Some Considerations about Winter Colony Losses in Italy According to the Coloss Questionnaire

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Simple Summary: The Italian beekeeping industry has grown steadily during the last decade, according to data from the national beekeeping registry. Nevertheless, winter colony losses remain a matter of concern for beekeepers in Italy, and administration of the questionnaire defined by the Coloss Association could contribute to a better understanding of this phenomenon. Since 2008, data on winter colony losses have been gathered through the Coloss questionnaire at European Union, European, and extra-European levels. In Italy, these data have been collected since 2008 in hard-copy form and online since 2019–2020. However, since the responding beekeepers did not represent all Italian regions over the years, the whole questionnaire administration period (2008–2021) could not be reasonably considered for analysis. Hence, we included only the periods 2014–2015 and 2020–2021. Among the possible causes of colony losses were queen-related problems, natural disasters, and dead or empty colonies, since these questions remained unchanged over time. We also took account of responses related to treatments against Varroa mite infestation.

Abstract: The Italian beekeeping industry has grown steadily during the last decade, according to data from the national beekeeping registry, which came into existence in February 2015. Winter colony losses remain a matter of concern for beekeepers in Italy, and administration of the questionnaire defined by the Coloss Association could contribute to a better understanding of this phenomenon. To evaluate the percentage trends over time in honeybee colony losses arising from various causes, we used the quasi-binomial generalized linear modelling (GzLM) approach, taking the year as an independent variable. We set our level of significance at 5% and performed the data analysis only for the seven regions that sent data continuously from 2014 to 2020. We considered the percentage of losses due to queen-related problems, natural disasters, and dead or empty colonies, given that these questions remained unchanged over the years. The survey also revealed that the percentage trend for respondents using drone brood removal showed a significant increase. In general, the percentage of colony losses due to queen-related problems remained lower than 8%, and the percentage of colony losses associated with natural disasters was very low (<2%). The mean percentages of losses due to dead or empty colonies ranged from 6 to 17% in the considered period. In addition, we took account of the responses relating to treatments against Varroa mite infestation, given the importance attributed to this honeybee parasite. Unlike the other variables, we calculated the percentages related to the types of beekeeper treatments against Varroa destructor based on the respondents, not on the colonies. What emerged was that almost every beekeeper used at least one type of treatment against V. destructor. In general, the trend of respondents appeared stable at 0.3% during the last four years.

Keywords: winter colony losses; Coloss questionnaire; honeybee; treatment; Varroa destructor

1. Introduction

The Italian beekeeping industry has grown steadily during the last decade, following the implementation of the national beekeeping registry, in accordance with the Min-
istry of Health Decrees of 4 December 2009 and 11 August 2014, which became fully operative in 2015 [1,2]. The Interdirectoril Decree (Ministries of Health and Agriculture) of 27 November 2017 further integrated the national beekeeping registry [3]. This system was actually developed in advance of, and is now in agreement with, Regulations 2019/2035 and 2022/1345 defining the rules governing establishments keeping terrestrial animals and with more recent Italian legislation [4–7]. The figures for the Italian national beekeeping registry as of 30 June 2022 are as follows: 71,104 beekeepers, 175,281 apiaries, 1,767,390 colonies, and 278,982 nuclei (referred to as swarms in the beekeeping registry). The average densities are 0.58 apiaries/km$^2$ and 0.23 beekeepers/km$^2$, with peaks of 1.6 apiaries/km$^2$ and 0.92 beekeepers/km$^2$ in the north. Most Central–Northern regions and some areas of Southern Italy seem the most suited to apiculture (https://www.vetinfo.it/j6_statistiche/#/report-pbi/45) (accessed on 4 October 2022).

The health status of honeybee colonies has been investigated across the Italian territory [8–11], focusing on nature reserves [12,13] and at European Union level [14,15], with a view to better understanding the phenomenon of colony losses and the possible risk factors involved. Spring honeybee losses were also investigated in Italy in spring 2008 in relation to the use of neonicotinoid-coated corn seeds [16–18]. Winter losses have been studied in many other countries in Europe [19–40] and beyond [41–48]. The possible impacts on colony losses of Varroa mite infestation and its control [49–51], as well as of weather conditions [52–56], have also been investigated.

