate and do not accumulate in the hives (Imdorf et al., 1996; Bogdanov et al., 2002). This lack of residue accumulation ensures that the hive products remain uncontaminated (Rosenkranz et al., 2010) and decreases the risk of resistance development in mites. In fact, after several decades of use, the alternative methods have not led to the development of resistance in mites, whereas such resistance arose within a few years of synthetic product use (Elzen et al., 2000; Maggi et al., 2011; Milani, 1999). Because of these advantages, as well as their proven efficacy when tested under controlled research conditions (Fries et al., 1991; Imdorf et al., 1996), organic acid-based concepts are recommended for controlling *V. destructor* in several countries (Switzerland (Imdorf et al., 2003; Charriere et al., 1997), Austria (Oberreiter and Brodschneider, 2020; Brodschneider et al., 2019), Denmark, Sweden, Netherlands, Germany (Genersch et al., 2010; van der Steen and Vejsnæs, 2021). However, it is not clear to what extent deviations from the recommended treatment regimen contribute to the recurrent winter colony losses recorded in these countries (Gray et al., 2020). Previous studies aimed at determining the role of *V. destructor* control in the maintenance of colony health were of short duration (Giacobino et al., 2015, 2016, 2017; Haber et al., 2019) and relied on beekeepers’ estimations of this role instead of standardized measurements through an adequate monitoring history at colony level (Beyer et al., 2018; Jacques et al., 2017; Haber et al., 2019). Moreover, none of these studies considered whether varroacidal treatments were implemented as recommended (Oberreiter and Brodschneider, 2020) or established a direct link between treatment and *V. destructor* infestation levels (Oberreiter and Brodschneider, 2020) or colony mortality (Giacobino et al., 2015, 2016, 2017; Haber et al., 2019). To better link the correct implementation in applying the alternative mite control concept (i.e., the application of the correct number of organic acid treatments at the correct time), with their intended goal of reducing *V. destructor* infestation rates and colony losses, we enrolled 30 Swiss hobby beekeepers. We asked them to record the number of treatment applications they performed and the time at which they were applied as well as to provide access to their colonies to trained field assistants for sample and data collection. These assistants recorded *V. destructor* infestation rates in each of the 10 colonies per apiary, colony survival over two consecutive winters, and the amount of brood reared in the colonies. The last parameter, together with the amount of honey harvested per apiary (López-Uribe and Simone-Finstrom, 2019), facilitated the assessment of potential negative side-effects of the treatments on brood survival (Tihelka et al., 2018; Elzen et al., 2004) and the identification of economic incentives potentially affecting compliance with recommendations. After colony losses of the first year were linked to compliance, we communicated the results to the beekeepers to monitor putative improvements in compliance and colony mortality in the second year. We tested the following hypotheses: (i) Lack of compliance with the recommended control concept decreases treatment efficacy, leading to increased *V. destructor* infestation rates of winter workers and colony mortality; (ii) Showing the link between compliance and colony mortality to beekeepers can increase compliance in the future; (iii) Reducing the number of treatment applications reduces the negative side-effects on colonies and leads to larger brood size; and (iv) Lack of compliance reduces apiary productivity. From the results, we derived suggestions to improve beekeeper compliance with the recommended treatment regimen for controlling *V. destructor*, with the aim of fostering the health of managed honey bees.

3.2 Material and methods

3.2.1 Beekeeper enrolment, study area, and experimental period

Colony monitoring was performed over two years (from August 2018 to April 2020) in 30 apiaries located in the Jura, Bern, and Vaud cantons, Switzerland. Eligible beekeepers were identified by calls to participate in the study relayed by the beekeeper associations in the cantons and through two information meetings. Beekeepers between the ages of 18 and 70 were eligible to participate. Enrollment was stopped after 30 participants were recruited for the study. The 30 apiaries hosted 10 colonies each. The type of apiculture performed by these volunteers was typical for Swiss beekeepers and, thus, this sample can be considered representative of the beekeeping community in this country. The beekeepers initially agreed to follow the recommended *V. destructor* treatment regimen. After the first year of study, the relationship between colony survival and implemented mite control was communicated to the beekeepers during a meeting session and through email. The 300 colonies (*Apis mellifera carnica*) used in this study were kept in Dadant and Swiss Bürki beehive systems and headed by queens between 1 and 1.5 years of age. All apiaries were monitored three times per year. The first visit occurred early August before the honey summer harvest and formic acid treatment. This was followed by a second visit in October before wintering and oxalic acid treatment and a third at the beginning of April of the following year, when colonies came out of the wintering period and started their development (Figure 6).

3.2.2 Data collection

3.2.2.1 Capped brood size

During the August and October visits, the amount of capped brood produced in the colonies was quantified to monitor the side-effects of the treatments applied. This quantification

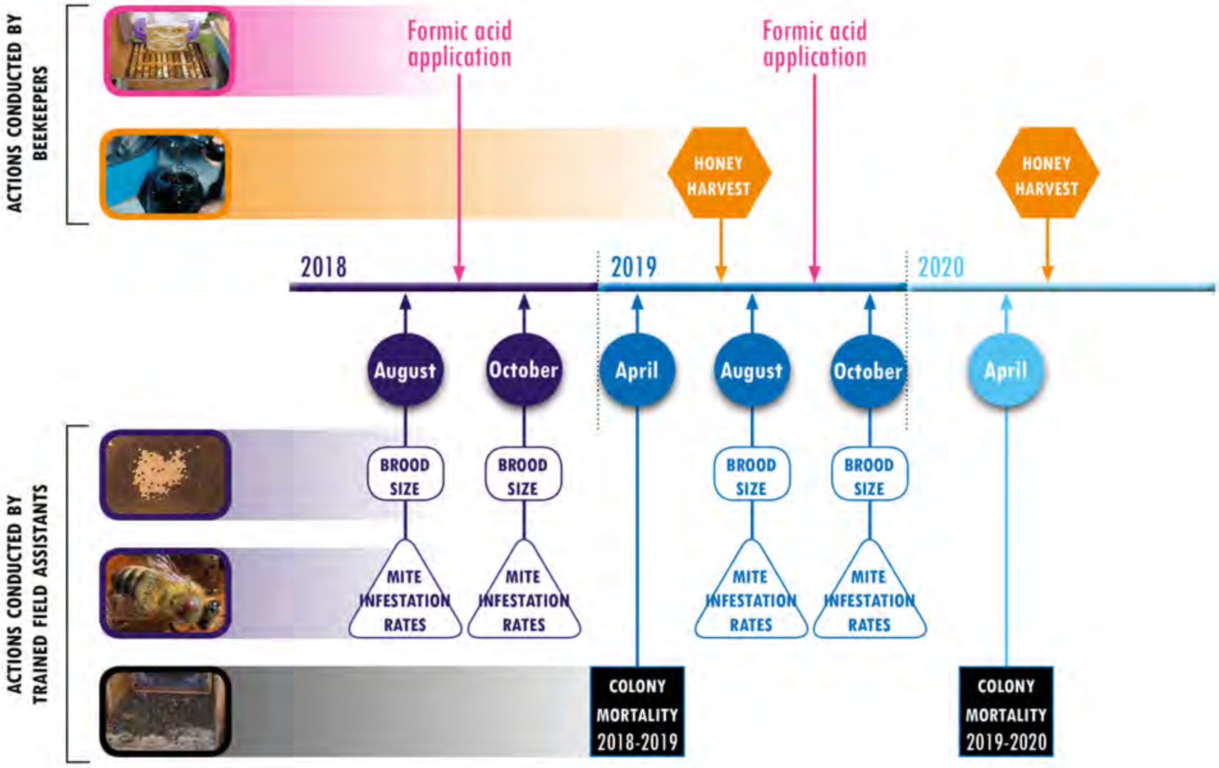

Figure 6: Summary of data collection indicating who from the beekeepers or the researchers performed a given action or measurement.

was obtained using the ColEval method (Hernandez et al., 2020). Briefly, a calibrated estimation of the percentage comb surface area occupied by capped brood was performed and, subsequently, converted into number of cells.

3.2.2.2 Honey harvest

The mass of honey harvested per apiary was recorded by the beekeepers each year (Figure 6) and used as a proxy for colony productivity and health.

3.2.2.3 *V. destructor* infestation rates

At the August and October visits (Figure 6), adult honey bee workers (300, SD = 50) were sampled from the open brood frames of each colony for *V. destructor* infestation rate assessment (Dietemann et al., 2013b; Lee et al., 2010). The samples were placed in plastic zip bags and kept on ice before being brought to the laboratory for storage at -20°C until analysis. Before analysis, the samples were weighted to determine the number of honey bees they contained. Each sample was then washed with soapy water to dislodge the mites for counting, following a standard protocol (Dietemann et al., 2013b). From these data, the number of mites per 100 workers was calculated (Dietemann et al., 2013b).

3.2.2.4 Colony mortality

Colony mortality was recorded in April, after the overwintering period (Figure 6). In case of colony losses, beekeepers replaced the dead colonies with nuclei prepared in the spring of the previous season, and new colony identity numbers were attributed to them.

3.2.3 *V. destructor* control regimen

In Switzerland, the recommended *V. destructor* treatment regimen includes three product applications. The first application of formic acid immediately after the honey harvest, between July 25th and August 10th, uses long-term dispensers. This is followed by a second application between August 25th and September 15th. Between November and December, when the colonies stop rearing brood, the application of oxalic acid is recommended. If more than five mites fall per day on the hives' bottom boards four weeks after this treatment, a second oxalic acid application is required (Apiservice, 2021). Several formic acid dispensers are available on the Swiss market [Apidea, FAM, Liebig, MAQS, or Nassenheider PRO (Apiservice, 2021)]. These models show a similar efficacy (Imdorf et al., 2003), and participating beekeepers were free to use any of them. Similarly, several equally efficacious oxalic acid application modes are available [spraying, trickling, or sublimation (Rosenkranz et al., 2010; van der Steen and Vejsnæs, 2021)], and beekeepers were also free to choose their preferred mode.

3.2.4 Classification of compliance according to the recommended *V. destructor* treatment regimen

To determine the influence of compliance on *V. destructor* infestation rates, brood size, honey harvest, and colony mortality, the beekeepers were asked to record the number of formic and oxalic acid applications, as well as the dates on which these were performed, through a mobile application (ApiNotes©). The number and timing of the treatment applications were used to determine compliance categories. The "compliant" category included beekeepers who correctly followed the control concept (i.e., who applied the correct number of treatments at the appropriate time). The "almost-compliant" category grouped beekeepers who did apply the required number of treatments but at inappropriate times. The "noncompliant" category characterized beekeepers who applied fewer treatments than recommended.

3.2.5 Statistical analysis

Our primary hypothesis was that colonies belonging to compliant beekeepers experience lower mortality compared to those belonging to almost-compliant and to noncompliant beekeepers. We ran a series of simulations to assess the sample size requirements. The simulations revealed that 30 apiaries with 10 colonies each (300 colonies in total) were sufficient to detect a true difference of 20% colony mortality per year in compliant beekeepers compared to the proportion of 40% in almost-compliant beekeepers with 80% power at 95% confidence level, assuming an intra-cluster (i.e., colonies clustered in apiaries) correlation coefficient of 0.2 and a similar number of beekeepers in each compliance category. The power to detect a difference between the complier and noncomplier groups was above 95%, assuming a true colony mortality of 50%. To analyze the effect of compliance on the variables measured and to verify our hypotheses, we used estimating and structural equation models (Overall and Tonidandel, 2004; Lefcheck and Freckleton, 2016; Pugesek et al., 2003). We combined these models because the estimating equation models rely on well-known regression models, which account for clustered observations (i.e., colonies in apiaries) and provide reliable estimates even if some assumptions are slightly violated, whereas the structural equation models, although relying on more complex assumptions, account for the complex relationships between variables. In these structural equation models, the same variable can be both an independent and a dependent variable, allowing the identification of possible causal–effect relationships. It thus becomes possible to analyze the *V. destructor* infestation rate as both an endpoint of the implementation of the control concept and as a cause for colony mortality. Generalized estimating equation models with an exchangeable correlation structure (Overall and Tonidandel, 2004) were thus used to analyze the effect of noncompliance (almost-compliant and noncompliant categories) on colony mortality, *V. destructor* infestation rates, and the number of capped brood cells in October, taking as reference the compliant category. These models used robust sandwich variance estimators to account for the correlations within clusters (i.e., colonies clustered in apiaries). For binary outcomes (mortality variable), we estimated the odds ratios using a logit-link function. Continuous outcomes (number of mites per adult bees and number of capped brood cells) were log-transformed prior to the analysis. Changes in compliance over the years were also assessed using a generalized estimating equation model for clustered ordinal data. The honey mass harvested did not follow any theoretical distribution. Therefore, the impact of compliance on this variable was analyzed with a conditional version of the nonparametric Kruskal–Wallis test.

These analyses were conducted in R version 4.0.3 using the "geepack" and 'coin' packages (R, 2018). Because organic acid application can affect the *V. destructor* infestation rates and colony brood size through both direct and indirect pathways simultaneously, we used generalized structural equation models (Lefcheck and Freckleton, 2016; Pugesek et al., 2003) to quantify the strength of the relationships in a single network and examine each of these pathways simultaneously while accounting for the correlations between multiple response variables (Grace et al., 2015). We present the results using a path diagram, together with the estimated coefficients for each path. Compliance was dummy-coded using "compliant" as the reference category, and colony mortality was modeled as a binary variable with a logit link function. All other variables were assumed to have approximately normally distributed error terms. The model was adjusted for year as a fixed effect and for colonies nested in apiaries as random effects. As generalized structural equation models do not provide standardized coefficients, we provided the unstandardized coefficients. Their interpretation is the same as in linear or logistic regression; that is, the coefficient on the path from a numeric variable toward a binary variable represents the log of the odds ratio associated with each unit increase in the variable at the start of the path. The model was implemented in Stata version 15.0 using the "gsem" command (StataCorp, 2015). No imputation of missing data was done. We assessed the validity of various model assumptions (normally distributed errors, approx. linear relationships, homoscedasticity, no influential outlier) by visual inspections of regression diagnostic plots (residual vs leverage and QQ plots).

3.3 Results

3.3.1 Compliance categories

Of the 30 beekeepers enrolled in this study, two did not provide sufficient information in 2018 and three in 2019. These cases were thus excluded from the analysis. All beekeepers applied winter oxalic acid treatments at the right time. Compliance was thus restricted to the number and timing of formic acid treatments performed (Table 2).

In 2018, 25% of the colonies were treated in compliance with the recommendations, 43% were treated in an almost-compliant manner, and 32% were treated in a noncompliant manner (Figure 7). A significantly higher percentage of colonies were treated according to compliant and almost-compliant regimens in 2019 with 26% in the compliant, 70% in

Table 2: Compliance categories defined according to the number of formic acid treatments performed and their application time.

Compliance categories	Number and timing of formic acid applications
Compliant	Two applications within the recommended period[a]
Almost-Compliant	Two applications but at least one outside the recommended period[a]
Noncompliant	Less than two applications

[a] The first application between July 25th and August 10th and the second application between August 25th and September 15th.

the almost-compliant, and only 4% in the noncompliant categories (p = 0.005, Figure 7). The improvement was mainly driven from 2018 noncompliant beekeepers becoming compliant or almost-compliant in 2019 and occurred despite more than half the 2018 compliant beekeepers reducing their compliance level to almost-compliant in the second year (Figure 7).

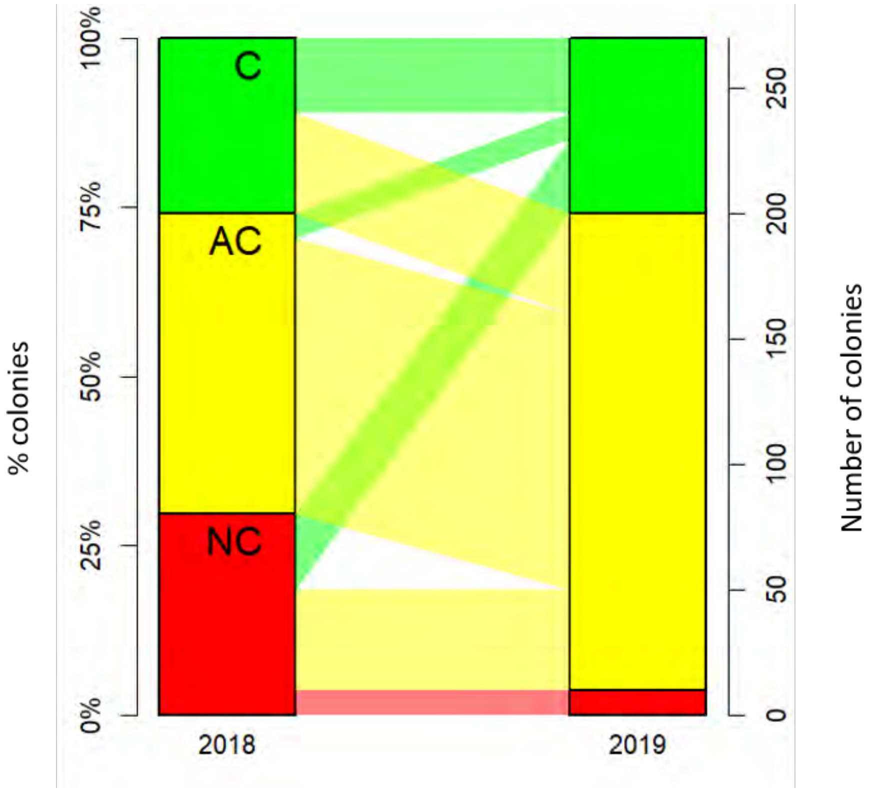

Figure 7: Changes in compliance categories between 2018 and 2019 expressed in percent and number of colonies treated according to the various compliance regimens.

3.3.2 Effect of compliance on honey bee colony mortality, *V. destructor* infestation rates, brood size, and honey harvest

3.3.2.1 Effect of compliance on honey bee colony mortality

Overall, 28% of the colonies died in 2018 and 15% died in 2019 (Tables 3 and 4). With 2% colonies lost, compliant beekeepers experienced a significantly lower mortality rate compared to the 20% almost-compliant beekeepers (odds ratio: 11.9, 95% CI: 2.6–55.2, $p = 0.002$) and to the 55% noncompliant beekeepers (odds ratio: 50.4, 95% CI: 9.7–262.5, $p < 0.0001$; Tables 3 and 4). For noncompliant beekeepers, the probability of colony loss increased rapidly with the infestation rate in October, with a 50% probability of death for an infestation of 10 mites per 100 adult honeybee workers (Figure 8).

3.3.2.2 Effect of compliance on *V. destructor* infestation rates

Overall, compliant beekeepers had a 26% lower mite infestation rate in October compared to August and this effect was more pronounced in 2018 than in 2019. In contrast, the mean infestation rate increased in the two other compliance categories (Table 3, Figure 9). The statistical model comparing infestation rates among compliance categories is presented in Table 4. We observed a marginally significant lower log number of mites in colonies of compliant beekeepers compared to the noncompliant category (differencelog_scale 0.6, 95% CI 0–1.2, $p = 0.07$). The difference between compliant and almost-compliant beekeepers was not significant (differencelog_scale 0.3, 95% CI -0.2 to 0.7, $p = 0.21$).

Table 3: Colony mortality, *V. destructor* infestation rate per 100 adult honey bee workers, capped brood cell number, queen age, and honey harvest for each compliance category in total and for each year separately.

	Compliant	**Almost-compliant**	**Non-compliant**	
N colonies	140	310	100	**Total**
Colony mortality* [%]	2	20	55	
***V. destructor* August [mean(SD)]**	3.3 (5.0)	4.6 (8.6)	3.8 (4.3)	
***V. destructor* October [mean(SD)]**	2.5 (3.9)	4.9 (9.8)	6.8 (9.8)	
Relative difference [%]	-26	5	77	
Brood cells August [mean(SD)]	8694 (4981)	9058 (5176)	8966 (5112)	
Brood cells October [mean(SD)]	2055 (1556)	1702 (1600)	1446 (1550)	
Relative difference [%]	-76	-81	-84	
Honey harvest (kg per apiary) [mean(SD)]	194.2 (101.4)	180.5 (137.6)	68.0 (87.2)	
Queen age (years) [mean(SD)]	1.0 (0.6)	1.0 (0.6)	0.9 (0.7)	
N colonies	70	120	90	**2018**
Colony mortality* [%]	3	19	60	
***V. destructor* August [mean(SD)]**	3.8 (5.9)	3.0 (5.3)	3.6 (4.2)	
***V. destructor* October [mean(SD)]**	2.4 (2.6)	5.7 (13.5)	7.5 (10.4)	
Relative difference [%]	-37	92	110	
Brood cells August [mean(SD)]	10,397 (4665)	9863 (4845)	8685 (5252)	
Brood cells October [mean(SD)]	2154 (1589)	1526 (1308)	1158 (1247)	
Relative difference [%]	-79	-85	-87	
Honey harvest (kg per apiary) [mean(SD)]	130.7 (55.9)	83.9 (38.2)	63.0 (94.4)	
Queen age (years) [mean(SD)]	0.9 (0.5)	1.0 (0.6)	0.8 (0.7)	
N colonies	70	190	10	**2019**
Colony mortality* [%]	1	21	10	
***V. destructor* August [mean(SD)]**	2.7 (3.6)	5.7 (10.0)	6.0 (4.0)	
***V. destructor* October [mean(SD)]**	2.6 (5.0)	4.4 (6.1)	2.6 (3.1)	
Relative difference [%]	-5	-23	-57	
Brood cells August [mean(SD)]	6991 (4726)	8556 (5323)	11,460 (2675)	
Brood cells October [mean(SD)]	1954 (1526)	1813 (1754)	3920 (1754)	
Relative difference [%]	-72	-79	-66	
Honey harvest (kg per apiary) [mean(SD)]	257.7 (99)	240.9 (143.4)	98.3 (NA)	
Queen age (years) [mean(SD)]	1.0 (0.6)	1.0 (0.6)	0.9 (0.5)	

* One colony with missing data in 2018 for almost-compliant category.

Table 4: Analysis of the effect of beekeepers' compliance with the recommended *V. destructor* control concept on colony mortality, *V. destructor* infestation rates (in mites per 100 adult honeybee workers in October), and amount of brood (in number of capped brood cells in October) using generalized estimating equation models.

Colony mortality	**Dead colonies**	**Odds ratio**	**95% CI**	**p**
Compliant	3/140	ref		
Almost-compliant	62/309	11.9	2.6-55.2	0.002
Non-compliant	55/100	50.4	9.7-262.5	<0.0001
2018	72/279	ref		
2019	41/270	0.76	0.3-1.7	0.49
***V. destructor* infestation rates**	**log(mites + 1)**	**Difference**	**95% CI**	**p**
Compliant	0.9	ref		
Almost-compliant	1.2	0.3	-0.2-0.7	0.21
Non-compliant	1.5	0.6	0-1.2	0.07
2018	1.2	ref		
2019	1.1	0.0	-0.3-0.3	0.89
Number of capped brood cells	**log(cells + 1)**	**Difference**	**95% CI**	**p**
Compliant	6.7	ref		
Almost-compliant	5.6	-1.1	-2.3-0.1	0.07
Non-compliant	5.2	-1.3	-2.5-0	0.04
2018	5.6	ref		
2019	6.1	0.4	-0.7-1.5	0.47

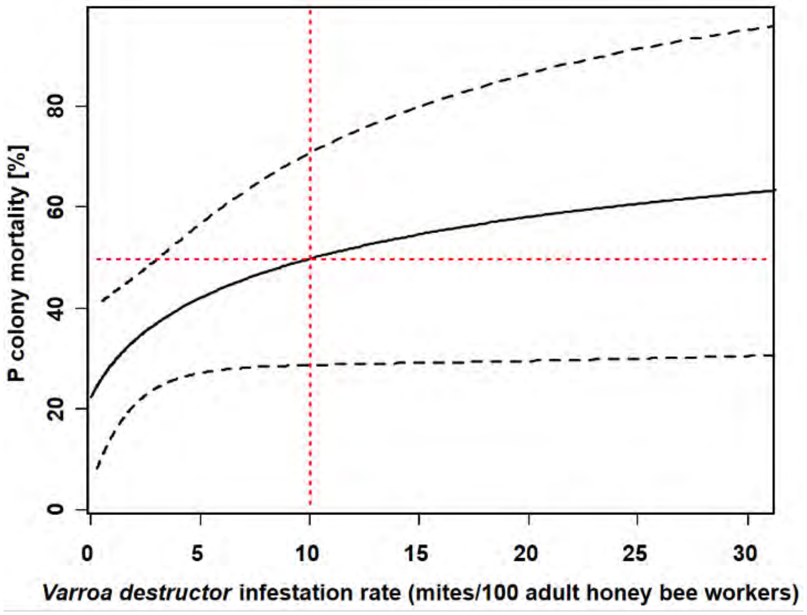

Figure 8: Probability of colony mortality depending on *Varroa destructor* infestation rate in October. The figure is a visualization of the predicted values resulting from a logistic regression in noncompliant beekeepers back-transformed to the probability scale. Dashed lines represent the 95% confidence band around the prediction line.

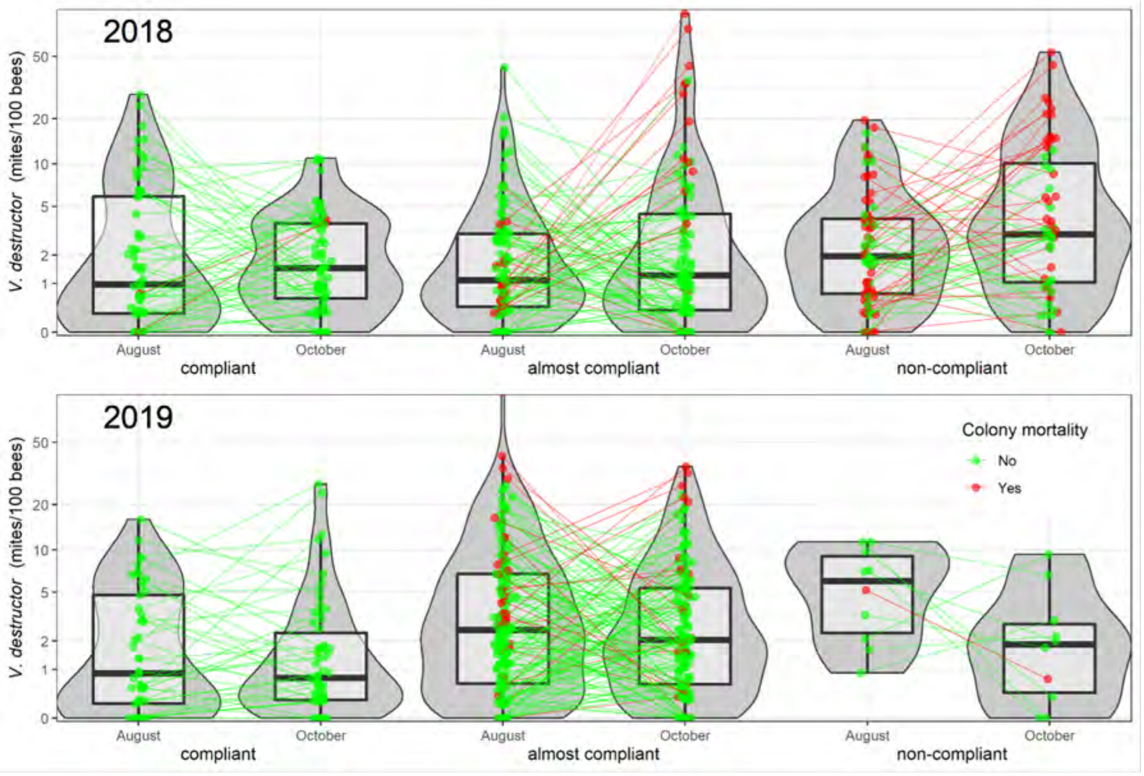

Figure 9: *Varroa destructor* infestation rates in August and October, before and after treatments, respectively, and colony mortality over the following winter according to compliance categories in 2018 and 2019. The violins-plots show the probability density curve of the infestation rate values, and boxplots indicate the median of the data (thick horizontal line) and the interquartile range (box).

3.3.2.3 Effect of compliance on brood size

We observed the highest mean number of capped brood cells in October in the colonies of compliant beekeepers (Table 3). This was marginally significantly higher than the mean value measured in colonies of almost-compliers (differencelog-scale = 1.1, 95% CI: -0.1 to 2.3, p = 0.07; Tables 3 and 4) and significantly higher than that in colonies of noncompliers (differencelog-scale = 1.3, 95% CI: 0.0–2.5, p = 0.04; Tables 3 and 4).

3.3.2.4 Effect of compliance on apiary productivity

Honey harvest differed significantly among compliance groups with mean yields of 194.1, 180, and 68 kg in compliant, almost-compliant, and noncompliant groups, respectively (Kruskal–Wallis Test, chi-squared = 6, p = 0.04).

3.3.2.5 Path analysis of simultaneous direct and indirect effects of compliance on *V. destructor* infestation rates, colony mortality, and brood size

The structural equation path model was defined according to the relationships between the variables measured, as depicted in Figure 10 (see also Appendix A.2.1). The infestation rate in October was not linked to that in early August before the treatments were applied but was affected by the compliance category. Almost-compliance and noncompliance were associated with a significant positive effect on *V. destructor* infestation rates in October (0.31 logarithm units for almost-compliant and 0.6 logarithm units for noncompliant cases). The log-transformed *V. destructor* infestation rate in October had a significant positive impact on colony mortality. The odds of colony death increased by 1.88 with each log unit increase in the *V. destructor* infestation rate in October. In addition, the infestation rate measured in October had a significant negative effect on the number of brood cells (-0.73 log units). In this month, brood size had no significant effect on colony mortality (Appendix A.2.1). The factor year had no significant effect on mite infestation rates in October but a significant effect on colony mortality and brood size.

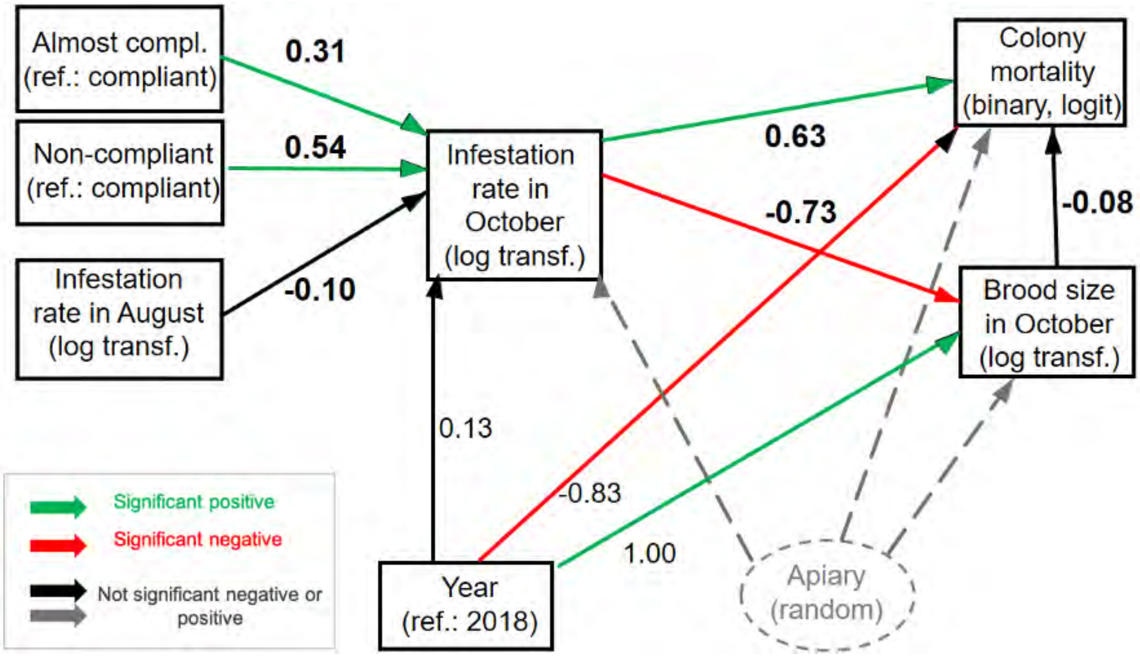

Figure 10: Direct and indirect effects of compliance categories on *Varroa destructor* mite infestation rates, colony mortality, and amount of brood generated by the structural equation model. The standardized regression coefficient for each path is given next to the corresponding arrow. The model was adjusted for clustering of colonies within apiaries (random effect) and year (fixed effect).

3.4 Discussion

We showed that compliance with the recommended *V. destructor* treatment regimen reduced infestation rates in the colonies, which drastically increased colony survival and apiary productivity. The potential negative side-effects of treatment application on the brood did not significantly decrease winter colony survival, but brood size was significantly negatively impacted by the lack of compliant treatment. Communicating the negative impact of noncompliance on colony survival and productivity to participating beekeepers increased compliance of a fraction of the noncompliant beekeepers in the following year. However, a decrease in compliance was observed in half of the previously compliant beekeepers.

3.4.1 Compliance effects on *V. destructor* infestation rates, brood size, and colony mortality

We showed a strong link between compliance with the recommended *V. destructor* control concept and colony mortality. The results from the generalized equation model showed that if treatment regimens only deviated slightly from the recommended concept (i.e., were almost-compliant), a colony had 10 times higher risks of dying compared to a compliant treatment (20% vs. 2% colony losses) and 25 times higher risks if the beekeeper was noncompliant (i.e., applied less than two treatments) (55% vs. 2% colony losses, odds ratio 11 and 50, respectively, Table 4). The decrease in mite infestation after treatment application (i.e., between August and October) was higher in the compliant group, confirming the superior efficacy of the recommended control concept. This lower infestation is likely to have contributed to the decreased colony losses experienced in this group. Despite the generalized equation modeling showing only a marginally infestation decrease in October comparing compliant to noncompliant groups (Table 4), the structural equation modeling showed a significant interaction effect (Figure 10). This effect indicates that an increase in *V. destructor* infestation rates in October due to deviations from the recommendations is directly linked to increased colony mortality (Figure 10). The negative coefficient between mite infestation rates in August and October (Figure 10, Appendix A.2.1) in structural equation modeling and the higher infestation rate in noncompliant treatment regimens compared to compliant ones (7 vs. 2.5 in mites per 100 adult workers, Table 3) indicate that formic acid applications decouple the number of mites in August from that in October. The number of mites measured in October was mainly determined by the level of compliance (Figure 10). In the case of noncompliance, an infestation rate of 10 mites per 100 adult honeybee workers in October led to a 50% chance of colony death over winter (Figure 8). This result is in line with the previous literature, according to which the mortality rates of colonies infested in the 10 to 20 mites per 100 workers range in autumn could reach 20% to 50% on average (Genersch et al., 2010; Liebig, 2001; Guzmán-Novoa et al., 2010). The analysis of the effect of *V. destructor* infestation on the amount of brood and colony mortality with the two models strongly supported the hypothesis that healthy winter honey bee workers, which were not parasitized during their pre-imaginal development, are crucial to ensure colony survival over winter. This was especially the case with the structural equation model, which allows for deriving the possible causal–effect relationships because of the model taking into account the complex relationships between the variables. Thus, our results represent the most tangible evidence, to date, that healthy winter honey bee workers are crucial to ensuring colony survival over winter. However, the effect can be smaller than our estimate because we cannot rule out the possibility that brood size itself also had a direct negative impact on infestation rates. The positive effect of high compliance on colony survival occurred despite the potential negative side-effects of formic acid on brood survival (Gregorc et al., 2004; Strachecka et al., 2012). In addition, a reduced number of treatment applications by noncompliant beekeepers did not lead to a higher amount of brood in their colonies compared to compliant beekeepers (Tables 3 and 4). Instead, they experienced a significant decrease in the amount of brood in October. This decrease was likely due to the higher number of mites infesting the colonies (Table 4, Figure 10). Thus, there is a stronger negative impact of *V. destructor* infestation than that of formic acid on the brood, infirming the hypothesis that the negative side-effects of the repeated formic acid applications (Tihelka et al., 2018) can exceed their positive effects. In addition, the amount of brood in October showed no noteworthy association with mortality (Figure 10). There is thus no benefit in refraining from applying two formic acid treatments as recommended. The importance of factors other than *V. destructor* infestation in causing colony mortality was indicated by significant effects of the factor year on brood size and colony mortality. The factor year includes the effect of variables not measured in our study. For example, interannual variations in weather can affect resource acquisition and brood rearing (Beyer et al., 2018; Bagheri and Mirzaie, 2019; Nürnberger et al., 2019), which in turn can affect the population dynamics of *V. destructor*, treatment effectiveness and thus colony mortality (Nürnberger et al., 2019; Calovi et al., 2021). However, no year effect on infestation rates was observed during our study (Figure 10), indicating the involvement of other variables. Although our results have clearly shown the importance of reducing *V. destructor* infestation rates with correctly implemented control methods to reduce colony losses, we have not considered the role of other possible causes of mortality. Further variables will be considered in a follow-up study by extending our measures and observations to following years and by investigating land-use factors (e.g., pesticide use, agricultural management, and resource availability) in the vicinity of the apiaries, as well as the effect of other pathogens such as viruses, bacteria, and fungi, with the aim of acquiring a more holistic view on the various causes for colony losses.

3.4.2 Promoting compliance and limitations of the control concept

The lack of compliance observed in a proportion of the participating beekeepers may be due to them being less experienced and lacking sufficient knowledge about the recommended treatment regimen. Several studies have shown that a beekeeper's training background and practices are the main factors promoting honey bee colonies' health (Jacques et al., 2017; Thoms et al., 2019). To improve these situations, authorities or associations in many countries strive to provide information and training to beekeepers (e.g., Switzerland, Germany, Austria, Sweden, Denmark, and Netherlands) (van der Steen and Vejsnæs, 2021). Our results, however, show that despite readily available information and training, compliance can be prone to self-interpretation. Compliance was increased in the framework of our experiment through a high personal involvement of beekeepers and ready access to hard data showing the consequences of ones' acts, even when occurring several months after the act itself. Personal involvement in the framework of a research project, with access to systematically acquired data can be considered informal training (Adams, 2018), and is an efficient means to improve colony health. However, such an approach may not be applicable to the wider beekeeping community. Improved compliance can be fostered by including results such as ours as an example of the consequence of deviating from the recommendations (i.e., an increased mortality risk) in formal training to make the latter less theoretical and more relatable to personal experience (Adams, 2018). An additional incentive to promote compliance with recommendations can be of an economic nature, through the main motivation of most beekeepers (i.e., the honey harvest) (López-Uribe and Simone-Finstrom, 2019). Compliant beekeepers benefitted from three times higher harvests than noncompliers, whereas the harvests of almost compliers were only marginally smaller than those of compliers (Table 3). Showing the positive economic effect of implementing the control concept as recommended is likely to motivate beekeepers to improve their *V. destructor* control strategies, despite the complexity of the recommended concept. Although the general level of compliance increased significantly from 2018 to 2019 (Figure 7; Appendix A.2.2), the proportion of fully compliant beekeepers remained stable over the two years. This was due to an increase in the compliance of the previously less-compliant beekeepers being compensated by a decrease in the level of compliance of the initially compliant beekeepers (Figure 7, Appendix A.2.2). This decrease is unlikely due to lack of knowledge or poor concept acceptance, since these participants were compliant in the first year. This decrease may be due to constraints in implementing the complex treatment regimen. These constraints should be identified to foster colony health, possibly with the help of the social sciences, but we can speculate that they originate from the need to apply treatments at a given time, determined by ambient temperatures (Rosenkranz et al., 2010; Steube et al., 2021). This timing might conflict with other commitments, especially for hobby beekeepers whose main activity might take precedence on the care of their honey bee colonies. The frequency of such conflict can be exacerbated by climate changes with increasing periods of extreme temperatures or increasing deviation from usual weather patterns (Steube et al., 2021), which do not allow formic acid application at the appropriate time, when it can reduce damages to the winter honey bee workers effectively. This phenomenon is suggested by the anecdotal reports gathered during this study. We occasionally observed a reluctance to apply the second formic acid treatment, which was described as too stressful for the colonies. A complicating factor was the recurrent summer heatwaves, which made the second application of formic acid more challenging to perform when the right conditions prevailed [application above 29°C led to excessive negative side-effects (Rosenkranz et al., 2010; Steube et al., 2021)]. To overcome this issue, some participating beekeepers implemented biotechnical methods [queen caging, brood interruption, and hyperthermia (Büchler et al., 2020; Apiservice, 2021)] to avoid the second application of formic acid, while others acted directly on the diffusion mode of the second formic acid application by modifying the evaporation quantity, possibly affecting the effectiveness of treatment. This clearly reveals the personal appropriation of the treatment concept against *V. destructor*. This phenomenon has also been observed in Austria with the application of unexpected *V. destructor* treatment regimens by beekeepers with detrimental effects on colony health (Oberreiter and Brodschneider, 2020). Further research is required to better understand the motivations and constraints faced by beekeepers that lead to a lack of compliance and increased colony losses. Given that, irrespective of the intention to comply, not all constraints can be overcome, identifying which elements of the concept are more crucial to ensure colony health can lead to a "next best strategy" as a compromise between realistic implementation in the field and promotion of colony health. Here, we showed that deviations from the recommended treatment application time (almost-compliant) led to fewer colony losses than renouncing to one of the formic acid applications altogether (noncompliant) and allowed honey harvests almost as high as those of compliant beekeepers. Concept formulation could, therefore, be adapted by setting the priority on performing two formic acid applications, even if the appropriate timing cannot be held precisely. This represents a short-term solution to mitigating colony losses due to *V. destructor*. However, monitoring the precise implementation of *V. destructor* control methods in the field can contribute to developing new and sufficiently effective concepts better adapted to a constantly evolving context, be it climate changes or changes in social trends and personal constraints. Our results also highlight the need to consider how *V. destructor* treatments are implemented (i.e., conformity to manufacturer instructions or compliance with recommendations) when surveying beekeepers to determine the role of management in colony health. All beekeepers in our study would have declared treating against the mite, but the data showed wide variations in treatment implementation and in their efficacy.

3.5 Conclusion

Although *V. destructor* is not the only cause for colony losses (Steinmann et al., 2015; Smith et al., 2013a; Van Esch et al., 2020), our results support the view that the correct implementation of varroacidal treatments drastically improves colony survival over winter. We also showed that improved communication of the negative consequences of deviations from the recommended treatment regimens can lead to improved compliance and calls for new paradigms in beekeeper training. Integrating principles of social sciences into training can foster the acceptance of, and compliance with, recommendations. Social sciences can also contribute to identifying the constraints inherent to the complexity of the alternative control methods, which seems to limit compliance. In case such constraints are unavoidable, our results suggest that performing the treatment applications at suboptimal dates results in fewer honey bee colony losses than renouncing a treatment altogether. Alternatively, constraints to treatment implementation can be reduced with the development of simpler yet effective treatments against *V. destructor*.

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Conflict of interest

The authors declare no conflict of interest.

Data statement

The datasets generated and analyzed during the current study are not publicly available due to their use in ongoing primary research, but subsections may be made available from the corresponding author upon reasonable request.

Chapter 4

Agroecological measures in meadows promote honey bee colony development and winter survival

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4 Agroecological measures in meadows promote honey bee colony development and winter survival

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Abstract

The homogenization of agricultural landscapes has led to a decrease in pollinator diversity and abundance. In response to this decline, farmers have implemented agroecological measures, which, in meadows, aim at providing more floral resources. These measures are the availability of unmown floral strips, delayed mowing and discouraging the use of the conditioner, a device known to harm insects. The aim of our study was to investigate the cascade of effects of these agroecological measures on honey bee colony development and winter survival. We (i) determined the effect of these measures on colony size during the nectar and pollen collecting season in spring and summer, (ii) evaluated the effect of spring and summer colony sizes on autumn size and (iii) described the effect of colony size in autumn on winter mortality. In this study, 300 honey bee colonies were monitored over three years in three cantons of Switzerland. Colony size was defined by numbers of brood cells and of adult workers. Honey bee colony size in summer and autumn were improved by agroecological measures on meadows and likely contributed to the increased overwintering success. This study is a first step towards the targeted identification of viable agroecological measures on temporary meadows that can be implemented to promote honey bee colonies health in the agricultural landscape.

Keywords: *agricultural landscapes, agroecological measures, agri-environmental schemes, Apis mellifera, colony size, honey bee, meadow management, winter colony losses*

4.1 Introduction

In response to the negative effects of intensive agriculture on pollinators and on overall biodiversity, agri-environmental schemes have been implemented since the 1990s in most European countries (Albrecht et al., 2007; Marja et al., 2019; Uthes and Matzdorf, 2013; Zingg et al., 2019). A variety of agroecological measures aim at providing additional food and nesting resources for pollinators, as the ecological service they provide increases productivity (Ricketts et al., 2008). The specific effects of landscape composition and floral resources on honey bee development and winter losses have been demonstrated using large-scale monitoring studies (Genersch et al., 2010; Kretzschmar et al., 2016; Kretzschmar and Frontero, 2017; Kuchling et al., 2018). The implementation of agroecological measures was shown to maintain diverse floral resources benefitting the development and survival of honey bee colonies during and after periods of scarcity (Alaux et al., 2017; Decourtye et al., 2010; Odoux et al., 2014; Requier et al., 2015; Wintermantel et al., 2019). Finding measures that can be implemented in meadows, and efficiently benefit honey bees, is a major challenge as meadows are a crucial part of fodder in dairy and crop production regions across Europe (Huyghe et al., 2015). The aim of our project was to identify, among a set of agroecological measures implemented by farmers in meadows, those effectively increasing floral resources to benefit honey bee health. The measures considered were leaving unmown floral strips, delayed mowing, and foregoing the use of conditioner devices while mowing. The conditioner hastens the drying of grass after mowing, thereby improving hay quality, but is harmful to bees and other insects visiting the flowers during mowing (Fluri and Frick, 2002; Humbert et al., 2009). These studies on conditioners observed dead insects in the field during mowing operations, but the impact on bee colonies has not been documented. We here tested the cascading effects of the implementation of these agroecological measures in proximity of the apiaries, in order to better understand the underlying mechanisms (Shakarian et al., 2015; Requier et al., 2017). This cascade included the effects of (i) the agroecological measures on colony size during spring and summer (June-July), at the time they are applied, (ii) spring and summer colony size on autumn colony size, and (iii) autumn colony size on winter mortality. For this, the size and winter survival of 300 honey bee colonies belonging to amateur beekeepers voluntarily participating in the project were monitored over three years at 30 apiaries. Two parameters of colony size were assessed: the number of adult bees and the number of capped brood cells from which young adults emerge. These parameters were chosen because they can respond differently to the agroecological measures (e.g., brood will not be affected directly by foregoing to the use of a conditioner when mowing, while adult honey bees will) and because their biological interdependence influences colony development and activity during nectar and pollen collecting season (Kretzschmar and Maisonnasse, 2022) as well as their survival over winter (Imdorf et al., 2010). Winter survival of honey bee colonies is also strongly affected by the ectoparasitic mite *Varroa destructor* (Hernandez et al., 2022). We thus considered the effect of infestation rates of adult honey bee workers by this parasite in October, at the time when their impact on colony health is greatest (Hernandez et al., 2022). By identifying effective agroecological measures or combinations of such measures in meadows, our results allow for the adjusting of meadow management to optimize honey bee colony health.

4.2 Material and methods

4.2.1 Sites and agroecological measures in meadows

The study was conducted over three successive years (from March 2018 to March 2021) and took place in the Cantons of Vaud, Jura and Bern in western Switzerland. The agricultural landscape of these regions is characterized by a mosaic of diverse elements such as meadows, arable fields and forests (Lachat, 2010). Legume plants (mainly white clover, red clover and alfalfa) are important sources of nectar and pollen and are frequently found in meadows (Agroscope, 2021; Ziaja et al., 2018). The Swiss Agricultural Policy defines three types of meadows: temporary, permanent and ecological (BLW 2004). Temporary meadows are included in crop rotation and last up to five years before being replaced by crops, whereas permanent meadows last for several decades. These two meadow types receive fertilizer, are mowed two to five times per year, and are occasionally used as pastures. For both meadow types, farmers individually choose their harvest dates and equipment. In contrast, in ecological meadows, the Swiss Agricultural Policy sets the earliest mowing dates. Moreover, ecological meadows receive little or no fertilizer.