Winter colony losses are a cause for concern for the beekeeping industry of Italy, and the first reports on this topic go back to 2007/2008 [57,58]. Honeybee colony losses recorded in Italy in the winter of 2007–2008 were 30–40% in the Northern part of the country and 10–30% in central and southern areas, based on oral reports from beekeepers’ associations. Data from anonymous questionnaires distributed and collected during beekeepers’ meetings confirmed these oral reports [58]. The limited availability of data on losses prevents a better understanding of the phenomenon and of causative factors. It does appear, however, that the reported losses are attributable mainly to inadequate and/or improper control of Varroa destructor infestation and interactions between the mite and other pathogens and only partially to inadequate apicultural techniques. The questionnaires used were processed locally and developed in the framework of COST Action FA0803 “COLOSS” (Prevention of honeybee COlony LOSSes). The latter was then formalized as the Coloss questionnaire, which constitutes an output of the Coloss Association (www.coloss.org) (accessed on 4 October 2022) [58]. Further improvement in the questionnaire’s administration and targeting of different specific topics each year could contribute to a better understanding of this phenomenon. Data on winter losses have been collected through the Coloss questionnaire at European Union, European, and extra-Europe levels since 2008 [59–64]. In Italy, these data have been gathered since 2008 (in hard-copy format and online since 2019–2020). However, since the responding beekeepers did not represent all Italian regions over the years, we could not reasonably consider the whole questionnaire administration period (2008–2021) for analysis. Hence, our analysis was limited to the periods 2014–2015 and 2020–2021.

The goal of our paper was to present the data collected through the Coloss questionnaire in Italy, quantify colony losses, and consider the possible causes. Among the collected data, we devoted attention to those related to treatments carried out by beekeepers against the parasitic mite V. destructor. Lastly, we considered critical issues encountered in conducting the Coloss survey in Italy, particularly in relation to the very limited participation recorded since the beginning of the monitoring project.

2. Materials and Methods

The Coloss questionnaire has been used in Italy since 2008 [58–64] to collect data on winter honeybee colony losses. It consists of about 27 questions which mainly require numerical entries and are closed-ended (single or multiple choice), except for a few open-ended questions. As the questionnaire has varied slightly from year to year, the number of
questions has also varied, from a minimum of 21 to a maximum of 28. Of these, about 15 were mandatory for continuation, while the remainder were optional. Only the questions listed in Table S1 were included in the present study. The others were deemed irrelevant to the aim of this study. A copy of the full questionnaire is available as Table S2.

Regarding questionnaire administration and data collection, until 2018–2019, the survey was disseminated via the website www.izsvenezie.it (accessed on 4 October 2022). Here, it was possible to download the questionnaire, fill it in on paper, and then email it for data collection purposes. The questionnaire was further advertised via an official note from the Ministry of Health emailed to national and local beekeepers’ associations and beekeeping magazines and distributed during beekeeping meetings. In 2019–2020, online entry was introduced. The questionnaire, which was computerized using the Lime Survey web application, could therefore be filled in directly online or downloaded and filled in on paper as in previous years. Data collection took place between the end of March and mid-June each year. An introductory text was placed both at the beginning of the hard copy of the questionnaire and online at the website www.izsvenezie.it (accessed on 4 October 2022). All participants were therefore fully informed about the research and its purpose.

No sensitive data were collected through the questionnaire; the only personal information requested was the post/zip code and name of the city/town/village near to the respondent’s apiary. Accordingly, the research did not require review or approval by the Ethics Committee. Nevertheless, we adopted specific participant protection procedures to comply with privacy policy. Since 2019–2020, participants in the online survey have been requested to consent to a privacy agreement through a checkbox at the beginning of the online questionnaire. Before 2018–2019, i.e., when only the hard-copy version of the questionnaire was available, response to the questionnaire was deemed to constitute consent, considering that participants were invited to take part in the survey on a voluntary basis. The data were registered anonymously and handled in accordance with the EU General Data Protection Regulation [65].

To verify the presence of any monotonic increasing or decreasing trends in the percentages by year for honeybee colony losses due to various causes, we used the quasi-binomial generalized linear modelling (GzLM) approach [51], taking the year as an independent variable. We used R version 3.2. (©2015) software. We set our level of significance at 5% and carried out the data analysis only for the seven regions that sent data continuously from 2014 to 2020. We considered the percentages of colony losses due to queen-related problems, natural disasters, and dead/empty colonies, since these questions remained unchanged over the years. In addition, we also analyzed responses related to treatments against Varroa mite infestation. Concerning V. destructor control methods, for each treatment, we calculated the percentage of apiaries that underwent treatments in the year in question, determining the trend per year only for treatments with the highest percentages.

3. Results

Table 1 presents the distributions of apiaries by region and year and of respondents with their colonies, as well as the percentages of respondents compared to the numbers of apiaries extracted from the Italian national beekeeping registry, according to the beekeepers’ answers to the Coloss questionnaire.
Table 1. Distributions of apiaries by region and year and of respondents with their colonies, along with the percentages of respondents compared to the numbers of apiaries extracted from the Italian national beekeeping registry.