Thirty volunteer beekeepers managing apiaries in the three cantons were enrolled in the study. Recruitment occurred during information sessions and volunteer selection was based on the following criteria: age of beekeeper (<70), apiary size (≥ 10 colonies), and distance to another selected beekeeper's apiary (≥ 5 km). The colonies in selected apiaries had not been equalized for size at the beginning of the monitoring period and the queens heading them were mostly unrelated. Each monitored apiary marked the center of a 2 km radius sector in which agroecological measures were considered to possibly affect the development and health of the colonies. The 2-km radius has been defined as an average foraging radius for honey bees (Steffan-Dewenter and Kuhn, 2003). Two sectors were located in canton Bern, eight in canton Jura and 20 in canton Vaud (Figure 11). Five of the Vaud sectors extend into the neighboring canton of Fribourg. Total sector area was 1'256 ha. Farmers owning meadows within the sectors were given the opportunity to apply agroecological measures that aimed at enhancing meadow exploitation by honey bees in return for subsidies. In temporary meadows, three measures were implemented, alone or in combination: (i) to forego conditioner use when mowing; (ii) to leave a strip unmown during each of the mowing operations performed between June 1st to August 31st (time of legume, i.e., white clover, red clover and alfalfa, flowering); (iii) to delay one mowing operation until legumes flowering ended. The combination of (ii) + (iii) was not considered as relevant because leaving an unmown strip in a late-mown meadow could produce hay with an insufficient quality for cattle feeding. In permanent meadows and ecological meadows, only foregoing to the use of a conditioner was proposed to the participants. The other measures were not relevant because ecological meadows already have specific rules regarding mowing dates, and because permanent meadows were assumed to have less legume flowers than temporary meadows. On average, meadows represented ca. 300 ha (24 % of total sector area; Table 5). Temporary and permanent meadows represented ca. 80% of meadows and were distributed across the landscape (see example in Figure 12).

Table 5: List of meadow types, corresponding agroecological measures, surface area and codes used in the analyses. Numbers indicate the average surface area across sectors and years and the proportion of the total sector area occupied by each element. The last column indicates how each element was coded in the models presented in supplementary material.

Meadow type	**Agroecological measure description**	**Average surface area in hectares**	**Average % of total sector surface area**	**Code used in analyses**
Temporary Meadows	Temporary meadows without agroecological measures	96	7.6	TM-no-aems
	Mowing without conditioner	20	1.5	TM-no-cond
	Unmown floral strip	1.30	0.1	TM-strip
	Delayed mowing	4.86	0.3	TM-delay
	Combination of mowing without conditioner use and leaving an unmown floral strip	2.6	0.2	TM-no-cond*strip
	Combination of mowing without conditioner and delaying mowing	5.33	0.42	TM-no-cond*delay
Permanent Meadows	Permanent meadows without agroecological measures	78.22	6.22	PM-no-aems
	Mowing without conditioner	21.8	1.73	PM-no-cond
Ecological Meadows	Ecological meadow without agroecological measure	52.8	4.12	EM-no-aems
	Mowing without conditioner	8.14	0.65	EM-no-cond

4.2.2 Data collection

Landscape data

Landscape data originated from public sources. Data for forest and urban areas as well as bodies of water from 2019 were obtained from Cantonal services of geographic information (Geoportal SIT-Jura, Geoportal Canton de Berne, ASIT-VD catalogue). All agricultural land-use types were provided by Cantonal services of agriculture (Jura, Bern, Vaud, Fribourg). Agricultural data provided the following information: field location, field surface area, crop type, and whether agroecological measures had been implemented. These datasets were used to quantify the total surface area per land-use type, within the 2-km-radius sectors around the monitored apiaries. Maps of land use around apiaries were computed through QGIS (QGIS 3.16.1-Hannover).

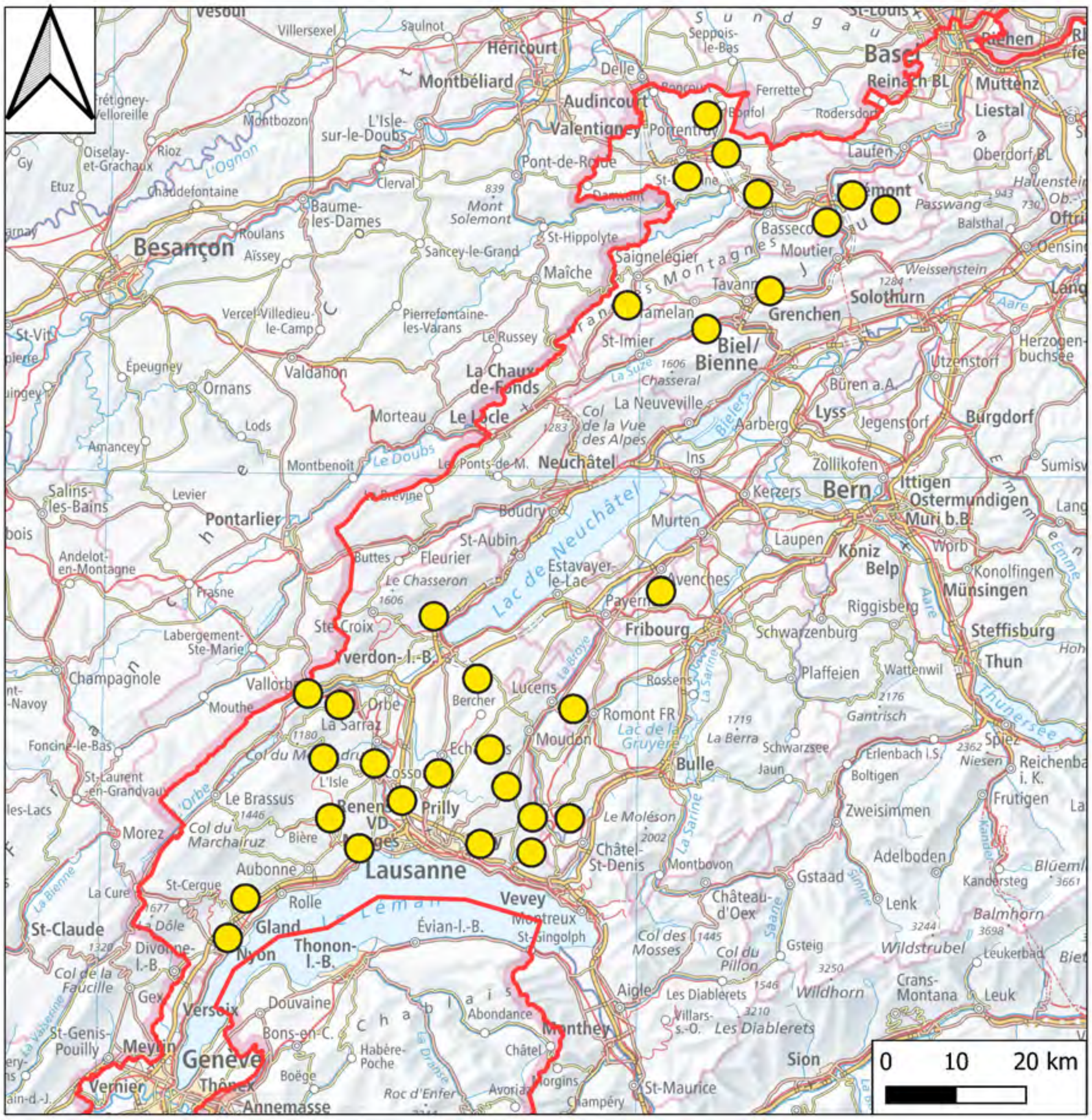

Figure 11: Locations (yellow dots) of the 30 monitoring sectors across western Switzerland.

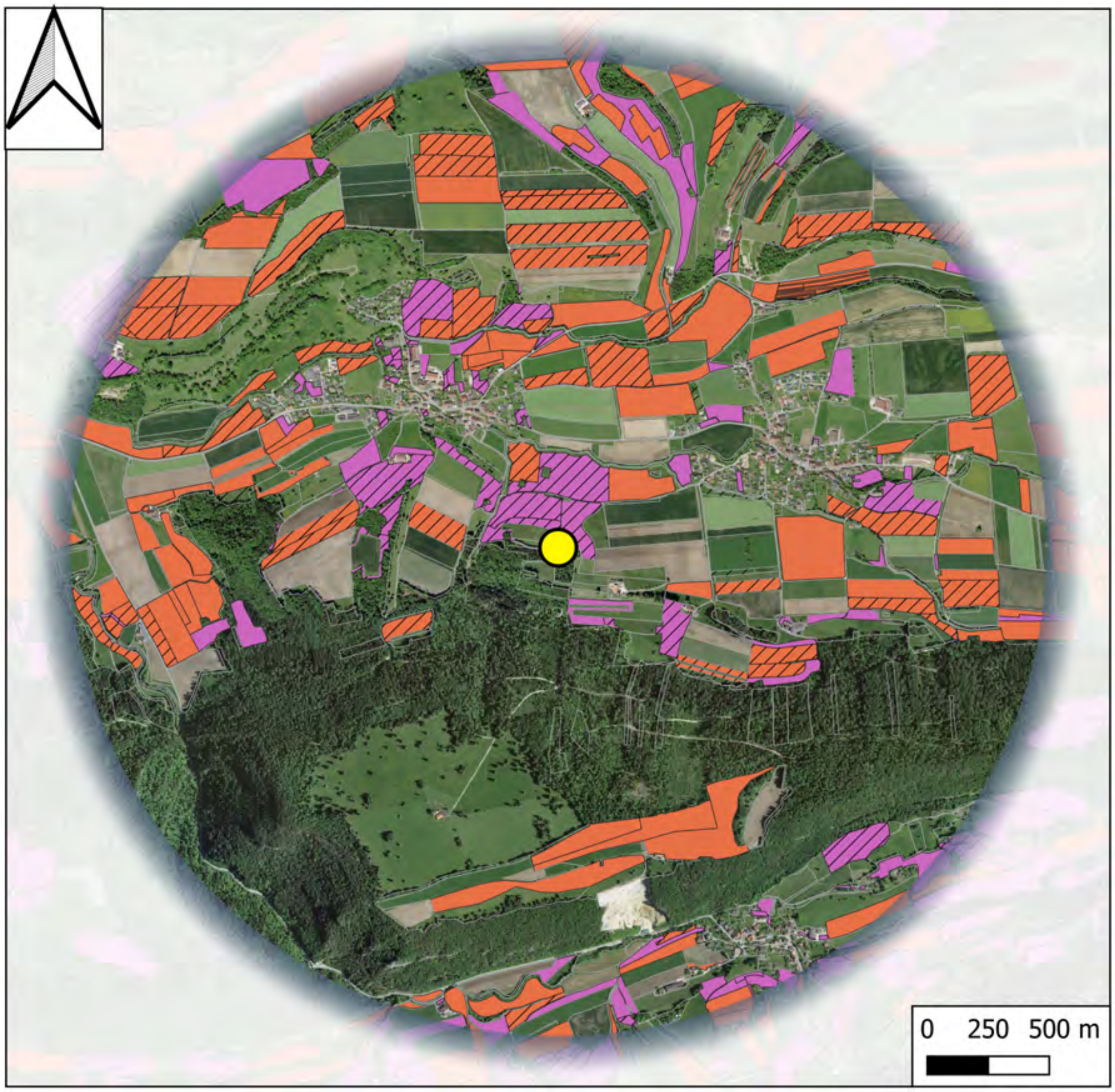

Figure 12: Example of one 2km radius study sector. Orange: temporary meadows; pink: permanent meadows. Dashes indicate meadows managed with agroecological measures as detailed in Table 5. Yellow dot: apiary location. Aerial photograph accessed via MapGeoAdmin-WMS on 03.03.2022.

Honey bee colonies and quantification of their size

Each of the 30 apiaries, containing 10 monitored colonies, was owned and managed by volunteer beekeepers following the local recommended practices (Apiservice, 2021). Beekeepers were allowed to split their colonies or adjust their size between April and June, after which they were instructed not to implement any measure which could interfere with our colony size assessment. To assess the effects of agroecological measures implemented in meadows on honey bee colony size, the number of adult honey bees and capped brood cells in the colonies were quantified in June, July and October. These three periods were chosen because June and July define the floral resource scarcity period (Requier et al., 2017) when agroecological measures are implemented, while colonies begin with winter preparations in October (Hernandez et al., 2022; Imdorf et al., 2010). The quantification of the two colony-size parameters was obtained using the ColEval method (Hernandez et al., 2020). Briefly, a calibrated estimation of the percentage of the comb surface area occupied by adult honey bees and by capped brood was performed and subsequently converted into number of adult individuals and brood cells. Adult workers and brood data for 2018 and 2019 were previously used in Hernandez et al. (2022).

Measure of V. destructor infestation rates in October

For the assessment of *V. destructor* infestation rates during the October apiary visit, adult honey bee workers (mean = 300, SD = 50) were sampled from open brood combs of each colony. The sample was placed in a plastic zip bag, stored on ice and brought to the laboratory where they were stored at -20°C until processing. During processing, each samples were first weighed to determine the number of adult honey bees they contained and then washed with soapy water following a standard protocol to dislodge mites for counting (Dietemann et al., 2013b) . From these data, the number of mites per 100 workers was calculated. The infestation rate data for 2018 and 2019 were previously used in Hernandez et al. (2022).

Colony mortality

Colony mortality was recorded after the overwintering period, i.e., at the beginning of April 2019, 2020, and 2021. Beekeepers replaced dead colonies each year with nuclei prepared in the spring of the previous season and attributed them new identification numbers.

4.2.3 Statistical analysis

Principal component analyses (PCA, package R ade4; R Core Team (2021)) were used for selecting the meadow types (including those without agroecological measures) that contributed most to the differentiation of the landscape structure in the sectors around the apiaries. Variables that best correlate with the first two dimensions of the PCA identify meadow types for which the surface areas were variable enough for their effect on colonies to be tested statistically. This step was necessary because the implementation of measures in the field was not controlled but dependent on the decisions of farmers volunteering for the study. We included temporary meadows without measures in the analysis as baseline to disentangle the effect of the resources provided by the varying surface areas of meadows themselves, and that of the meadows subjected to agroecological measures. PCA analyses were run on the three years separately. Meadow types that contributed more than the average contribution of inertia (10%) of the first two eigenvalues were selected for model construction (Abdi and Williams, 2010). Generalized linear mixed models (GLMM, R package lme4) with Gaussian distribution (with Identity as link function) were used to investigate the relationship between the highest contributing agroecological measures in meadows identified by PCA and honey bee colony size in June and July. The variable apiary was used as random effect. The same GLMM modelling method (Gaussian distribution, Identity as link function and apiary as random effect) was applied to meadows without agroecological measures to identify a possible direct effect of meadows per se independently of the agroecological measure implemented. The effect of summer colony size on autumn colony size was evaluated with the same GLMM modelling method (Gaussian distribution, Identity as link function and apiary as random effect). The effects of colony size and *V. destructor* infestation rates (alone and of their interaction) in autumn on winter mortality were investigated with a Bernoulli model (i.e., a GLMM with a binomial distribution, a logit function as link and apiary as random effect). We combined these variables in a single model because they act in concert on colony health, but we also modelled them separately to assess their individual effects on the response variable, winter mortality. The evaluation of the p-value (significant p-value ≤ 0.05) of the variables in the models was estimated with Satterthwaide approximation (Kuznetsova et al., 2017). Because the area of implementation of agroecological measures changed over years, and because intense variations in yearly climatic conditions are common, GLMMs were applied on the three study years separately, instead of adding the factor "year" as a random effect in a global model. Additionally, the random effect of "year" is partially absorbed by the random effect of "apiary" (Kretzschmar et al., 2016).

4.3 Results

4.3.1 Identification of the contribution of agroecological measures to the meadow structures surrounding the apiaries

PCA analyses showed that the distribution pattern of the meadow types (temporary, permanent and ecological) was consistent from year to year over the three years. The meadow types showing the most variable surface area within the sectors over the 3 years were: temporary meadow mowed without conditioner, temporary meadow with floral strips, the combination of the latter two, and temporary meadow mowed without conditioner after flowering, i.e., with delayed mowing (see Appendix A.3.1). Because of this consistency, the meadow types used for further analysis were selected based on a new PCA run on the

pooled data of the three years (See Appendix A.3.2). As observed in the correlation circle in this PCA, temporary meadows mowed without conditioner, as a single measure or in combination with floral strips or with delayed mowing, and temporary meadows with floral strips as a single measure are strongly correlated with dimension 1 (inertia = 28.6%) and contributed to more than 93.6% of the distribution of apiaries on the plane defined by the first two dimensions (Figure 13 and Appendix A.3.3). Permanent meadows mowed without conditioner and permanent meadows without measures, as well as to a lesser extent, temporary meadows with delayed mowing correlated with dimension 2 (inertia = 22.0%) and contributed to 77.2% of the total inertia (Appendix A.3.2). Ecological meadows and temporary meadows without measures correlated with dimension 3 (inertia = 15.4%) with respective contributions of 40.2 and 25.1% (Appendix A.3.2). These results suggested focusing the analysis on temporary meadows with and without agroecological measures, on temporary meadows with floral strips and on temporary meadows mowed without conditioner as a single measure or in combination with floral strips or with delayed mowing.
The surface area of temporary meadows mowed without conditioner increased from 17.6 ha/sector in 2018 to 22.9 ha/sector in 2020, whereas the surface area of temporary meadows without measures slightly decreased in 2020 (Table 6). The same pattern was observed for delayed mowing without conditioner, while the changes in surface areas of floral strips combined with mowing without conditioner were more variable, with no marked tendency (Table 6).

Table 6: Total surface area (in ha) of the agroecological measures implemented in temporary meadows in the 30 study sectors over the three-year study.

	2018	**2019**	**2020**
Temporary meadow mowed without conditioner	17.60	19.46	22.91
Temporary meadow with floral strips	1.56	0.94	1.41
Temporary meadow with delayed mowing	5.50	5.28	3.80
Temporary meadow mowed without conditioner and floral strips	2.98	3.39	1.43
Temporary meadow mowed without conditioner and delayed mowing	5.14	5.45	5.40
Temporary meadow without agroecological measures	99.60	106.78	81.61

The four measures were applied over the three years with small variations in the surface

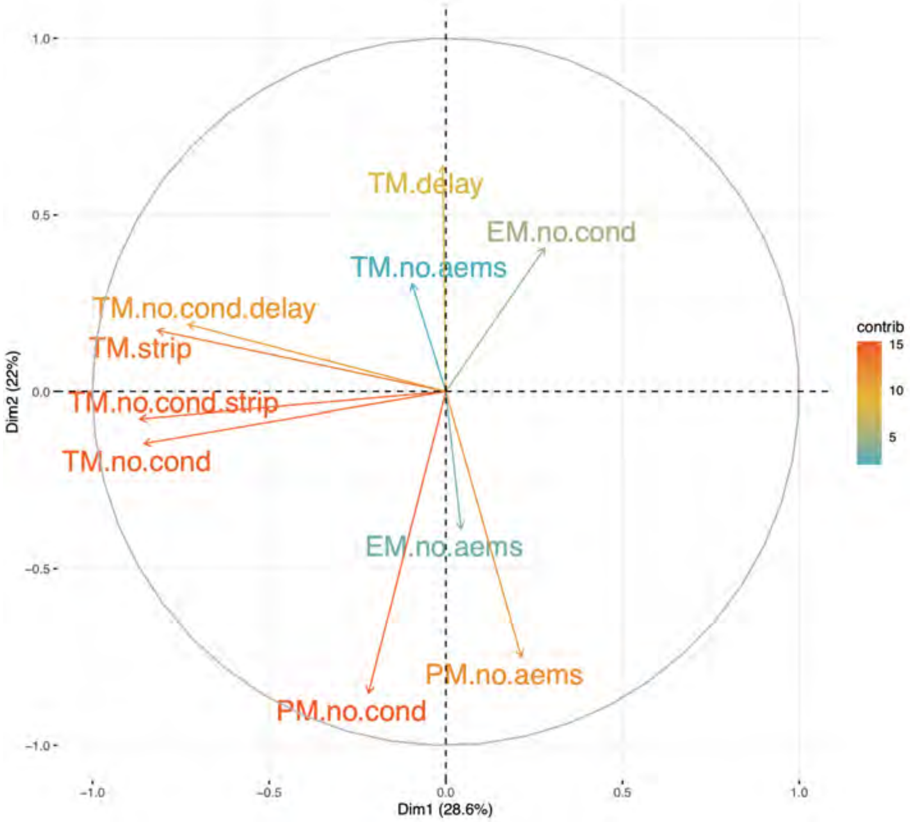

Figure 13: Correlation circle of the distribution of meadow types with and without agroecological measures according to dimensions 1 and 2 of the PCA. Percentage of inertia for dimension 1 and dimension 2 are given in parentheses. TM-no-aems= Temporary meadows without agroecological measures; TM-no- cond= Temporary meadows mowed without conditioner; TM-strip= Temporary meadows with unmown floral strip; TM-delay= Temporary meadows with delayed mowing; TM-no-cond.strip= Temporary meadows mowed without conditioner and with unmown floral strip; TM-no-cond.delay= Temporary meadows with delayed mowing without conditioner; PM-no-aems= Permanent meadows without agroecological measures; PM-no-cond= Permanent meadows mowed without conditioner; EM-no- aems= Ecological meadow without agroecological measure; EM-no-cond= Ecological meadow mowed without conditioner.

areas of meadows. Their total contribution to the first dimension of the PCA was almost constant (91.1%, 93.4% and 92.0% for 2018, 2019 and 2020, respectively) (see Appendix A.3.4).

4.3.2 Effect of the agroecological measures in temporary meadows on spring and summer colony size

In 2018, but not in the other years, a significant positive effect of temporary meadows without measures on the number of adult honey bee workers in June was observed (p-value= 0.047 with R2.fixed = 0.05; See Appendix A.3.5 in the associated Dryad repository). Despite the significant p-value, the determination coefficient for the fixed effect R2.fixed was below 0.10, indicating a relatively small effect. Temporary meadows mowed without conditioner also showed a slight positive, albeit non-significant, effect on the number of adult honey bee workers in June 2018 (p-value=0.082 with R2.fixed = 0.09; See Appendix A.3.5 in the associated Dryad repository). In 2019, there was a slight significant positive effect on the number of adult bees in June associated with the mowing without conditioner combined with the presence of floral strips (p-value = 0.04 with R2.fixed =0.06), and a trend for the effect of delayed mowing (p-value = 0.06 with R2.fixed = 0.05; Appendix A.3.5 in the associated Dryad repository). In June 2020, July 2018, 2019 and 2020 there was no significant effect of any agroecological measure on the number of adult workers. There was neither a significant effect of the selected agroecological measures on the amount of brood in June and July 2018, nor in June 2019 (all p-values > 0.05; Appendix A.3.5 in the associated Dryad repository). By contrast, a significant positive effect on brood was observed at the end of July 2019 when mowing without conditioner alone and combined with floral strips or with delayed mowing, were applied (p-values ranging from 0.02 to 0.002 with R2.fixed ranging from 0.10 to 0.22; See Appendix A.3.5 in the associated Dryad repository) In July 2019, the effect of mowing without conditioner was also significant when temporary meadows without measures were added as an additional variable in the model (p-value=0.016 with R2.fixed=0.14). The same pattern was observed in July, but not in June, of 2020, with a significant positive effect of temporary meadows mowed without conditioner (p-value=0.022; R2.fixed=0.08) and of delayed mowing (p-value=0.035; R2.fixed=0.07).

4.3.3 Effect of the spring and summer colony size on the number of adult honey bees and brood in autumn

In June and July for all years, the number of adult honey bees was correlated with the number of capped brood cells (See Appendix A.3.7, in the associated Dryad repository). For each of the three years, the number of adult workers in July had a significant positive effect on colony size in October (p-values<0.04 with R2.fixed between 0.08 and 0.04; See Appendix A.3.6 in the associated Dryad repository). However, the number of adult workers in June did not significantly correlate with colony size in October (See Appendix A.3.6 in the associated Dryad repository). The positive effects of the amount of brood in June and in July on colony size in October was occasionally significant only in 2019 and 2020 and never in 2018 (See Appendix A.3.6 in the associated Dryad repository).

4.3.4 Effects of colony size in autumn and *V. destructor* on winter mortality

When the number of adult honey bees and capped brood cells were considered in a mortality model, the two variables had divergent effects. The number of adults had a significant positive effect on survival in the three years (p-value<0.005; See Appendix A.3.7 in the associated Dryad repository). By contrast, the number of capped brood cells had no effect in 2018 and 2020, whereas in 2019, a significant positive effect on survival was observed (See Appendix A.3.7 in the associated Dryad repository). For each of the three study years, *V. destructor* infestation rates in autumn, when considered as the single variable in a mortality model, was significantly associated with higher winter mortality (p-value<0.009; R2.fixed>0.03; See Appendix A.3.7 in the associated Dryad repository). When the number of adult honey bees, capped brood cells and the *V. destructor* infestation rates were included as variables, only the positive effect of the number of adult honey bees on colony survival in 2019 and 2020 remained significant (p-value$=0.028$ and p-value$=0.001$, respectively). In 2018, a positive trend was observed for adult honey bees (p-value$=0.07$; See Appendix A.3.7 in the associated Dryad repository). There was no significant interaction between *V. destructor* infestation rates and colony size (p-value>0.310; See Appendix A.3.7 in the associated Dryad repository).

4.4 Discussion

The objectives of our study were to evaluate the effects of agroecological measures applied to meadows on honey bee colonies development and survival. For this, we evaluated whether the surface areas of meadows under agroecological management in the flight range of the colonies were associated with increased summer and autumn colony sizes and decreased winter colony mortality. Colony size in July was positively influenced by three agroecological measures on temporary meadows (i.e., mowing without conditioner, leaving floral strips unmown and delayed mowing combined with mowing without conditioner). In July, the amount of brood and even more so the number of adult honey bees, had a positive effect on colony size in autumn. In turn, colony size in autumn, again mainly the number of adult honey bees, was associated with better colony overwintering. We thus gathered evidence that the agroecological measures applied to temporary meadows promote the development of the colonies positively, which increases their probability of survival over winter (Figure 14).

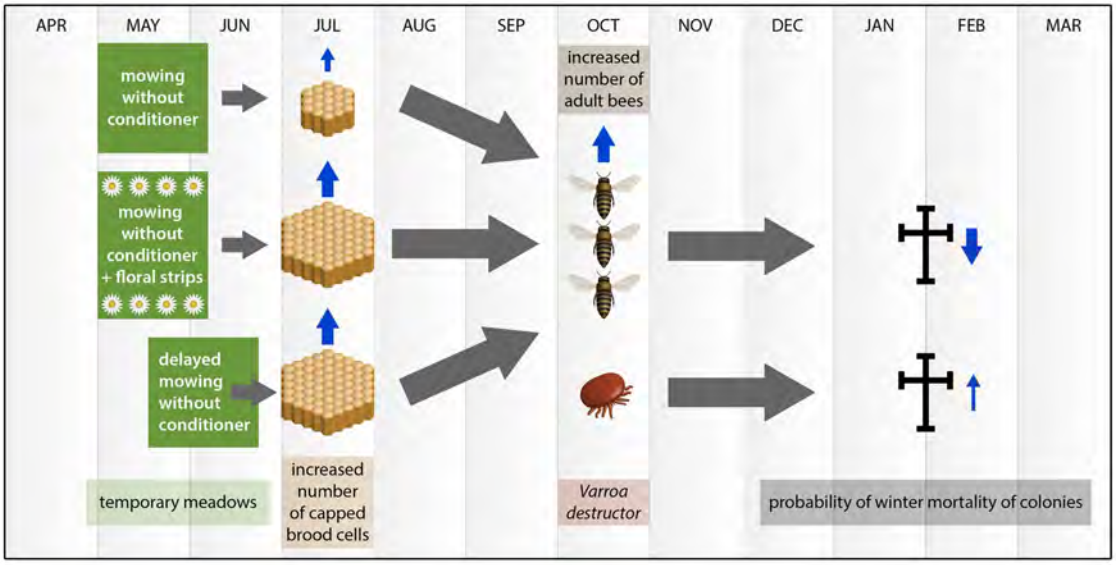

Figure 14: Graphical summary of the main cascading beneficial effects on colony development and winter survival of the three agroecological measures on temporary meadows.

Selected agroecological measures in temporary and permanent meadows around apiaries

Four agroecological measures implemented in temporary meadows contributed most to the first two dimensions of the yearly PCAs (with an average contribution of 92 % to dimensions 1; Appendix A.3.4). Despite changes in surface area (Table 6), the contributions of these four measures were consistent over the three years of observations and targeted temporary meadows. These measures were the mowing without conditioner alone or in combination with delayed mowing or with leaving unmown floral strips, and the floral strips alone. A beneficial effect of temporary meadows without agroecological measures on colony size was observed only in 2018 and not in 2019 and 2020. These results suggest that the resources normally available in meadows were not at the origin of the beneficial effects observed, at least not for 2019 and 2020, and that these effects indeed resulted from the agroecological measures implemented (See Appendix A.3.5 in the associated Dryad repository). The agroecological measures were less frequently employed in permanent meadows (Table 5). Permanent meadows on which no conditioner was used or without any measures were strongly correlated with dimension 2, which means that the areas of the permanent meadows over the 30 sectors around apiaries, varied independently from those of the temporary meadows (Appendix A.3.2). Additionally, their contributions to land-use structure were lower than that of temporary meadows (Appendix A.3.4).

Effects of selected agroecological measures in temporary meadows on colony size

The mowing of temporary meadows without conditioner, the delayed mowing without conditioner and the presence of floral strips in meadows mowed without conditioner had significant positive effects on brood size in July 2019 and 2020, but not in 2018 (See Appendix A.3.5 in the associated Dryad repository). This pattern may be related to an insufficient surface area on which these agroecological measures were implemented in the first year and to their annual increase above an effective threshold in the following years (of 5.4 % in 2019 and 6.7 % in 2020; calculated from data in Table 6). It is therefore possible that if these surface areas further increased, their beneficial effects on honey bee colony size would also rise. Despite low values of the coefficients of correlation R2, it is possible to approximate the effect of these measures on the number of capped brood cells. The number of capped brood cells could increase from a thousand to almost three thousand for every 10-hectare increase in surface area on which the various measures were implemented. Another explanation for the fact that we did not detect an effect in 2018 could be due to suboptimal beekeeping practices, in particular regarding the treatments against *V. destructor* (Hernandez et al., 2022; Correia, 2021) . The negative effects of excessive *V. destructor* infestation rates on brood production (Hernandez et al., 2022) could have masked the effects of the agroecological measures.

We could find no significant effect of foregoing the use of a conditioner while mowing on the number of adult workers in June or July, despite the fact that forager bees are directly exposed to the conditioner, whereas brood cells are not at all exposed to it. Mowing without conditioner thus likely affected the amount of brood indirectly. Foregoing the use of this device could have resulted in higher numbers of foragers returning to the colony with resources, which benefited brood rearing by the colonies. This measure might alleviate the need for colonies to compensate for foragers killed by conditioner (Fluri and Frick, 2002), thus allowing the honey bee nurses to continue caring for the brood instead of exiting the hive to forage. Indeed, a lack of foragers promotes the behavioral maturation of nurses, which start foraging to maintain colony resources (Eyer et al., 2017; Johnson, 2010; Sagili et al., 2011). A positive effect on the number of adult bees in June 2019 and on the amount of brood in July 2019 and 2020 was detected when the mowing of temporary meadows without conditioner was combined with the presence of floral strips or with delayed mowing. This effect was most certainly due to the floral resources that these agroecological measures provided, increasing the availability of pollen and nectar for the colonies. These results suggest that the effect of mowing without conditioner were enhanced when combined with the floral strips or with delayed mowing. Delayed mowing appeared as effective as floral strips at promoting honey bee colony size (See Appendix A.3.5 in the associated Dryad repository). As a result of the high correlation between the number of brood cells and adult honey bees, we can consider that, despite some non-significant relationships in our models (See Appendix A.3.5 in the associated Dryad repository), the positive effect of agroecological measures observed on capped brood cells in July implied the same effect on adult honey bee numbers. Consequently, we can assume that agroecological measures also had a positive effect on colony size in July.

Colony size in the spring is influenced by several interrelated factors (i.e., climate, genetics), and therefore differences in colony dynamics may mask the positive effect of the agroecological measures that could act as levers reinforcing colony development in the spring and summer. Despite the high number of uncontrolled factors in our field study involving volunteers (e.g., unforeseeable but necessary beekeeping actions at the end of summer, such as feeding, colonies merging, or queen replacement), which could influence colony development, we observed a positive effect of the colony size in July on the colony size in October, before wintering (See Appendix A.3.6 in the associated Dryad repository). This relationship was slightly decreased by high *V. destructor* infestation rates, which were possibly due to suboptimal treatments between late summer and autumn (See Appendix A.3.7 in the associated Dryad repository). This result indicates that effective *V. destructor* treatments are crucial to ensure honey bee colony health and to allow colonies to benefit from agroecological measures implemented in agricultural landscapes.

Colony size and winter survival

The size of a colony in autumn had an important influence on its overwintering survival probability (See Appendix A.3.7 in the associated Dryad repository). The number of adult honey bees in autumn had a significant negative effect on the mortality probability, i.e., a high number of adult bees in October led to low winter mortality. We observed that when *V. destructor* infestation rates in October, which are known to be an important determinant of colony survival over winter (Hernandez et al., 2022; Giacobino et al., 2015), are added to the model, the effect of the number of adult honey bees remained significant (See Appendix A.3.7 in the associated Dryad repository). The number of adult honey bees in autumn thus appears to be as important as the *V. destructor* infestation rates before winter in determining colony survival over the cold season (See Appendix A.3.7 in the associated Dryad repository). It is thus likely that agroecological measures, which lead to an increase in the number of honey bees during the flowering season would subsequently increase colony resilience to infestations by this parasite in autumn. However, this effect is unlikely to be sufficient to counter high infestation rates due to suboptimal varroacidal treatments for example (Hernandez et al., 2022).

The effect of the number of capped brood cells on colony survival was more variable. This effect was only significantly positive in 2019 (See Appendix A.3.7 in the associated Dryad repository) and became non-significant when the variable *V. destructor* infestation rates in October was added to the model (See Appendix A.3.7 in the associated Dryad repository). This variability may be due to beekeepers replacing queens or to natural queen replacements in September. The associated interruption of brood production would thus bias the measurement of colony size in October. This variability may also be due to yearly climatically induced variations in the timing of brood production decline towards the end of summer (Kretzschmar and Frontero, 2017; Odoux et al., 2014).

Robustness of the effects of agroecological measures to climatic variations
It is important to consider that the observations were made over three years (2018, 2019 and 2020) with different climatic conditions and, consequently, different colony development patterns. Annual climatic variations affect the availability of floral resources (pollen and nectar) and hence the amount of brood that can be reared by the colonies and in turn the number of emerging adult workers (Kretzschmar and Frontero, 2017; Odoux et al., 2014). The cascade of beneficial effects of the agroecological measures on colony size in summer and autumn and on winter mortality was, however, consistent across the three years. Our findings thus suggest that these agroecological measures may be robust against climatic variations in the range of those experienced during the three study years.

4.5 Conclusion

Our data indicate that colony size in July, i.e., the number of adults and capped brood cells, was enhanced by the agroecological measures implemented in temporary meadows, i.e.: mowing without conditioner, floral strips and delayed mowing. A high number of adult and immature honey bees in July led to a large colony size in autumn. In turn, the high number of workers populating colonies ahead of winter increased colony survival over the cold season. The number of workers at this time of the year was, however, negatively affected by high *V. destructor* infestations rates, which can be prevented by well implemented varroacidal treatments (Hernandez et al., 2022). Although implemented on relatively small surface areas (approximately 4 % of the sectors of 2 km radius around apiaries), we found evidence that agroecological measures in temporary meadows had significant effects on the improvement of the honey bee colonies development and survival. The detection of a link – although slight – between agroecological measures and colony strength is noteworthy given the variety of factors interacting with colonies development and health (beekeeping management, climatic conditions, pathogens, etc.). Further evaluation of the effects of agroecological measures on honey bees could be complemented by an evaluation of their cost-efficiency and by extending such monitoring efforts to designs with more controlled conditions and to other type of meadows (permanent and ecological). Our results advocate for an expansion of measures on temporary meadows, and especially of their combination, which enhanced the effect of single measures. They also show that both beekeepers and farmers can contribute to increased honey bee colony health and suggest that concerted actions promoting the well-being of these pollinators can benefit both parties.

Supplementary Materials

Open Research Statement, data associated with this paper will be permanently archived in Dryad, and is available private-for-peer review at:
https://datadryad.org/stash/share/pP33f3mvJfe9ANWwEnEKVwLbAb1oToa2Xv0xdsuBwtg

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Authors contribution

Conceptualization: JH, YDV, AA, VD, AK; Data Curation: JH, YDV; Formal Analysis: JH, AK; Methodology: AK, JH, YDV; Supervision: AA, VD, AK; Writing - Original Draft Preparation: JH, YDV, AK; Writing - Review & Editing: AA, VD.

Conflict of interest

The authors declare no conflict of interest.

Data statement

The datasets generated during the study are archived in the Dryad repository.

Chapter 5

Agroecological measures in meadows provide an additional supply of floral resources in crop-dominated landscapes

Hernandez, J.., Varennes, YD., Aebi, A., Dietemann, V., & Kretzschmar, A (*in prep.*). Agroecological measures in meadows provide an additional supply of floral resources in crop-dominated landscapes

5 Agroecological measures in meadows provide an additional supply of floral resources in crop-dominated landscapes (unpublished data)

5.1 Introduction

Agricultural intensification severely threatens biodiversity due to increased land-use and reduced habitat diversity for pollinators (Requier, 2013). As a result of habitat loss and a lack of floral resources for these pollinators, agroecological measures can be implemented (Albrecht et al., 2007; Nicholls and Altieri, 2013). To promote honey bee colony health within the agricultural landscape, viable agroecological measures have been identified on temporary meadows (See chapter 4). As a result, a cascade of beneficial effects of three agroecological measures has been demonstrated on colony size in summer and autumn as well as winter survival (See Figure 14). Now, we would like to understand in which landscape context these agroecological measures will be the most efficient for maintaining honey bee colony strength because spatial and temporal stress on floral resources affects colony development (Horn et al., 2016) .

In the context of our study, the landscapes were mostly composed of field crops (annual, perennial crops) and grasslands (pastures and different types of meadows management) (See chapter 4) (Lachat, 2010). Therefore, differences in the phenology and composition of flowering plants in these landscape types are to be expected, which might affect colony development differently. Areas of large mass flowering crops during spring (mainly represented by oilseed rape in this study context) are followed by long periods of low food resources in the landscape, especially in summer (Decourtye et al., 2010; Couvillon et al., 2014; Requier et al., 2015). It is then important for honey bees to find alternative plants to cover their needs during the low resource period. Although beekeepers can feed them supplements (pollen and syrup) to compensate for this lack of resources, it is important for the proper development of the colony to have access to diversified natural sources of nectar and pollen (Di Pasquale et al., 2013), as supplements do not offer the same nutritional quality (Pedersen and Omholt, 1993). So, the implementation of agroecological measures in field crop landscapes would benefit honey bee colonies development during the summer forage dearth. In fact, the floral resources provided by agroecological measures are supposed to partly compensate for the lack of floral resources depending on the landscape structure (Krimmer et al., 2019; Scheper et al., 2013). As part of this study, agroecological measures, which are present in two landscape types (landscape mainly composed of field crops and landscape mainly composed of grasslands), will be examined to determine if they contribute to supplying additional floral resources for honey bees in these respective landscapes. To assess the resources available to honey bees in these landscapes, we used an estimate of weight gain dynamics for each colony that describes the foraging on and collection of these floral resources. The effect of the landscape can therefore be interpreted by its effect on weight gain by interpreting an increase in weight gain as an increase in the available floral resources. We considered the weight gain as a criterion synthesizing three factors: the resource's availability, the colony size, and the colony health status. The weight gain also depends on the weather conditions, influencing the plants' phenology and the foragers' exit out of the hive. We used rainfall and temperature climatic data, to better understand the variations in weight gain per year. Therefore, the calculation of weight gain allowed us to see how much the foragers collect in their environment under climatic conditions. This study was carried out over three years (2019, 2020, and 2021) and focused on two key nectar influx periods of the beekeeping harvest season, i.e., spring and summer. We looked at the seasonality of resources availability (oilseed rape in spring and grasslands in summer) on colony weight gain. The effect of the size of the area implemented with agroecological measures in different types of landscapes (field crops and grasslands) on colony weight gain.

5.2 Materials and Methods

5.2.1 Landscape data and agroecological measures in meadows

We selected apiaries sector for which three years of complete data were available (2019-2021) and whose location did not change (See methodological part in Chapter 4). Twenty-one apiaries out of 30 corresponded to these criteria. The landscape within 2 km around monitored apiaries (Steffan-Dewenter and Kuhn, 2003) was described by the area occupied by different land uses. All agricultural land-use types were provided by Cantonal services of agriculture (Jura, Bern, Vaud, Fribourg). Agricultural data provided the following information: field location, field surface area, crop type, and whether meadow-related agroecological measures had been implemented (see methodological part in 4). The thirty-three land use variables used to describe landscape units were grouped in six categories to simplify the landscape variables as follows: semi-natural areas (extensive meadows, fallow land, forest) / perennial crop (orchards and vineyards) / oleaginous crops (oilseed rape and sunflower) / annual crops (cereals, corn, beetroot, etc.) / meadows with agroecological measures / grasslands including meadows without agroecological measure / (Table 7).We consider separately the grasslands with and without measures to see the effect of the agroecological measures. Then, principal component analysis (PCA) outputs were used to perform hierarchical ascendent classification (HAC), in order to define groups with comparable land uses. The PCA on the six variables allowed to show the contributions of each variable (See appendix A.4.1 and appendix A.4.2). The two land uses with the smallest contribution were grouped with the variables with similar land use having high contributions:
-The variable “other grasslands” has been grouped with the variable “seminatural”
-The variable “perennial crop” has been grouped with “annual crops”
The HAC on these four new defined variables, yielded a broad classification of apiary sectors in two main groups constantly over years (See appendix A.4.3). The composition of landscapes of these two groups is shown in Figure 15. As we analyzed the composition

of these two groups, we found that one consisted primarily of field crops and the other of grasslands (Figure 15).

Table 7: Variables used to describe land-use in sectors around monitored apiaries.

Variables used	**Land-use composition**	**Grouping of land-use after PCA and HAC classification method for the "field crops" (G1) and "grassland" (G2) groups used in Figure 1**
Seminatural land-use	*forest,* *hedgerows,* *extensive meadows*	*Grasslands excluding agroecological meadows (“grass_ excl_ aem”)*
Other grasslands	*grassland including meadows without agroecological measures*	
Oleaginous crops	*sunflower and oilseed rape*	*Oleaginous crops (“oilseed”)*
Annual crops	*cereals, maize,* *and various other annual crops*	*Annual crops (“annual”)*
Perennial crops	*vineyards and orchards*	
Meadows with agroecological measures	*permanent and temporary meadows with agroecological measures*	*Meadows with agroecological measures (“aems”)*

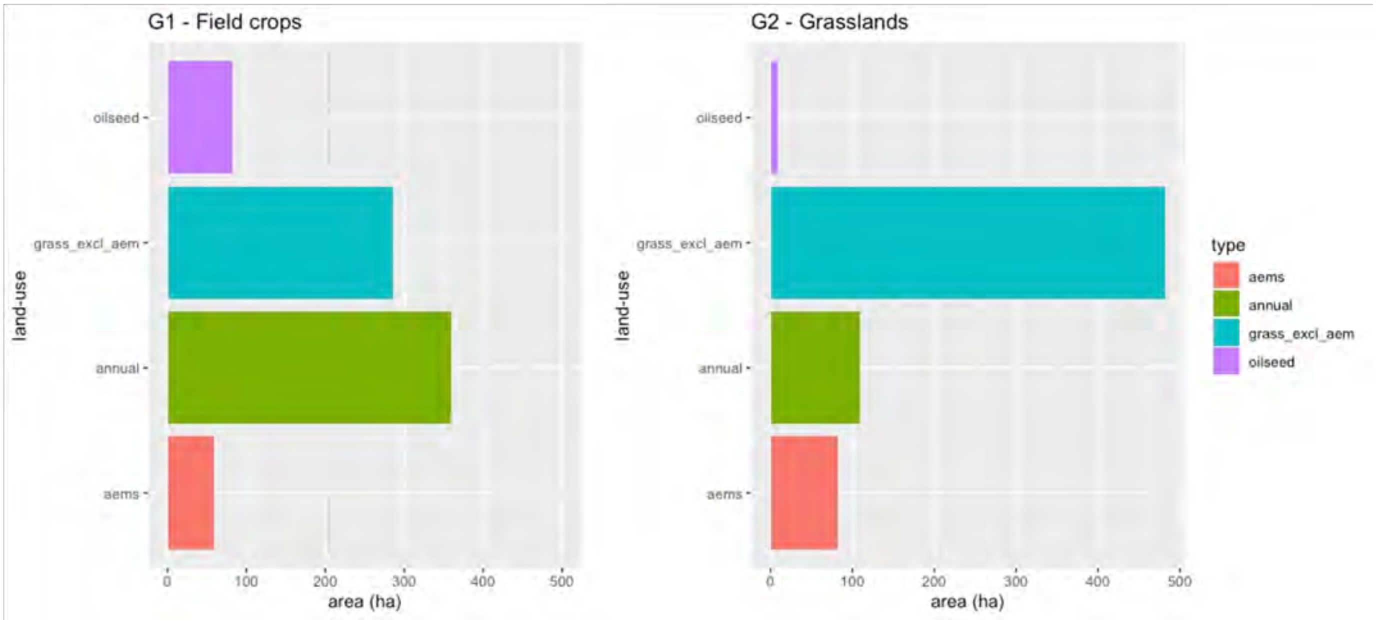


Figure 15: Detailed land use for field crops (group G1) and grasslands (group G2). (Aems= Meadows with agroecological measures; Annual = Annual crops; grass_excl_aem= Grasslands excluding agroecological meadows; oilseed= Oleaginous crops).

5.2.2 Nectar flow periods

There is a first period of nectar flow between the first of April and mid-June (around June 20th), which is referred to as the "spring nectar flow". A second period of nectar flow occurs from the end of June to the end of July (after June 20th to the 31st of July), which is referred to as the "summer nectar flow". These dates correspond to the periods of honey harvest by the beekeepers that comes from our data.

5.2.3 Weight data collection

Honey bee colony weight is measured using automatic scales (Youbee©) installed under five hives per apiary. Weight measurements were taken every ten minutes and were sent to the Youbee© platform every four hours. In this database, the weight data was raw, and a cleaning procedure was necessary (due to beekeepers handling influencing weight by temporarily adding or removing elements to the hives or to data transmission problem, see below). The weight data collection requires the use of the R program and loading the "RMariaDB" package, which allows defining a precise period of time during the season to calculate the weight gain per colony (spring period from the 1st of April to the 20th of

June, and summer period from the 20th of June to the 31st of July). A SQL (a language designed specifically for interacting with a database) function generates the connection with Youbee© database and the selected weight sequence was loaded. This connection generates a data file with the defined periods of time.

5.2.4 Data cleaning and linear segmented model for weight gain variation with time

The scale manufacturer has established the regular data format as follows: one weight measurement every 10 minutes. The raw data from electronic scales can be affected by various sources of errors: random electronic noise, beekeeper operations, transmission failure, missing data for various time periods. In order to obtain clean data suitable for modelling, successive cleaning steps were applied (step of ten minutes). A first filter eliminated the variation of weight greater or lesser than 1 kg as more than 99% of the data are included in this interval (see appendix A.4.4). A second filter was applied to cope with the missing data when the scale did not register weight data for a period of time greater than one step of 10 minutes. These periods could last from just a step missing to several days. In this case, the number of steps during which the scale was "silent" was calculated; the maximum of nectar influx during 10 hours of potential foraging per day was fixed at 6 kg (Kretzschmar and Maisonnasse, 2022) which corresponds to 100 g/step of 10 minutes. The weight variation during this "silent" period was then calculated and tested against the maximum potential weight variation. If the variation is higher than the maximum, the observed variation is rejected because it may include weight increasing by others causes than nectar foraging. A similar correction was applied for decreasing if the period of missing data depicted decreasing weight variation. From corrected data, a segmented linear regression (Muggeo, 2003, 2017) and an adjustment (package "selgmented") were applied on the curves of weight gain. The linear segmentation results in a series of breaking points that delimit a series of linear segments (N breaking points delimit N-1 linear segment). The number of breaking points was fixed by the lower value of the BIC criterium of the fitting procedure. The position of each breaking points was described by two coordinates: x as a date, y as a weight. For any period of time, the maximum weight gain was estimated by the highest value of the y-coordinates of the breaking points included in this period of time (See an illustration of segmented curve in Appendix A.4.5). The total weight gain was measured at the colony level. In the statistical tests used for evaluating the difference in weight gain between groups, the outliers less or more than 2.5 standard deviation have been excluded (Deaton and Kozel, 2005). Because intense variations in yearly climatic conditions are common, the data representations (using boxplots) were applied on the three study years separately.

5.2.5 Weather data

The cumulative precipitations and the mean season temperatures at apiary scale were used as these are relevant variables likely to influence honeybee foraging activity and colony development (Odoux et al., 2014; Clarke and Robert, 2018). The mean season temperature was calculated as the average of mean daily temperature over the overall periods (for spring and summer nectar flow defined in 5.2.2). The meteorological data for the Jura, Bern and Vaud cantons used has been recovered from the national Swiss meteorological station (federal office of the meteorological and climatology "MétéoSuisse").

5.3 Results

5.3.1 Average weight gain by apiary and landscape types in spring and summer

In spring 2019 and 2021, the weight gain was higher in field crops than in grasslands (field crops (G1) mean=13.2 for both year and grasslands (G2) mean =6.6 and 5.4 in 2019 and 2021 respectively; Figure 16). In 2020, the average weight gain was non significant between grasslands and field crops and had a larger quartiles dispersion (from 15kg to 30kg for grasslands compared to 17kg to 23kg for field crops; Figure 16). In summer 2019 and 2020, the weight gain was higher in grasslands than field crops (grasslands (G2) mean =7.8 and 17kg in 2019 and 2020 respectively; and field crops (G1) mean =7.3 and 13.7 in 2019 and 2020, respectively; Figure 16). In 2021 the average weight gain was higher in field crops than grasslands, but with a larger dispersion (from 2kg to 5kg for field crops(G1) than 2kg to 3kg for grasslands(G2); Figure 16).

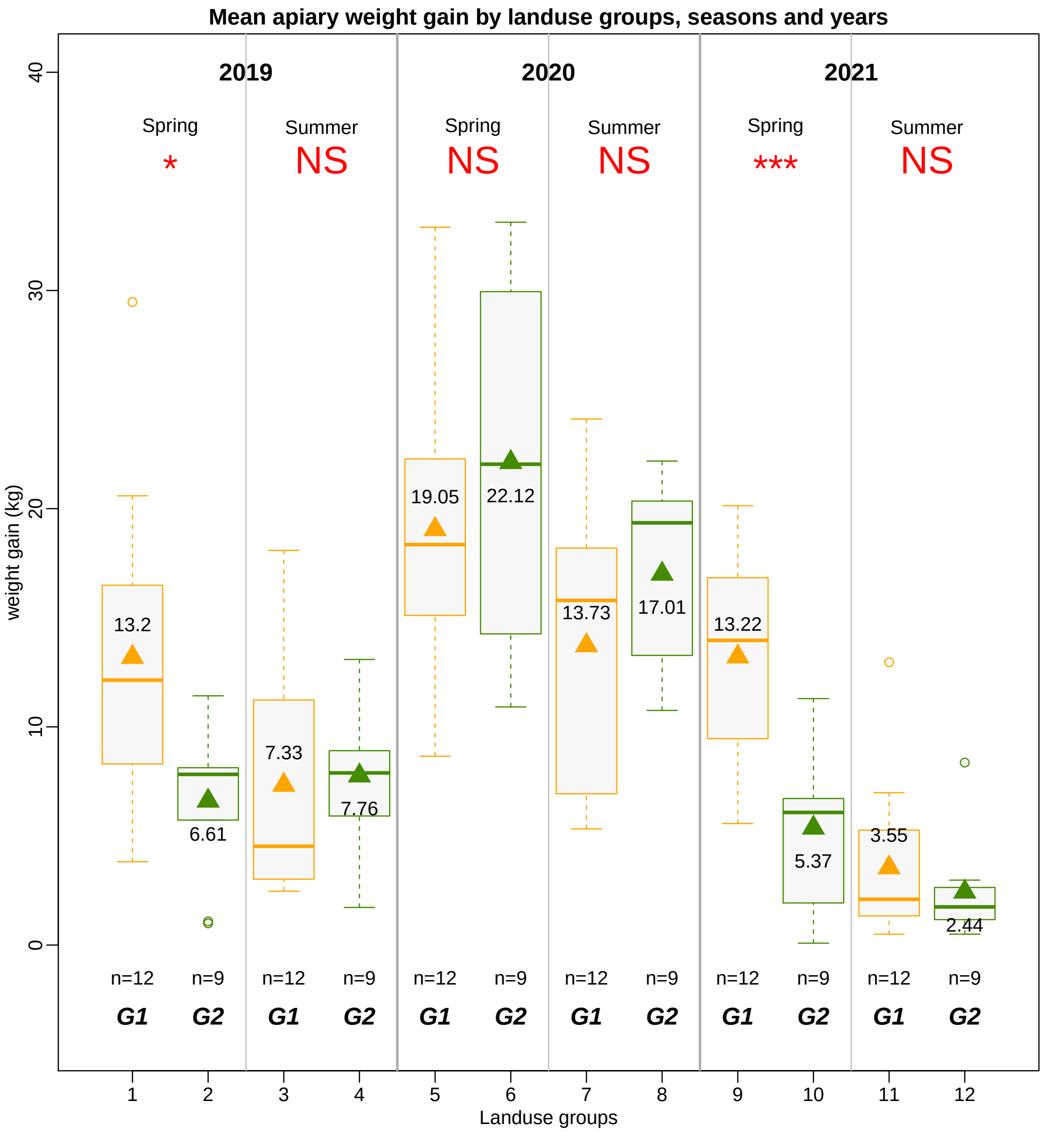


Figure 16: Average weight gain variations for fields crops and grasslands groups in spring and summer nectar flow (Triangles = mean of weight gain of apiary by group; the middle bar of the boxplots = median of group apiary weight gain; “n” = the number of apiaries in each group; yellow boxplots correspond to group 1 “field crops” and green boxplots corresponds to group 2 “grasslands”).

5.3.2 Effect of the intensity of agroecological measures on weight gain according to landscape types

To verify if the agroecological measures were normally distributed, we examined the size distribution of the areas including grassland with the implementation of agroecological measures. This distribution showed two distinct groups, i.e., sectors with less than 76 hectares and sectors with more than 76 hectares grasslands area with agroecological measures (Figure 17).

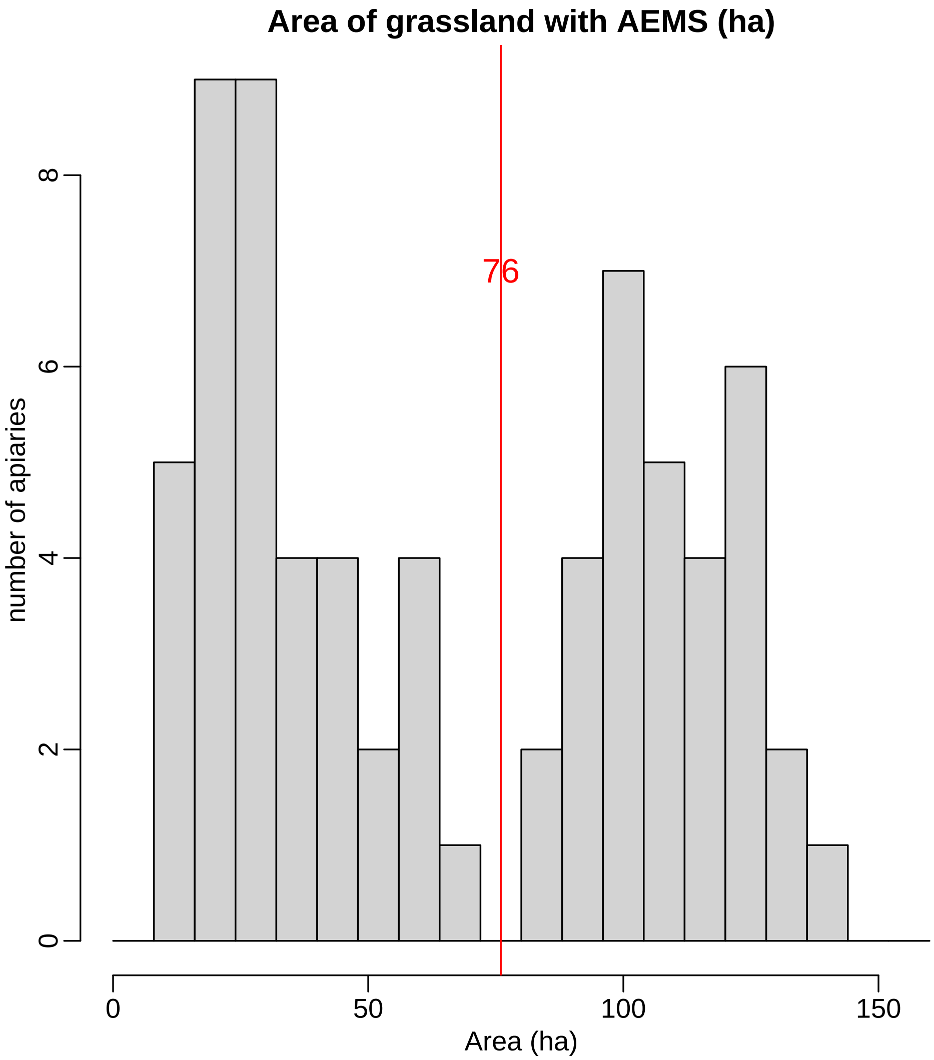
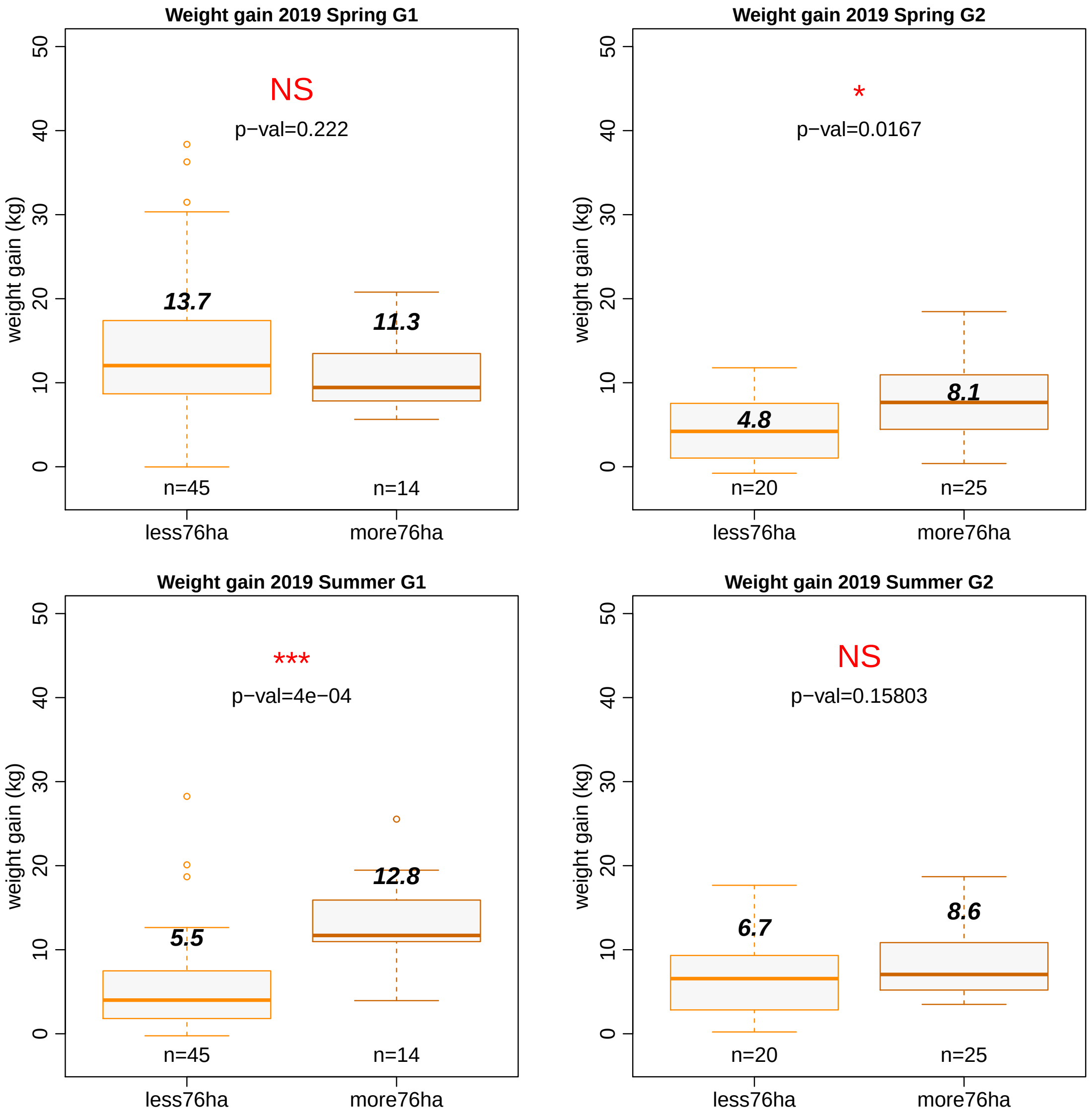
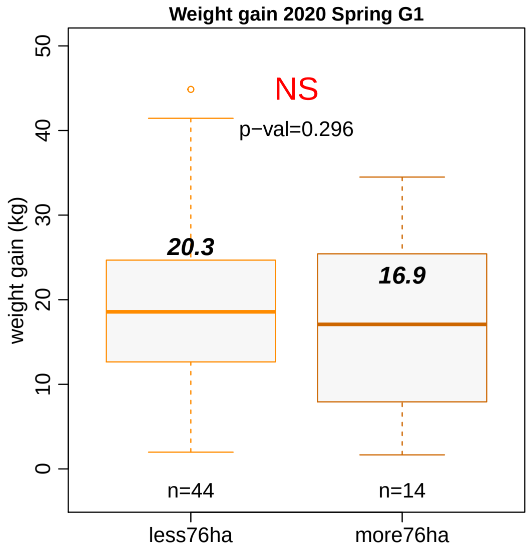


Figure 17: Distribution frequency of the area of grasslands with agroecological measures.

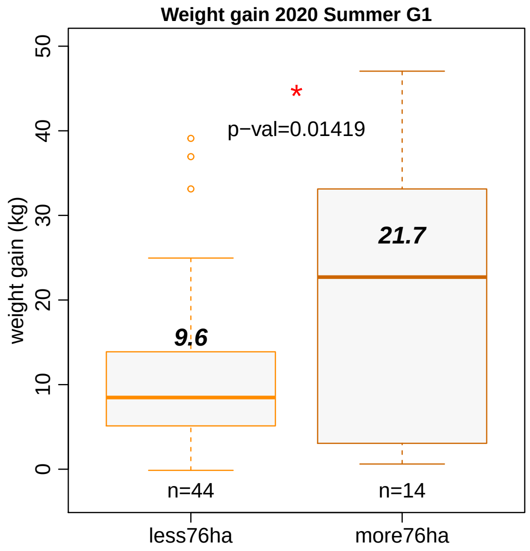
In spring 2019 and 2021, the weight gain was significantly higher in areas with more than 76 hectares of grassland with the agroecological measures implemented for the group "grasslands" (G2) (p-value=0.01 in 2019 and p-value=0.003 in 2021; Figure 18).
In summer of each year, the weight gain was significantly higher in areas with more than 76 hectares of grassland with the agroecological measures implemented for the group "field

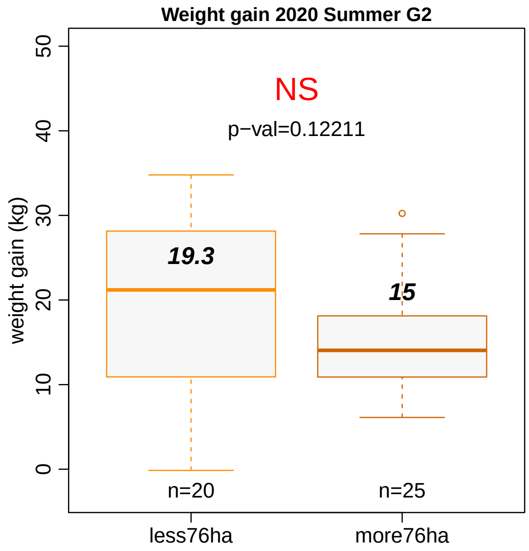
crops" (G1) (p-value=4e-04 in 2019; p-value=0.014 in 2020 and p-value=0.04 in 2021; Figure 18).




Weight gain 2020 Spring G1
weight gain (kg)
0
10
20
30
40
50
NS
p−val=0.296
20.3
16.9
n=44
n=14
less76ha
more76ha


Weight gain 2020 Spring G2
weight gain (kg)
0
10
20
30
40
50
NS
p−val=0.437
23.9
21.2
n=20
n=25
less76ha
more76ha


Weight gain 2020 Summer G1
weight gain (kg)
0
10
20
30
40
50
*
p−val=0.01419
9.6
21.7
n=44
n=14
less76ha
more76ha


Weight gain 2020 Summer G2
weight gain (kg)
0
10
20
30
40
50
NS
p−val=0.12211
19.3
15
n=20
n=25
less76ha
more76ha

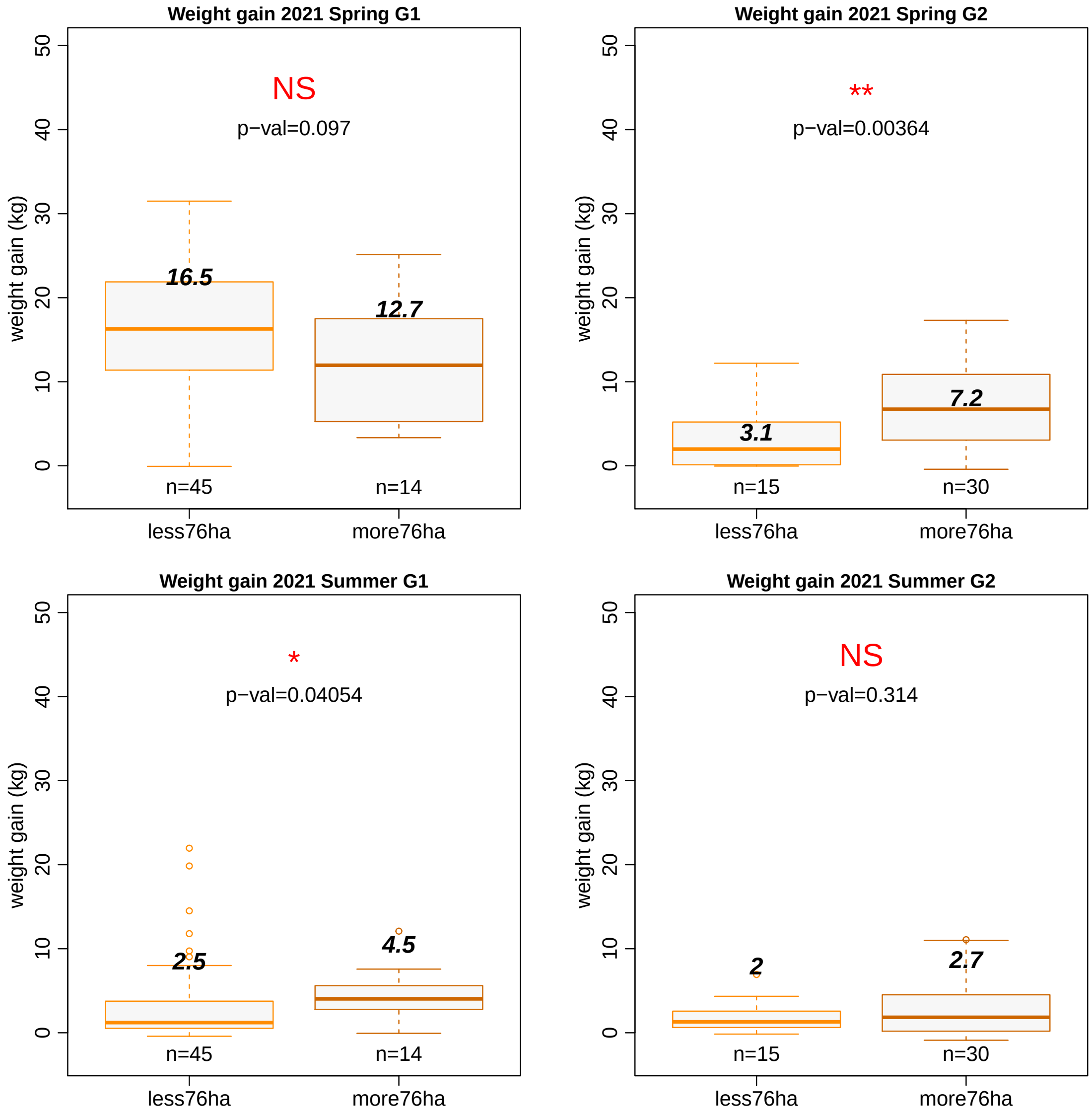


Figure 18: Boxplots showing the weight gains by colony for field crop (G1) and grassland (G2) groups for the different classes (more or less 76 hectares of agroecological measures) for each year of the two defined period (spring and summer). (n=colony number; *= level of significance; NS=non significant; the numbers in bold on the boxplots indicate the average)

5.3.3 Temperatures and precipitations effect on colony weight gain

The variation of mean season temperature among the 21 apiaries in spring, did not change significantly in 2019 and 2021 (means=12.20°C and 12.02°C, respectively). This distribution is significantly higher in 2020 (mean=13.3°C) (See appendix A.4.6). There is no effect of temperature on weight gain in 2019 and 2020 but there was an effect of temperature on weight gain in 2021 in spring (p-value=0.01) and summer (p-value=0.04) (See appendix A.4.7). At apiary scale, the weight gain in 2021 for spring and summer increased significantly with temperature in 2021 ; this effect of temperature was not observed in 2019 and 2020 (See appendix A.4.7). In contrast, the increase of temperature between spring and summer had no global effect when the 21 apiaries were considered together each year due to the effect of shortening in resources in summer and the strong effect of rain in summer (See appendix A.4.6).

We examined the role of the cumulative precipitations on weight gain in spring and summer for the three years of the study. The cumulative precipitations in the summer of 2021 reduced foraging activity of honey bees; to a lesser extent, it also limited nectar influx on oilseed in spring 2019 and 2021 (Figure 19). Mainly, in 2021, the summer was dominated by heavy rainfall and the total average of weight gain was reduced.

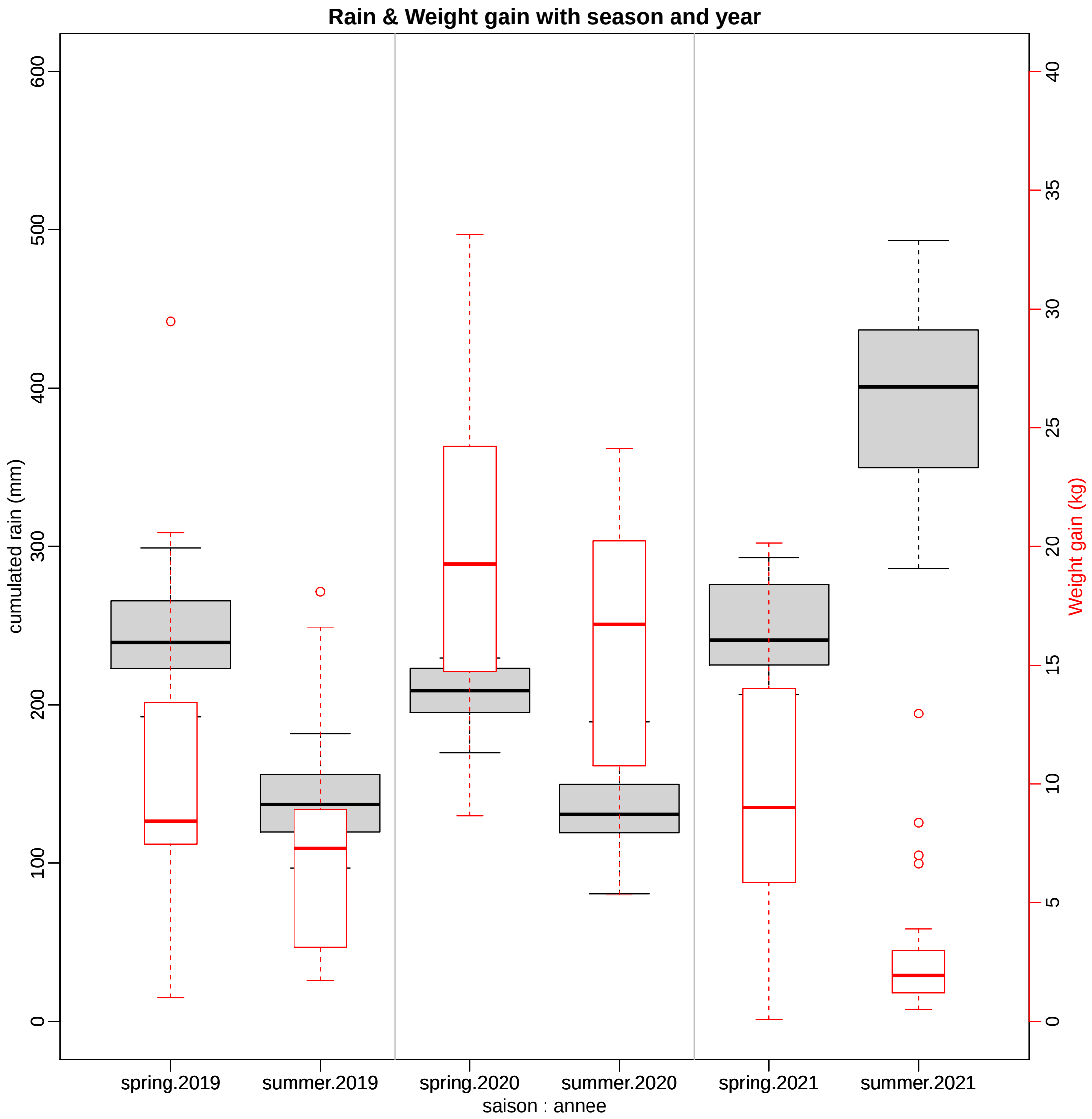


Figure 19: Average of cumulative rainfall (grey) and average of weight gain by apiary (red) in spring and summer.

5.4 Discussion

We can see that landscape management with the implementation of agroecological measures can improve the availability of floral resources (nectar flow) in summer in crop dominated landscapes. For all three years, whichever the intensity of nectar flow (variation in the quantity of weight gain), the positive effect of more than 76 ha of grassland with agroecological measures implemented is mainly observable in the sectors where « crops » are dominant (Figure 18) . These sectors, which have less grasslands and semi-natural habitats are limited in food supply in summer (Cole et al., 2017); the agroecological measures seem to provide an efficient additional food supply. This shows that in these agricultural landscapes, agroecological measures can provide an additional nectar source to honey bees. The colony weight gain variable allowed us to establish this observation in crop dominated landscape. However, weight gain is not directly related to nectar availability. Indeed, weight gain is the result of both, the availability of the resource (common to all colonies) and the ability of colonies to find for it (which explains why not all colonies follow the same pattern) These results allow us to better understand in which landscape context to effectively integrate these agroecological measures to improve the health of colonies through nectar flows. Indeed, the response of agroecological measures can be modulated according to the type of landscape in which they are implemented as shown in Scheper's study (Scheper et al., 2013). Our results also showed that in the grassland dominated landscape the fact of having sectors with more 76 hectares of agroecological remained not significant in summer but was significant in spring 2019 and 2021. This can be explained by the fact that in mild years (from a climatic point of view, as in our study in 2020), there is a continuous influx of nectar into the environment. This may explain why in 2020, the differences in weight gain between the landscape groups are not significant. The bees most likely found nectar in both low and high agroecological measure areas in spring. The differences in weight gain (nectar influx) are probably less marked in grassland landscapes, offering a regular supply of flowers between spring and summer; this may explain these lesser differences in the grassland dominated landscape in summer compared to field crop dominated landscapes.

Legume plants (mainly white clover, red clover, and alfalfa) are important sources of nectar and pollen and are frequently found in grasslands (Agroscope, 2021; Ziaja et al., 2018); leading to a more continuous supply over time for pollinators compared to mass flowering crops. This is in line with the results of other studies which showed that the response of pollinators to agroecological measures is modulated by landscape context and farmland type, with a greater positive response in croplands compared to grasslands (Scheper et al., 2013; Batáry et al., 2011). Through viable and effective agroecological measures (Chapter 4), farmers can manage the landscape to provide a supplement of floral resources for honey bees.

Our results also illustrate that climatic conditions directly influence the activity of the foragers and therefore the weight gain (resources) brought back to the hive and can directly influence colony development. Some years can be strongly affected by unfavorable weather conditions for the foragers. In our study, we see that in 2021, the high cumulative precipitations did not allow for a sufficient honey harvest and thus a sufficient stock for the bees before winter (Corbet, 1990).

Our study shows the importance of targeting the implementation of agroecological measures around field crop landscapes to optimize the supply of floral resources, which will provide a supply when resources are limited in the environment, especially in summer (Couvillon et al., 2014; Requier et al., 2015). However, we also see that in spring, in grassland dominated landscapes, the density of measures had a positive trend, but less exacerbated than in crop dominated landscapes in summer. This reflects that despite the years (i.e. climatic conditions), in summer, the landscape is limited in floral resources and this can be compensated by an additional contribution of agroecological measures.

Chapter 6
Discussion and Conclusion

6 Discussion and Conclusion

This thesis presents original results on the effect of agroecological measures implemented in temporary meadows on honey bee colony development and survival. To monitor the development of the colonies, a field method was developed and implemented (Chapter 3). Then, a part of our results deals with the conditions of the respect of the treatment protocol against the *V. destructor* implemented by the beekeepers. These results showed that compliance treatment regimen reduced infestation rates in the colonies, which increased colony survival (Figure 20A). After monitoring the compliance of beekeepers with varroa treatments control, which allowed us to describe and to measure statistically the Varroa infestation rates as a factor influencing colony activity, we were able to demonstrate the effect of three agroecological measures on honey bee colonies development and survival. We showed that colony size in July was positively influenced by three agroecological measures on temporary meadows (i.e., mowing without conditioner, leaving floral strips unmown and delayed mowing combined with mowing without conditioner). The colony size in summer (July), acted on autumn colony size leading to a to better colony overwintering (Figure 20B). We thus gathered evidence that the agroecological measures applied to temporary meadows promote the development of the colonies positively, which increases their probability of survival over winter.

These results of this study have identified three viable agroecological measures on temporary meadows that can be implemented to promote honey bee colonies health in the agricultural landscape. As a continuation of this result, we investigated in which landscape types these measures will be more useful in the poorer landscape in terms of floral resources for honey bee colonies. We have identified that the agroecological measures positively influence the summer nectar flow in field crop-dominated landscapes (Figure 20C).

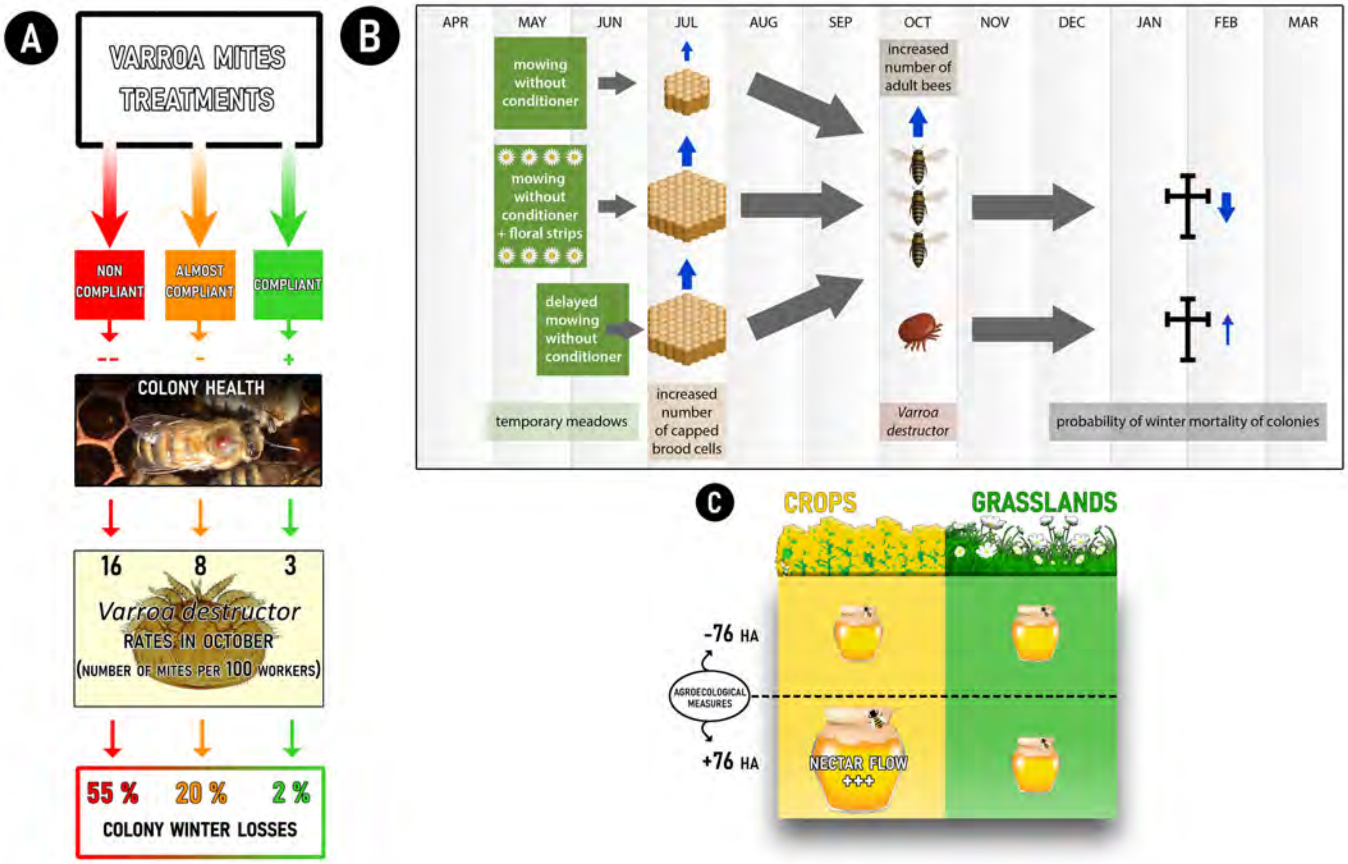


Figure 20: Main thesis results, (A) treatments regimens against *Varroa destructor*, (B) Positive effect of three agroecological measures implemented in temporary meadows on honey bee colony development and survival, (C) Positive effect of agroecological surfaces for the summer nectar flow in field crop dominated landscapes.

Beekeeping practices and V. destructor: importance of optimal treatments and perspectives

As many studies showed and as we have also identified in our study, apiary management with varroa control is important for colony survival (Genersch et al., 2010; Steinhauer et al., 2018; Giacobino et al., 2016; Hernandez et al., 2022). The apiary management by beekeepers can explain high varroa infestation levels in honey bee colonies (Steinhauer et al., 2018; Giacobino et al., 2016; Hernandez et al., 2022). In fact, the *V. destructor* infestation rates measurements before winter, i.e., in October, predicts the colony's chances of survival

during wintering and varroa treatment should be performed in a way to achieve a low adult workers bee infestation rate in autumn (Genersch et al., 2010). However, treatment implementation constraints can be identified and can be various. Indeed, the need to apply treatments at a given time, determined by ambient temperatures or personal commitments, can lead to a conflict in implementing these varroacidal treatments. Additionally, the weather effects on honey bees and *V. destructor*, as well as climatic variations or the mite resistance emergence, will change the effectiveness and treatments practices (Smoliński et al., 2021; Brodschneider et al., 2022). Climate changes could also lead to an advanced start of brood-rearing activity after overwintering and could increase the negative impact of the *V. destructor* in the colonies (Nürnberger et al., 2019). In an attempt to deal with climate change, new and effective methods of varroa control appear, as the method of queen caging in combination with oxalic acid treatment, creating an egg-laying break, which allows to stop the reproduction of varroa mites in the brood (Gregorc et al., 2017; Büchler et al., 2020). Beekeepers will therefore try to adapt their practices by implementing new emerging treatment methods and move towards local selection of resistant bees which could be an additional tool in the fight against varroa (Guichard et al., 2020a).
Nevertheless, *V. destructor* is not the only cause of colony losses, but it is a combination of several causes that increase the probability of colony mortality (Steinhauer et al., 2018). Causes of honey bee colony losses are complex and interconnected, with *V. destructor* and related viruses, pesticides, beekeeping management and nutrition, among the most common drivers (Goulson et al., 2015). Recent study showed that the varroa can interact negatively with the neonicotinoids exposure on honey bee longevity (Straub et al., 2019). This synergistic interaction may explain the increase in colony losses even though beekeepers try to control the *V. destructor*. The chronic pesticide exposure in intensive agricultural landscapes combined with pathogens can then cause collateral effects on colonies (Alaux et al., 2010; Straub et al., 2019). Furthermore, a lack of floral resources in agricultural landscapes causes nutritional stress for honey bees, which can interact even more negatively with pesticides presence reducing honey bee colony survival (Tosi et al., 2017). The floral resources provided by the landscapes are consequently very important to allow the colonies to thrive and overcome the pressure of different biotic and abiotic agents (Alaux et al., 2010; Tosi et al., 2017; Requier et al., 2019). These studies show that the interactions between biotic and abiotic agents can threaten honey bee colonies. Therefore, efforts should be focused on developing sustainable agroecosystem management schemes that provide floral resources and reduce the use of pesticides, and on the requirement to find sustainable solutions to control *V. destructor* in the near future.

Beneficial cascading effect of agroecological measures in temporary meadows

To better understand the consequences agroecological measures may have on honey bees development and survival, we identified a cascading effect of the implementation of specific agroecological measures in proximity of the apiaries (Chapter 4). This cascade of effects of three agroecological measures showed a beneficial effect on colony size in summer (July), that act on autumn colony size leading to a positive effect on colony winter survival. Furthermore, our study also showed that colony size in autumn (number of bees) appeared to be as important as the *V. destructor* infestation rates before winter in determining colony survival over the cold season (Chapter 4). These results show that the environment with the sufficient supply of floral resources provided by the agroecological measures is an important lever to improve the colony strength before the winter, without forgetting that beekeepers have also important levers in their hands by reinforcing their colonies and by treating them properly against the varroa mite in order to ensure a maximum overwintering success (Figure 20B).

Detailing the effects of these agroecological measures, the delayed mowing and the presence of floral strips combined with the no-conditioner use in temporary meadows showed a significant effect on the size of colonies in summer. These measures aim to increase the amount of floral resources in summer and to be less harmful to bees during mowing. Indeed, other studies have also demonstrated that the establishment of flower strips in terms of the number of flower species sown was positively correlated with pollinator abundance (Decourtye et al., 2010; Scheper et al., 2013; Albrecht et al., 2020), and that floral strip networks would link habitats and help counteract the vicious cycle of limited floral resources in the environment by providing essential food resources throughout the year for bees (Buhk et al., 2018). Delayed mowing also provides an additional nutritional resource, with the blooming of clover, which provides honey bees with an abundant source of nectar and pollen in summer. Increased use of clover in grasslands is a low-cost way to improve the food supply for pollinators while supporting agricultural productivity, creating a win-win scenario (Harris and Ratnieks, 2021). All these results show the potential of implementing these floral measures in meadows to benefit honey bees. Moreover, as grasslands cover 70% of the Swiss landscape, improving their density (i.e. more agroecological measures implemented per hectare) and floristic diversity has a huge potential to benefit pollinators in general. Our results therefore argue for an expansion (i.e. increase in surfaces on which measures are applied) of agroecological measures on temporary meadows, and especially of their combination to promote honey bee health in the Swiss landscape context. The recommendations of these measures may be proposed at national level for farmers by the Swiss federal office for agriculture.

Compensation of the lack of floral resources in the agricultural landscape can be done by agroecological measures implemented in summer to provide honey bee colony development

The managing of agricultural landscapes through agroecological measures to support biodiversity and ecosystem services is a key objective of sustainable agriculture (Martin et al., 2019; Albrecht et al., 2020). To improve pollination service on farms, the implementation of semi-natural habitats such as floral strips or grass strips most often along the edges of crops improves yields (Martin et al., 2019). However, few aspects are known about the overall effects of the landscape arrangements in agricultural systems. Indeed, landscape composition (percentage of different land use types) and structure (size and spatial arrangement of land use patches) contributes to promoting functional biodiversity and ecosystem services and could be influencing honey bee winter mortality (Kuchling et al., 2018; Martin et al., 2019). Martin et al. (2019) study showed that landscape configuration (i.e., edge density in cropland) contributes to promoting pollinator biodiversity and pollination. Also, another study showed that the effects of agroecological measures depend mainly on the landscape context and ecological contrast in which they are implemented (Scheper et al., 2013). The results of this study show that pollinator response to agroecological measures is moderated by landscape context, namely with more positive responses in cultivated land (compared to grassland). In the case of our study (Chapter 5), we found that the density of agroecological measures implemented in a crop dominated landscape had a positive effect on the weight gain of honey bee colonies during the summer. These results show that a continuous supply of pollen and nectar in field crop systems helps to maintain honey bee populations and thus their pollination service (Horn et al., 2021). Field crop systems are often composed of crop large mass flowerings (such as oilseed rape), that are important resources for pollinators as well as for agriculture (with the pollination service provided by bees increasing yields) (Odoux et al., 2012) . Indeed, these flowerings in spring allow to stimulate the queen eggs-laying and contribute to colony development (Horn et al., 2016). After these massive blooms, depending on the structure of the landscape, a period of dearth for pollinators occurs (Requier, 2013). During this dearth period, honey bees must cover a larger foraging area than in spring to find floral resources (Couvillon et al., 2014). Weed species, as well as those implemented in grasslands via agroecological measures, can therefore provide an important part of the pollen and nectar brought to the hive during this summer resources shortage (Decourtye et al., 2010; Requier et al., 2015). Based on this study, we can therefore observe that increasing and implementing food resources for honey bees through agroecological measures in meadows compensates for the lack of floral resources in summer around field crops landscape in Switzerland. However, despite these results, it should be kept in mind that climate change will alter plant phenology and resource availability in the landscape. For example, in severe drought, floral resources can be depleted (as in the case of grasslands that may wither), directly impacting the colonies nutritional needs. In this case, gardens could also provide pollinators with pollen and nectar, increasing resource connectivity in the landscapes (Goddard et al., 2010).

Implication of the implementation of floral resources through agroecological measures for maintain honey bee strength for beekeeping activities and pollinators biodiversity in agricultural landscape

The availability of nutritional resources (quantity, quality, and diversity) can be highly variable over season in intensive agricultural systems (Louveaux, 1959; Requier et al., 2015;

Di Pasquale et al., 2016). To limit beekeepers from artificially feeding their colonies despite increasingly less resilient agroecosystems, it is necessary to increase the supply of floral resources in the environment during dearth period. Implementing entomophilous floral species such as clovers (*Trifolium sp.*), alfalfa (*Medicago sp.*), or cultivated sainfoin (*Onobrychis sp.*) in landscapes is an effective solution to provide additional floral resources that can be proposed by agroecological measures (Decourtye et al., 2010; Rollin, 2013). Moreover, instead of monofloral pollen, honey bee colonies need polyfloral pollen (Schmidt, 1984) but also, the pollen resource diversity provide better immunity to biotic agents (Alaux et al., 2010; Foley et al., 2012; Di Pasquale et al., 2013). The diversification of floral offer through the implementation of agroecological measures in the landscape as well as the conservation of semi-natural habitats (i.e. forests, meadows, hedgerows and floral strips) is therefore crucial for the successful maintenance of honey bee colonies and to ensure ecosystem services (Martin et al., 2019). It is therefore important to have these types of environments around field crops to provide additional floral diversity for pollinators.

It is also important to note that conservation objectives must be reconciled to safeguard honey bees and wild bees. One study has shown that simply increasing floral resources is not necessarily an effective strategy for achieving conservation goals for pollinators; but it is essential to identify the floral resource needs of different pollinator groups carefully (Sutter et al., 2017). Because as far as nutritional needs are concerned, wild bees are rather specialists in floral resources and have a smaller foraging radius than domestic bees. Key plant species (such as *O. vulgare* and *A. millefolium*) have shown a real interest shared by wild and domestic bees. It is, therefore, essential to promote these key plant species in agri-environmental measures, which aim to encourage the supply of floral resources for the different target bee groups (Sutter et al., 2017, 2018; Rollin et al., 2013). It is necessary to find a compromise so that the agroecological measures implemented also benefit wild bees, even though climate change will favor the reduction of ecological niches for these species, which may prove more complicated for their resilience.

While providing adequate floral resources in crop-dominated landscapes is crucial to maintaining pollinators, the same is true regarding the reduction of crop protection inputs in field crops. Herbicides and fertilizers can indirectly affect bees by decreasing the floral resources available in the field; as well as insecticides that are frequently sprayed on large flowering field crops to control pests, thus exposing directly foraging bees (Decourtye et al., 2019; Requier et al., 2019). Particularly systemic insecticides permeate all plant tissues and contaminate bees' sources of nectar and pollen and can also be found on adventitious floral species (Poquet et al., 2016). Pesticide exposure can have consequences on the behavior of foragers and their spatial orientation, but also synergistic interaction effects between agrochemicals and biotic agents such as *V. destructor* and *Nosema spp.*, all of which have an important impact on bee colony dynamics (Alaux et al., 2010; Blanken et al., 2015; Siviter et al., 2021; Traynor et al., 2021); endangering both beekeeping with the weakening of colonies and contamination of hive products (Calatayud-Vernich et al., 2018), but also affecting the pollination service provided by honey bees for agricultural production

(Henry et al., 2012). It is therefore necessary to reduce the use of phytosanitary products and enhance floral diversity among agricultural landscape to ensure the sustainability of pollinators and maintain agricultural yields and beekeeping activities.

Management of a long-term monitoring survey and perspectives

The experimental design of this project allowed us to achieve the challenging objectives and recommend specific agroecological measures to be implemented on the field. Indeed, the "Agriculture and pollinators" field study was a challenging and multidisciplinary project involving several partners including research, academic and agricultural institutions, and collaboration with 30 local beekeepers and 1300 participating farmers. At the end of the fieldwork, more than 4800 honey bee colonies assessments were performed with the ColEval method by five trained field assistants that received theoretical and practical training in order to calibrate their estimations (reducing under and over estimation) for a precise honey colonies size evaluation. As many samples for counting and quantifying *V. destructor*, viruses and other pathogens were taken during each colony visit by field assistants. From a beekeeping point of view, we developed a database platform ApiNotes© to record the apiary management practices for better understanding the colonies development during the whole year. Each beekeeper was requested to work according to the good apicultural practice guide and any deviations were traceable in ApiNotes©. To ensure this rigor, we regularly reminded them the monitoring protocol and informed them that failing to comply will result in a loss of power to detect the effects of agroecological measures on their colonies development. Thanks to the information recorded by the beekeepers in ApiNotes©, we were able to identify deviations from the respect of the treatments against the *V. destructor*. Indeed, at the end of the first year of the project, we evaluated the infestation rates of the colonies and found that the values obtained were excessive. Our attention was therefore focused on the respect of the recommendations of control against varroa implemented by the partner beekeepers (Chapter 3). At landscape level, thirty-three landscape description variables for each sector were described. Concerning the data identification of agroecological measures, they can change every year, according to the Swiss federal office of agriculture (OFAG) decision to encourage more farmers to participate and promote citizen science for this project. But it was also possible that farmers stopped implementing these measures. In fact, these decisions could have complicated our data analyzes and might not allow us to see any significant effects of these agroecological measures. However, we noticed that the percentage of agroecological measures taken by farmers did not vary much from one year to the next and we were able to detect an effect of agroecological measures over three years (Chapter 4).

As a result, this study produced considerable amounts of biological and field data. Proper data management was therefore crucial to optimize data collection, data entry and data security. It was therefore necessary to produce clear and comprehensible data files with the implementation of a codebook describing all the variables in order to allow further analyses with possible external collaborations. Given the scale and number of people involved, the "Agriculture and pollinators" field study was well conducted and highlighted a many of advantages of conducting large-scale collaborative projects. The challenges encountered in designing projects of this scale may require the establishment of common planning methods to best conduct these field study monitoring and involve social sciences to identify some constraints to their implementation (Hodge et al., 2022; Hernandez et al., 2022; Correia, 2021).

It should be noted that it is difficult to set up an ideal monitoring device without any constraints. Real field conditions are confronted with uncontrollable meteorological events, personal constraints of the participating beekeepers who may not respect the protocol, or various accidents that may render the data unusable. The real field conditions will thus always have more difficult management aspects than controlled laboratory conditions. But the field studies allow the detection of sometimes slight but significant effects of specific factors given the variability involved in these real-field conditions. This means that these field surveys allow the statistical weight of different factors to be assessed. Indeed, some factors carry much of the variability related to environmental conditions. Evaluating various factors (such as, colony development, sanitary conditions, beekeeping practices, landscape,...) over a long-term monitoring could minimize the variability each year and at a specific location (region, defined areas) of one of these factors. Therefore, to assess the different stressors affecting pollinators, large-scale field studies are increasingly being conducted to obtain realistic descriptions of the typical honey bee and wild bee environments, aiming to generate more knowledge about bee conservation and biology. Our monitoring has taken us one step closer to understanding landscape management through agroecological measures by establishing their effects (slight but significant) on honey bee colony development. Actual recommendations can therefore be made through large-scale field surveys.

This project is considered as a long field surveys monitoring, which required an important effort for general coordination, for the supervision of the participating beekeepers, and for and the data management. However, the cooperation with all the partners worked out quite well and without any significant conflicts. We appreciated the involvement of each beekeeper in the interest of the project as well as in the realization of the protocol. This allowed us to respond the objectives of this thesis and more widely to the objectives of the project concerning the effect of agroecological measures that may be proposed at the Swiss national level by the policy makers. The results of this thesis, through the articles produced, have made it possible to give practical messages to the different actors of the beekeeping and agricultural fields. This close collaboration between scientist, technicians, beekeepers, and farmers is necessary for long term monitoring in this specific field surveys.

Conclusion

The articles presented in this thesis manuscript have shown how to monitor the honey bee colony development over a large-scale territory in Switzerland to evaluate the effect of a set of agroecological measures. As a result of our study, important factors have been identified as essential to maintaining honey bee populations, i.e., the varroa control through compliance with treatments, the implementation of agroecological measures in meadows, as well as the supply of floral resources through these measures in field crops. Based on a large set of experimental field data, the results of our study confirmed that the negative effect of *Varroa destructor* on honey bee colony can be prevented by well implemented varroacidal treatment. It is therefore essential in this type of participatory monitoring research that beekeepers follow the protocols to prevent this factor from becoming totally dominant and masking the other effects on colony health – i.e., here, the evaluation of specific agroecological measures on colony development and winter survival. Our study identified from a set of agroecological measures, three targeted measures implemented on temporary meadows that have shown positive cascading effects on the development and survival of colonies. These agroecological measures concerns the mowing without conditioner, the implementation of floral resource strips, and the delayed mowing combined without conditioner. This result indicate that the effects of the measures are detectable (although some effects are slight) despite the many other factors that play a role in colonies activity (such as beekeeping management, climatic conditions, pathogens, pesticides, etc.).

Furthermore, as suggested by other studies, it is important to consider the agricultural systems in which these agroecological measures will be implemented in order to optimize the food supply to maintain honey bee populations (Scheper et al., 2013). Our results show that targeted agroecological measures implemented around field crops in summer (when there is a lack of floral resources in the environment) are beneficial for colony weight gain and ensure their successful development.

Therefore, these agroecological measures bring an additional supply of floral resources in field crops areas, making it possible to alleviate the need for feeding with poor quality feeds by beekeepers sometimes must do for maintaining their colonies in periods of shortage. Also, our results illustrate how, each year, the agroecological measures can play a role in colony weight gain and that the season in which they play this role can change yearly due to weather variations. Based on the knowledge gathered under these field-real conditions through the research project "Agriculture and pollinators", farmers can therefore implement agroecological measures in their fields and thus contribute to maintain honey bee populations and beekeeping. The results provided by this thesis work will allow to promote management strategies for agricultural systems including these agroecological measures in order to maintain honey bee colony health to assure pollination services and agricultural yields in the Swiss landscape context.

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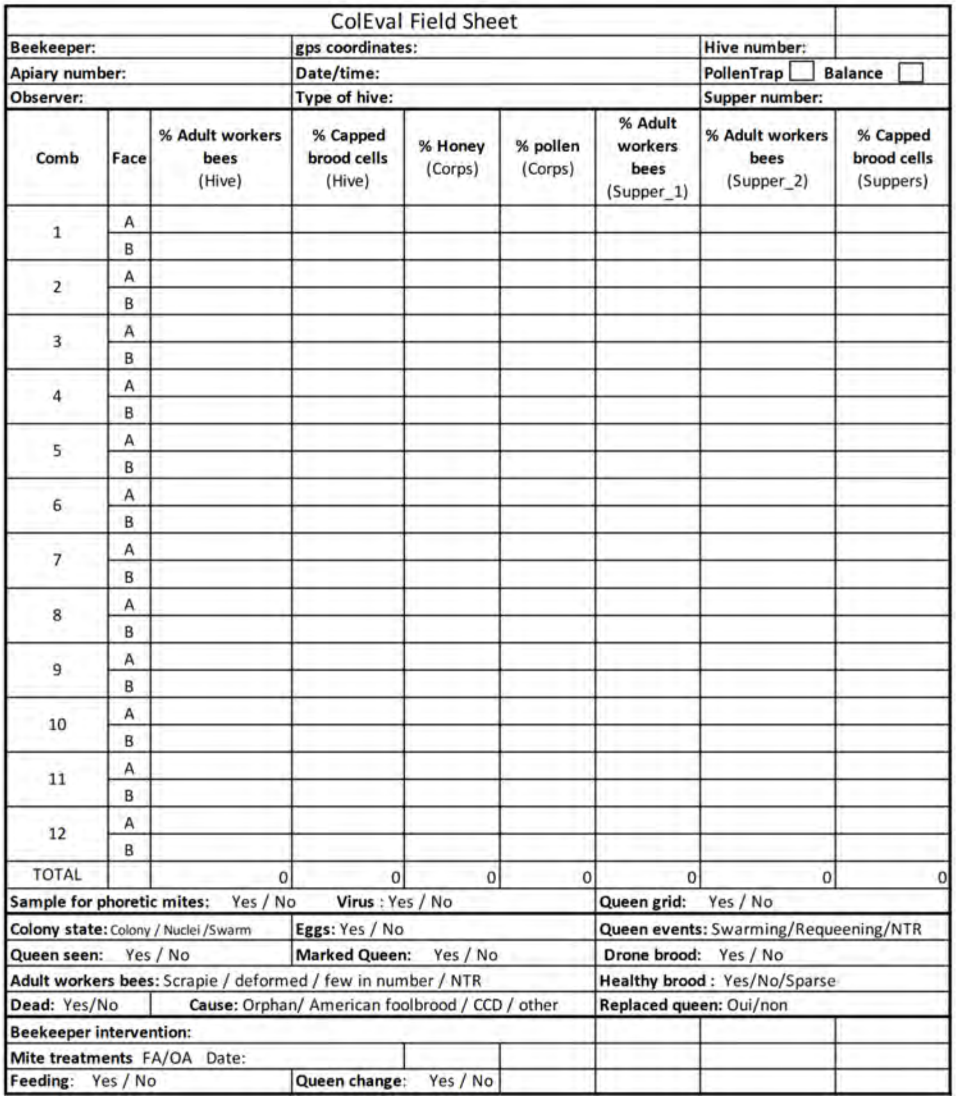
A Appendix

A.1 Appendices of Chapter 2

The files corresponding to the Appendices A.1.1, A.1.2, A.1.3 are available at http://www.mdpi.com/2075-4450/11/1/41/s1

A.1.1 Field spreadsheet



ColEval Field Sheet

Beekeeper: **gps coordinates:** **Hive number:**
Apiary number: **Date/time:** **PollenTrap** ☐ **Balance** ☐
Observer: **Type of hive:** **Supper number:**

Comb	**Face**	**% Adult workers bees** (Hive)	**% Capped brood cells** (Hive)	**% Honey** (Corps)	**% pollen** (Corps)	**% Adult workers bees** (Supper_1)	**% Adult workers bees** (Supper_2)	**% Capped brood cells** (Suppers)
1	A							
	B							
2	A							
	B							
3	A							
	B							
4	A							
	B							
5	A							
	B							
6	A							
	B							
7	A							
	B							
8	A							
	B							
9	A							
	B							
10	A							
	B							
11	A							
	B							
12	A							
	B							
TOTAL		0	0	0	0	0	0	0

Sample for phoretic mites: Yes / No **Virus** : Yes / No **Queen grid:** Yes / No
Colony state: Colony / Nuclei /Swarm **Eggs:** Yes / No **Queen events:** Swarming/Requeening/NTR
Queen seen: Yes / No **Marked Queen:** Yes / No **Drone brood:** Yes / No
Adult workers bees: Scrapie / deformed / few in number / NTR **Healthy brood :** Yes/No/Sparse
Dead: Yes/No **Cause:** Orphan/ American foolbrood / CCD / other **Replaced queen:** Oui/non
Beekeeper intervention:
Mite treatments FA/OA Date:
Feeding: Yes / No **Queen change**: Yes / No

A.1.2 Data spreadsheet variables description

variable name	**description**
coderucher	number of apiary
numruche	number of colony
anneeRuche	year of colony
year year	of measurement
datecoleval	date of collection
visites	number of visits
heurecoleva	l time of collection morning of afternoon
evaluateur	person who collected
typeCorps	hive format
typeHausse	honey supers format
nbreHausse	number of honey supers
etatColonie	type of colony
sumPCabeilleTotal	sum of percent of bees hive
sumPHabeilleTotal	sum of percent of bees into the honey supers
nbrAbeilleTotal	number of bees
sumPcouvfermeTotal	sum of percent of brood cells
nbrCellCouvFermeTotal	number of brood cells
sumPCmiel	sum of percent of honey hive
dm2miel	area of honey
sumPCpollen	sum of percent of pollen hive
dm2pollen	area of pollen

Estimated values of the coefficient to transform percentage of comb coverage into number of brood cells and adult workers or into area of honey/nectar and pollen. Values given for 1%.

REFERENCES	**corpsD**	**hausseD**	corpsL	**corpsS**
abeille/1%	14	7	11	12
cellule/1%	40	20	30.2	33
surface/1%	0.1134	0.055	0.0903	0.098

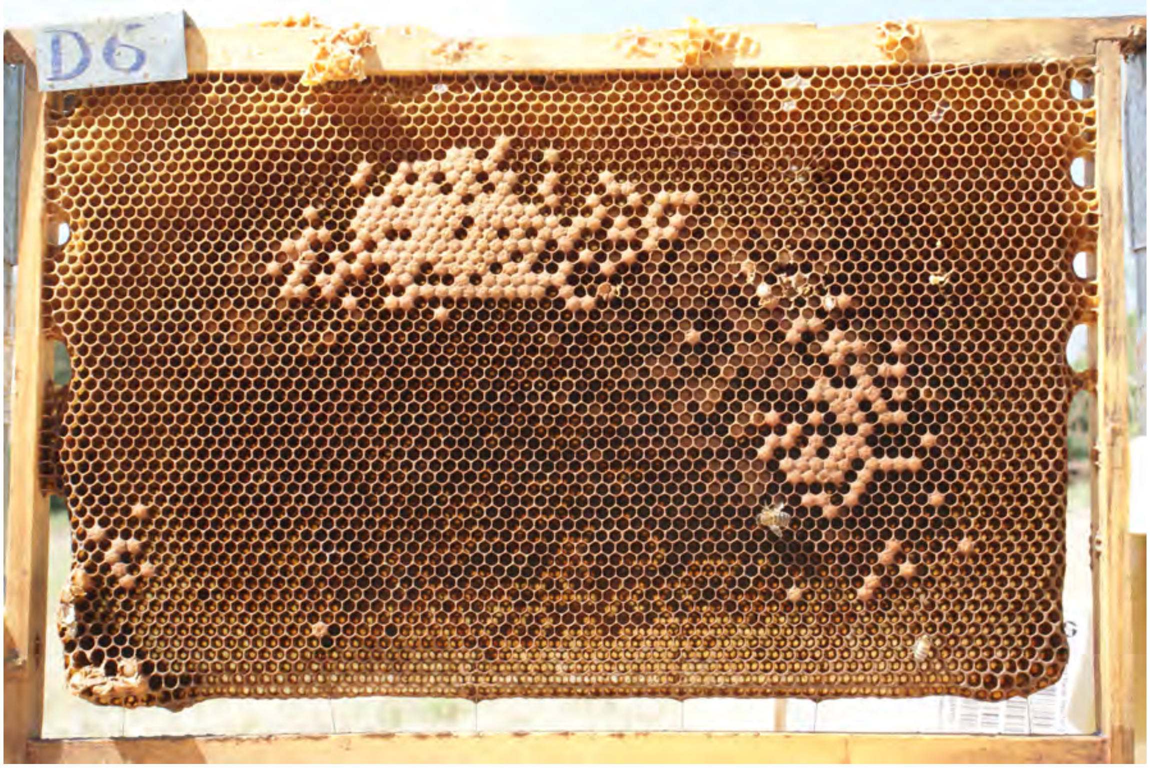
Estimated values of the coefficient to transform percentage of comb coverage into number of brood cells and adult workers or into area of honey/nectar and pollen. Values given for 1%. With the additional corrective procedure (x1.8 only for adult worker bees).

REFERENCES	**corpsD**	**hausseD**	**corpsL**	**corpsS**
abeille/1%	25.5	12.75	19.8	22
cellule/1%	40	20	30.2	33
surface/1%	0.1134	0.055	0.0903	0.098

Note

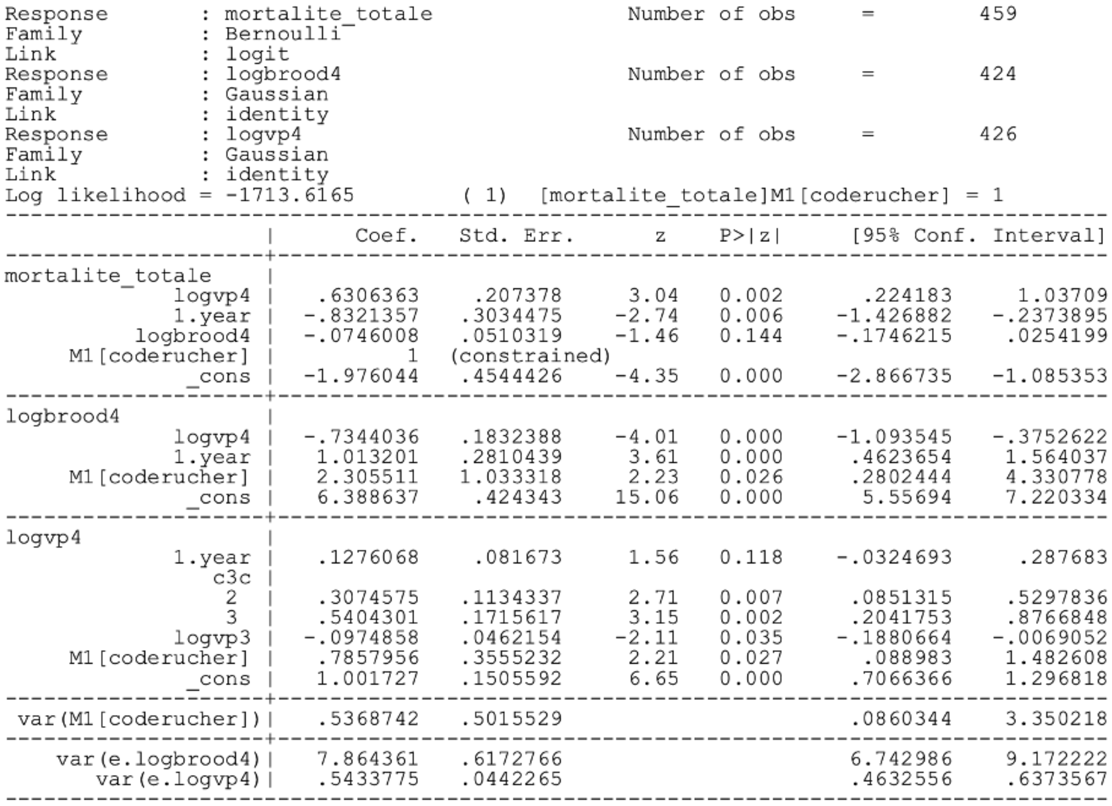
the number of male brood cells is obtained by multiplying the 1% of worker brood by 0.75

A.1.3 Brood comb picture



A.2 Appendices of Chapter 3

A.2.1 Structural Equation Model results table



```
Response       : mortalite_totale             Number of obs     =        459
Family         : Bernoulli
Link           : logit
Response       : logbrood4                    Number of obs     =        424
Family         : Gaussian
Link           : identity
Response       : logvp4                       Number of obs     =        426
Family         : Gaussian
Link           : identity
Log likelihood = -1713.6165        ( 1)  [mortalite_totale]M1[coderucher] = 1
------------------------------------------------------------------------------------
                   |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------------+----------------------------------------------------------------
mortalite_totale   |
            logvp4 |   .6306363    .207378     3.04   0.002      .224183     1.03709
            1.year |  -.8321357   .3034475    -2.74   0.006    -1.426882   -.2373895
         logbrood4 |  -.0746008   .0510319    -1.46   0.144    -.1746215    .0254199
    M1[coderucher] |          1  (constrained)
             _cons |  -1.976044   .4544426    -4.35   0.000    -2.866735   -1.085353
-------------------+----------------------------------------------------------------
logbrood4          |
            logvp4 |  -.7344036   .1832388    -4.01   0.000    -1.093545   -.3752622
            1.year |   1.013201   .2810439     3.61   0.000     .4623654    1.564037
    M1[coderucher] |   2.305511   1.033318     2.23   0.026     .2802444    4.330778
             _cons |   6.388637    .424343    15.06   0.000      5.55694    7.220334
-------------------+----------------------------------------------------------------
logvp4             |
            1.year |   .1276068    .081673     1.56   0.118    -.0324693     .287683
               c3c |
                 2 |   .3074575   .1134337     2.71   0.007     .0851315    .5297836
                 3 |   .5404301   .1715617     3.15   0.002     .2041753    .8766848
            logvp3 |  -.0974858   .0462154    -2.11   0.035    -.1880664   -.0069052
    M1[coderucher] |   .7857956   .3555232     2.21   0.027      .088983    1.482608
             _cons |   1.001727   .1505592     6.65   0.000     .7066366    1.296818
-------------------+----------------------------------------------------------------
 var(M1[coderucher])|  .5368742   .5015529                      .0860344    3.350218
-------------------+----------------------------------------------------------------
   var(e.logbrood4)|   7.864361   .6172766                      6.742986    9.172222
      var(e.logvp4)|   .5433775   .0442265                      .4632556    .6373567
------------------------------------------------------------------------------------
```

A.2.2 Statistical test of changes from 2018 and 2019 in compliance categories

Melded binomial test for difference, mid-p version alternative hypothesis: true difference is not equal to 0 (R's "exact2x2" package)

Compliant category

sample 2018: 70/270, sample 2019: 70/270 proportion 2018 = 0.259, proportion 2019 = 0.259, p-value = 1 Estimates (difference 2019-2018): 0.0; 95

Almost-Compliant category

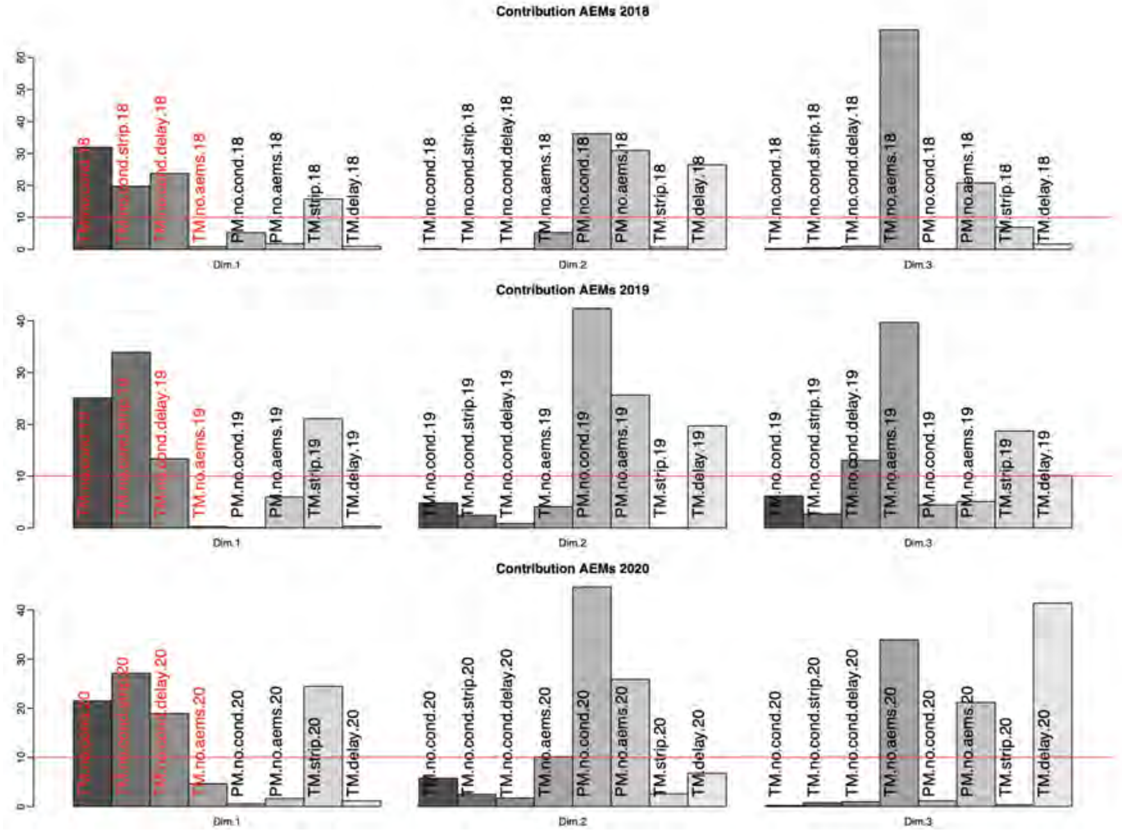
sample 2018: 120/270, sample 2019: 190/270 proportion 2018 = 0.444, proportion 2019 = 0.704, p-value <0.0001 Estimate (difference 2019-2018): 0.259; 95

Non-Compliant category

sample 2018: 90/270, sample 2019: 10/270 proportion 2018 = 0.333, proportion 2019 = 0.037, p-value <0.0001 Estimate (difference 2019-2018): -0.296; 95

A.3 Appendices of Chapter 4

A.3.1 Contribution of PCA dimension 1, dimension 2 and dimension 3 for the selected agroecological measures by year



Signification of measures codes : TM-no-aems= Temporary meadows without agroecological measures; TM-no-cond= Temporary meadows mowed without conditioner; TM-strip= Temporary meadows with unmown floral strip; TM-delay= Temporary meadows with delayed mowing; TM-no-cond.strip= Temporary meadows mowed without conditioner and with unmown floral strip; TM-no-cond.delay= Temporary meadows with delayed mowing without conditioner; PM-no-aems= Permanent meadows without agroecological measures; PM-no-cond= Permanent meadows mowed without conditioner; EM-no-aems= Ecological meadow without agroecological measure; EM-no-cond= Ecological meadow mowed without conditioner.

A.3.2 Contribution of PCA dimension 1, dimension 2 and dimension 3 for the selected agroecological measures over the 3 year

	Contribution		
Agroecological measure codes	**Dimension 1**	**Dimension 2**	**Dimension 3**
TM-no-cond	25.499	0.968	0.399
TM-strip	23.296	1.358	1.785
TM-no-cond*strip	23.211	0.266	0.628
TM-no-cond*delay	18.596	1.637	0.640
TM-delay	0.002	18.560	1.524
TM-no-aems	0.319	4.235	25.079
PM-no-cond	1.675	33.030	0.352
PM-no-aems	1.596	25.591	11.232
EM-no-cond	2.738	7.720	18.195
EM-no-aems	0.064	6.860	40.161

A.3.3 Eigenvalues and inertia of each PCA dimension for 2018, 2019 and 2020

	Dimension1	Dimension2	Dimension3	Dimension4	Dimension5
2018 – Eigenvalues (total Inertia=10)	2.85	2.01	1.50	1.00	0.88
2018 – Cumulative Inertia	28.51	48.70	63.79	73.84	82.66
2019 – Eigenvalues (total Inertia=10)	2.35	2.15	1.53	1.18	0.92
2019 – Cumulative Inertia	25.39	45.08	60.40	72.25	81.89
2020– Eigenvalues (total Inertia=10)	2.55	2.09	1.38	1.19	0.81
2020 – Cumulative Inertia	25.55	46.47	60.31	72.27	80.40

A.3.4 Contribution values of the PCA dimension 1, dimension 2, and dimension 3 for the selected agroecological by year. See Table 1 for the signification of measure codes.

	Dimension 1	Dimension 2	Dimension 3
TM-no-cond _2018	31.94	0.20	0.28
TM-no-cond*strip _2018	19.69	0.003	0.57
TM-no-cond*delay _2018	23.74	0.20	1.01
TM-strip _2018	15.74	0.75	6.96
TM-no-aems _2018	0.87	5.26	68.62
TM-delay _2018	0.96	26.43	1.68
PM-no-cond _2018	5.23	36.21	0.11
PM-no-aems _2018	1.83	30.93	20.78
Average of the contributions of the first four AEMs for 2018	**91.11**		

	Dimension 1	Dimension 2	Dimension 3
TM-no-cond _2019	25.08	4.84	6.17
TM-no-cond*strip _2019	33.92	2.43	2.70
TM-no-cond*delay _2019	13.33	0.87	13.07
TM-strip _2019	21.08	0.01	18.71
TM-no-aems _2019	0.26	4.11	39.70
TM-delay _2019	0.36	19.70	10.11
PM-no-cond _2019	0.032	42.39	4.44
PM-no-aems _2019	5.95	25.65	5.10
Average of the contributions of the first four AEMs for 2019	**93.40**		

	Dimension 1	Dimension 2	Dimension 3
TM-no-cond _2020	21.48	5.75	0.19
TM-no-cond*strip _2020	27.15	2.48	0.76
TM-no-cond*delay _2020	18.93	1.74	0.99
TM-strip _2020	24.48	2.57	0.34
TM-no-aems _2020	4.66	10.11	33.96
TM-delay _2020	1.13	6.78	41.41
PM-no-cond _2020	0.55	44.69	1.161
PM-no-aems _2020	1.63	25.87	21.19
Average of the contributions of the first four AEMs for 2020	**92.03**		

A.3.5 Effects of the agroecological measures in temporary meadows on honey bee colony size during spring and summer (June-July)

File *S4_ Tables.txt* available under the following Dryad link:
https://datadryad.org/stash/share/pP33f3mvJfe9ANWwEnEKVwLbAb1oToa2Xv0xdsuBwtg

A.3.6 Effect of the spring and summer colony size (June and July) on the number of bees and brood in autumn colonies (October) and the Relationship between brood cells and number of bees in June and July

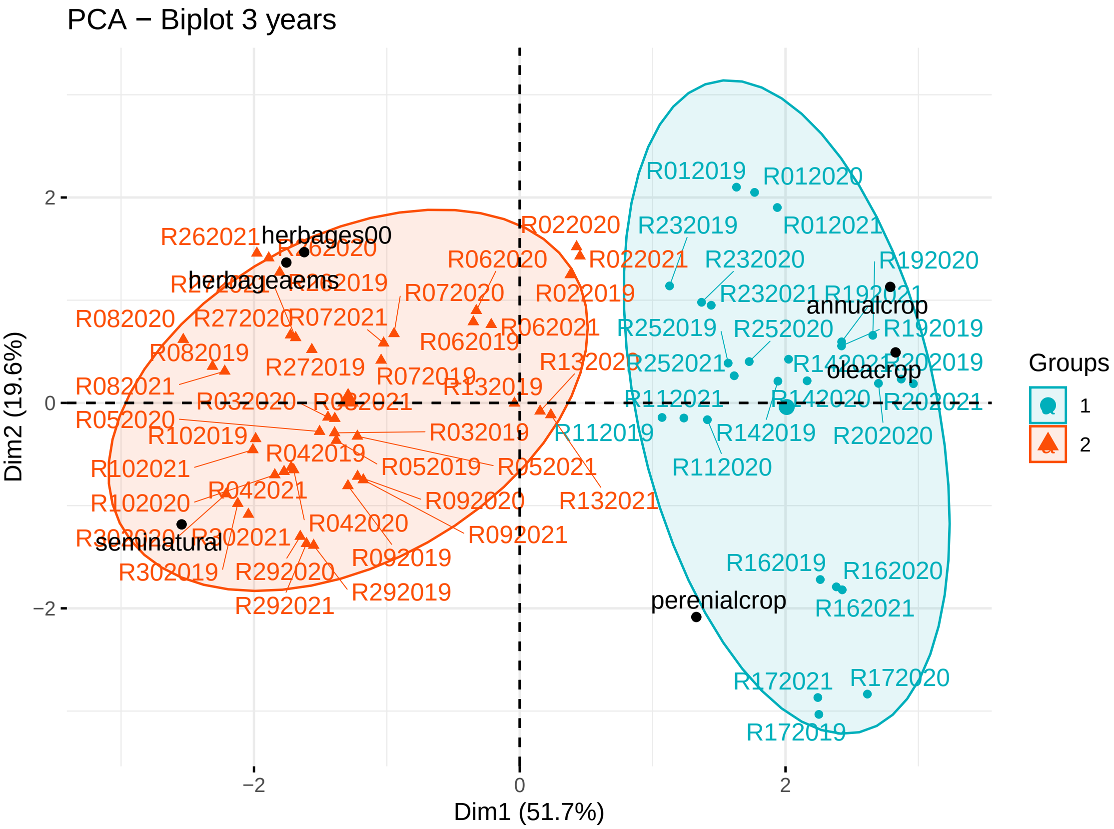
File *S5_ Tables.txt* available under the following Dryad link:
https://datadryad.org/stash/share/pP33f3mvJfe9ANWwEnEKVwLbAb1oToa2Xv0xdsuBwtg

A.3.7 Effect of colony size on winter survival

File *S6_ Tables.txt* available under the following Dryad link:
https://datadryad.org/stash/share/pP33f3mvJfe9ANWwEnEKVwLbAb1oToa2Xv0xdsuBwtg

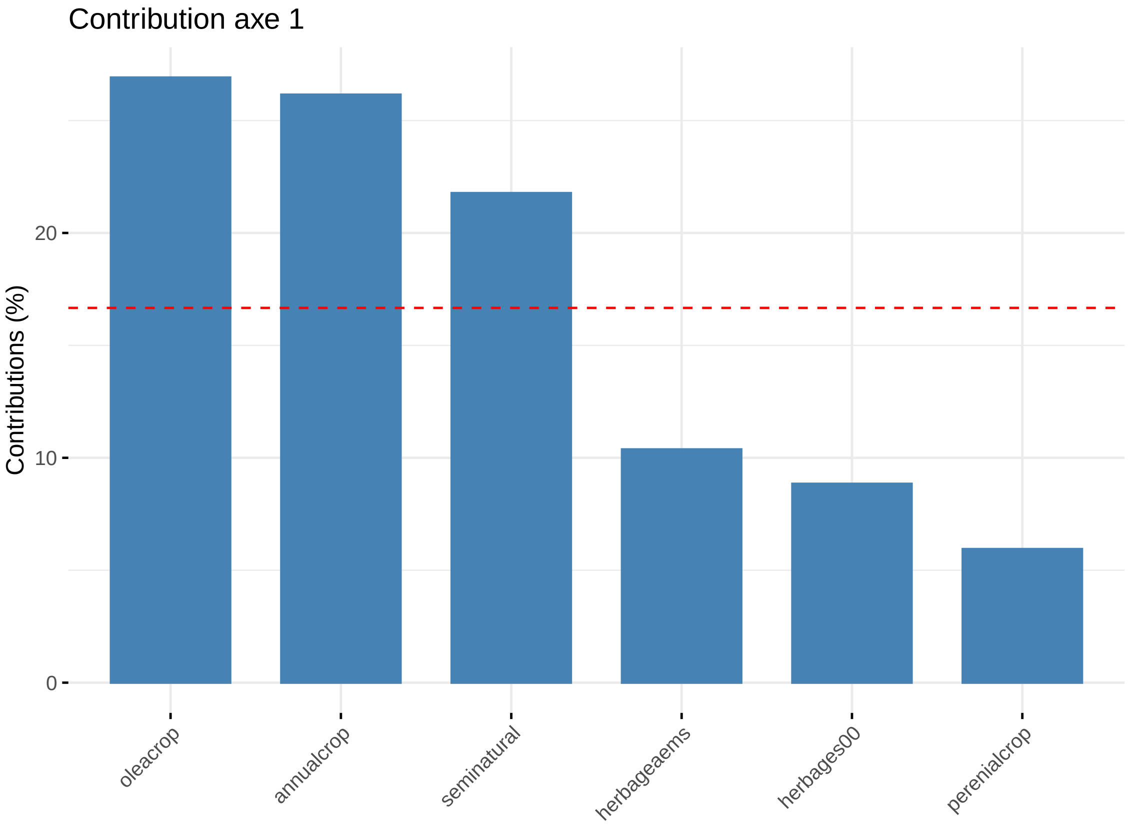
A.4 Appendices of Chapter 5

A.4.1 PCA - Biplot representation



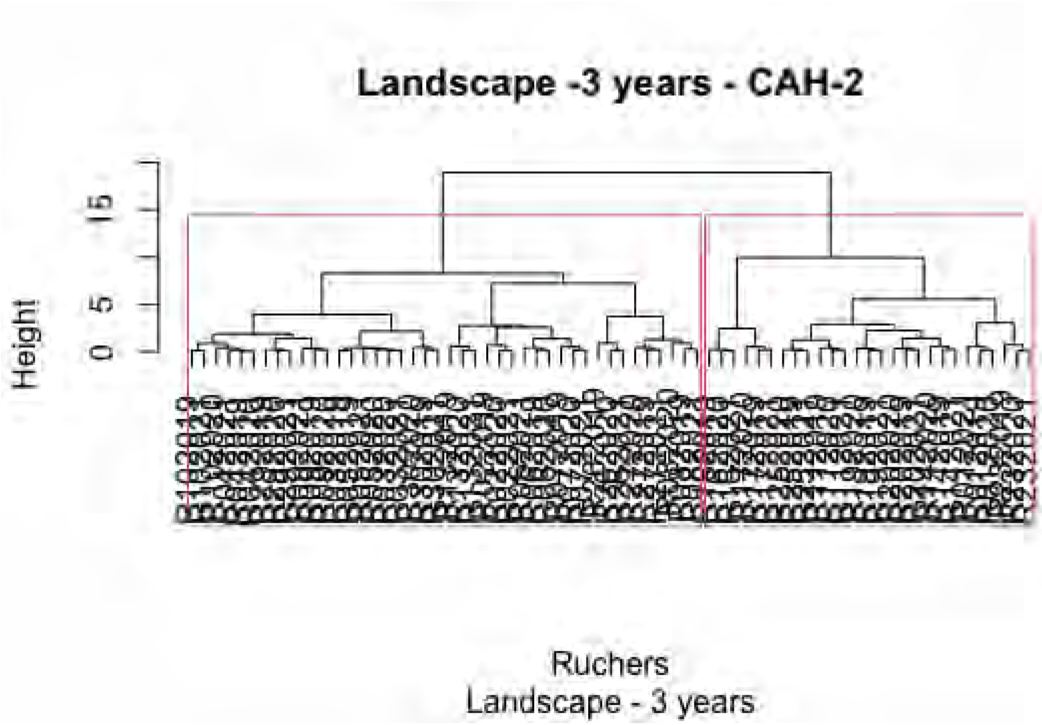
Visualization of PCA results on the six land-use variables via the biplot. The biplot represent the projection of the apiary sectors and landscape variables for the three-study year (confidence level=90 %).

A.4.2 PCA contributions



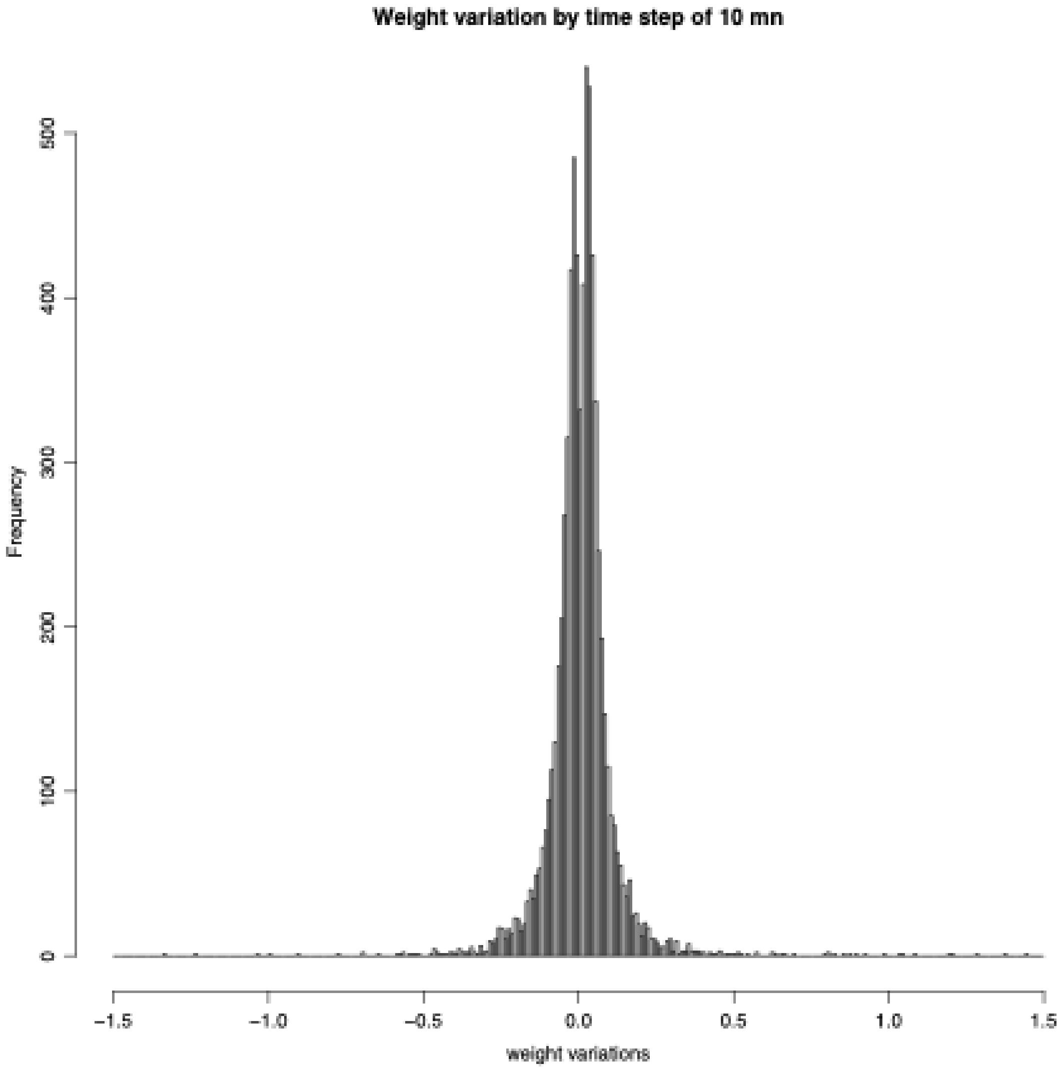
PCA contribution of axis 1 (in percentage) of the six main variables of land-use.

A.4.3 Hierarchical ascendant classification (HAC) representation

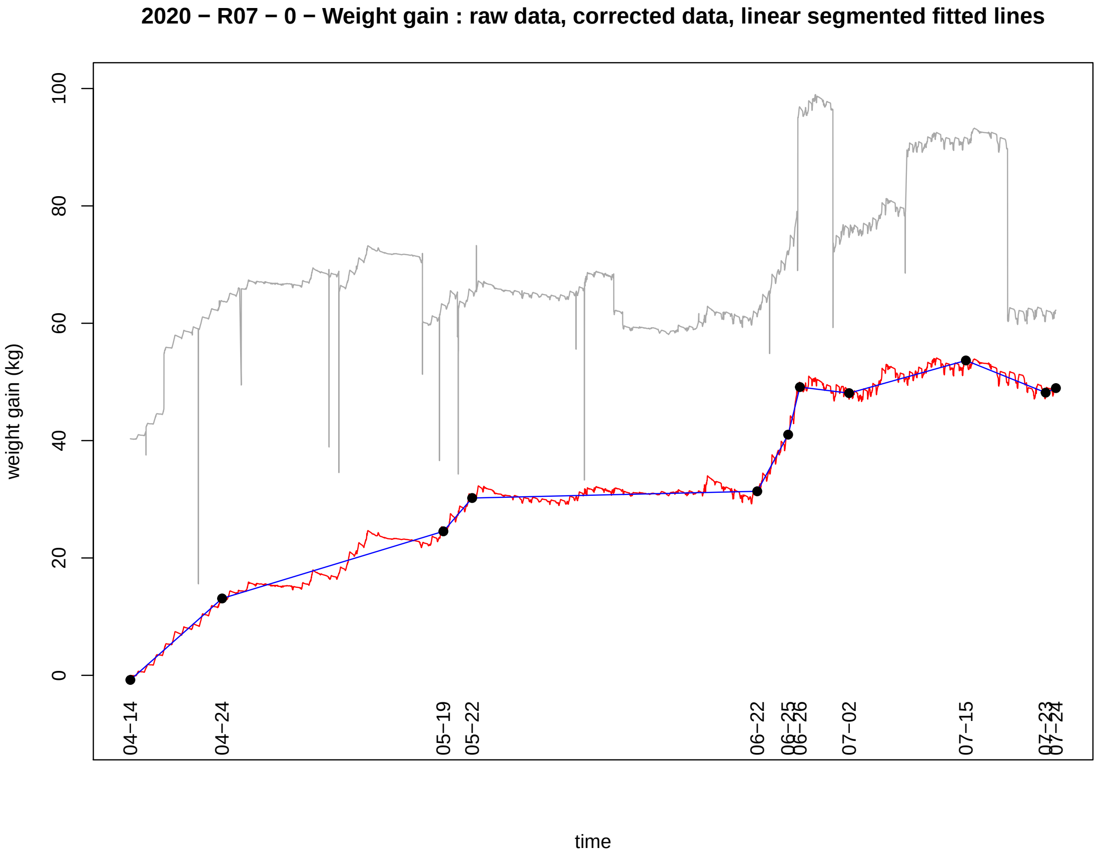


Hierarchical ascendant classification (HAC) of the apiary sector for the three-study year.

A.4.4 Weight variations in kg by time step of ten minutes

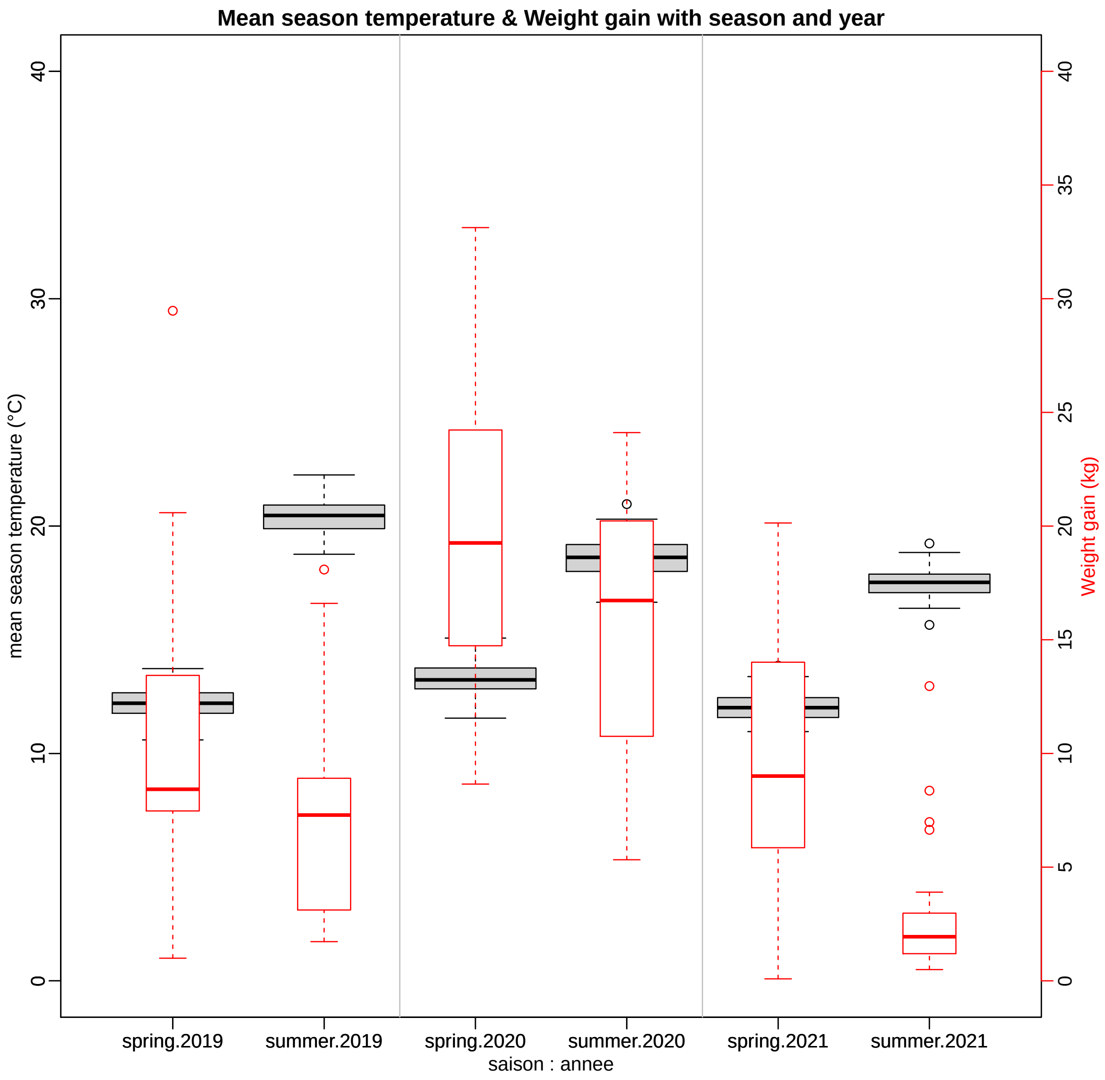


A.4.5 Weight gain : raw data, corrected data and linear segmented fitted lines



Example of segmented curve of weight gain from one colony. The grey curve illustrates the raw data, and the red curve illustrates the corrected data. The segmented linear fitting curve is shown in blue on the corrected weight curve. The time is given in month and day.

A.4.6 Mean season temperature and weight gain in spring and summer for each year



Mean daily temperature and average of weight gain by apiary in spring and summer (in red = the average weight gain and in grey=the mean daily temperature).

A.4.7 Coefficients of linear models of the average apiary weight gain in function of mean apiary temperature in spring and summer for each year.

Year	Coefs	Spring	Summer
2019	alpha	2.99	0.21
	beta	-26.1	3.31
	p.val	0.127	0.878
	r.squared	0.071	-0.05
2020	alpha	-2.167	-2.06
	beta	49.21	53.99
	p.val	0.296	0.14
	r.squared	0.008	0.067
2021	alpha	3.71	1.67
	beta	-34.7	-26.43
	p.val	0.010 *	0.044 *
	r.squared	0.26	0.15

B Thesis-related publications and communications

B.1 Publications

Published articles in peer-reviewed journals

2022

Hernandez, J., Varennes, YD., Aebi, A., Dietemann, V., & Kretzschmar, A. (2022). Agroecological measures in meadows promote honey bee colony development and winter survival. *Ecosphere*, accepted on 20 oct.2022

Hernandez, J., Hattendorf, J., Aebi, A., & Dietemann, V. (2022). Compliance with recommended *Varroa destructor* treatment regimens improves the survival of honey bee colonies over winter. *Research in Veterinary Science*, 144, 1-10.

2020

Hernandez, J., Maisonnasse, A., Cousin, M., Beri, C., Le Quintrec, C., Bouetard, A., ... & Kretzschmar, A. (2020). ColEval: Honeybee colony structure EVALuation for field surveys. *Insects*, 11(1), 41.

2019

Sutter, L., Aebi, A., Buchwalder, G., Caballe., Dietemann, V., Girardin, O., **Hernandez, J.**, Jacopin Bucher, E., Mayor, P., Menetrier, V., Praz, C., Varennes., YD. (2019). Agriculteurs, apiculteurs et chercheurs unis pour la sauvegarde des pollinisateurs. Rech. Agron. Suisse 2019, 10, 424–429.

2018

Alaux, C., Soubeyrand, S., Prado, A., Peruzzi, M., Maisonnasse, A., Vallon, J., **Hernandez, J.**, Jourdan, P., Le Conte, Y. (2018). Measuring biological age to assess colony demographics in honeybees. *PLoS ONE* 13(12): e0209192.

Article in preparation

2022

Hernandez, J.., Varennes, YD., Aebi, A., Dietemann, V., & Kretzschmar, A (in prep.). Agroecological measures in meadows provide an additional supply of floral resources in crop-dominated landscapes

Popular articles

Rollin, O. & **Hernandez, J.** (2022). Acides organiques contre Varroa : du respect des recommandations dépend la survie des colonies. *Abeilles&Cie*, Mars-Avril 2022

Hernandez, J., Aebi, A. & Dietemann, V (2022). Le respect des recommandations de traitements contre le Varroa améliore la survie des colonies pendant l'hiver. *Revue Suisse d'Apiculture*, Juillet 2022

Hernandez, J., Aebi, A. & Dietemann, V (2022). Lutte contre le varroa : Le respect des recommandations de traitements contre le Varroa améliore la survie des colonies pendant l'hiver. *La Santé de l'abeille FNOSAD*, août 2022

B.2 Communications

Contributions to international congress

- 29 July-02 August 2019 - 15th European Ecological Federation Congress - Ecology across borders: embedding ecology in sustainable development goals, Lisbon, Portugal (Poster presentation).

Communications at technical days or public general conferences

- Conference to present the project « Agriculture & Pollinisateurs » and presentation and introduction to the ColEval method – Journée du Service Sanitaire Apicole (SSA) - Saignelégier (October 2019).
- Conference for beekeeping associations in Payerne (August 2021) and in Yverdon (April 2022).
- Interview to produce a general public article on honey bee health in collaboration with the Imperial College London: Fighting Hive Pests – *Anthroposphere*, June 2022 https://www.anthroposphere.co.uk/post/fighting-hive-pests/
- Conference for the Swiss honey bee day - Lyss (July 2022).