| Region               | 2014–2015 * | 2015–2016 | 2016–2017 | 2017–2018 | 2018–2019 | 2019–2020 | 2020–2021 |
|----------------------|-------------|-----------|-----------|-----------|-----------|-----------|-----------|
|                      | N. of Apiaries ** | N. of Respondents (N. of Colonies/Respondents) | % of Respondents/Apiaries | N. of Apiaries ** | N. of Respondents (N. of Colonies/Respondents) | % of Respondents/Apiaries | N. of Apiaries ** | N. of Respondents (N. of Colonies/Respondents) | % of Respondents/Apiaries | N. of Apiaries ** | N. of Respondents (N. of Colonies/Respondents) | % of Respondents/Apiaries | N. of Apiaries ** | N. of Respondents (N. of Colonies/Respondents) | % of Respondents/Apiaries |
| Emilia Romagna       | 2 (27)      | 8526 (145) | 0.1       | 10,184 (1063) | 0.1 | 12,740 (162) | 0.0 | 14,598 (688) | 0.1 | 15,711 (2971) | 0.1 |
| Friuli Venezia Giulia| 22 (2443)   | 2686 (36)  | 0.1       | 3086 (1134) | 0.1 | 3406 (22) | 0.1 | 3689 (104) | 0.3 | 4065 (400) | 0.7 | 4332 (1099) | 1.1 |
| Lombardia            | 7 (128)     | 10,108 (156) | 1.5 | 12,752 (1867) | 1.2 | 14,398 (1046) | 0.3 | 15,306 (7156) | 0.0 | 17,303 (13166) | 0.1 | 19,087 (7957) | 0.4 | 44 (6327) | 0.4 |
| Piemonte             | 1 (10)      | 13,012 (30) | 0.0 | 16,777 (5193) | 0.0 | 18,855 (5644) | 0.1 | 21,309 (479) | 0.1 | 23,900 (2648) | 0.1 | 25,629 (1546) | 0.0 | 55 (1735) | 0.6 |
| Sicilia              | 1 (125)     | 4790 (195) | 0.1 | 5986 (1423) | 0.1 | 7105 (3115) | 0.0 | 8437 (2582) | 0.1 | 10,398 (290) | 0.1 | 11,447 (1099) | 0.1 |
| Trentino Alto Adige  | 9 (154)     | 5795 (53) | 0.0 | 6780 (1266) | 0.0 | 7571 (566) | 0.3 | 8296 (936) | 0.5 | 9064 (1691) | 0.9 | 9818 (55) | 9818 (55) |
| Veneto               | 62 (1777)   | 8711 (205) | 0.9 | 10,113 (2105) | 0.9 | 12,468 (1546) | 0.9 | 14,155 (1978) | 0.9 | 15,830 (1148) | 0.5 |
| Total                | 104 (4664)  | 53,628 (333) | 0.6 | 65,678 (8106) | 0.4 | 74,129 (202) | 0.3 | 82,245 (8456) | 0.3 | 93,483 (5661) | 0.3 | 101,854 (22804) | 0.3 |

* Not available since the Italian national beekeeping registry was not yet operating. ** Extracted from the Italian national beekeeping registry.
With the sole exception of Lombardia, Veneto, and Trentino Alto Adige, the number of respondents was quite low. The distribution of apiaries per region and year is based on data from the Italian national beekeeping registry (https://www.vetinfo.it/j6_statistiche/#/report-list/26) (accessed on 4 October 2022). Percentages >1 are highlighted in bold, and, in general, all percentages were very low, considering that the average density was 0.58 apiaries/km², with peaks of 1.6 apiaries/km² in the north of the country. In Friuli Venezia Giulia region (Northeast Italy), the percentage trend was found to be increasing, but appeared to be declining in the Veneto region. In general, the trend appears to have stabilized at 0.3% over the last four years (Table 1). The percentage numbers of respondents per apiary give an indication of respondent densities in the regions and in the country, based on registered establishments of managed honeybee colonies [4–7]. We considered the percentages of colony losses due to queen-related problems, natural disasters, and dead or empty colonies, since these questions remained unchanged over the years. The data are presented in Table S3.

Based on quasi-binomial generalized linear modelling (GzLM), the percentage trend for colony losses arising from queen-related problems appeared not to be significant (p-value = 0.628), as supported by the graphical analysis (Figure 1A).

![Graphs showing percentage trends for different types of colony losses.](image)

(A)  (B)  (C)  (D)

**Figure 1.** (A) Percentage of colony losses due to queen-related problems. (B) Percentage of colony losses due to natural disasters. (C) Percentage of losses arising from dead or empty colonies. (D) Percentage of treatments against *V. destructor* carried out by respondents.

The trend for colony losses due to natural disasters was not significant (p-value = 0.297), as supported by the graphical analysis (Figure 1B). In the case of losses from dead or empty colonies (Figure 1C), the trend was not significant (p-value = 0.594).

Unlike the other previously considered variables, we based calculations of the percentages of types of beekeeper treatments against *V. destructor* on the respondents and not on the colonies. This revealed that almost every beekeeper used at least one type of treatment...
against *V. destructor* (Figure 1D), which is in agreement with the annual Italian national program for *V. destructor* control ([www.izsvenezie.it](http://www.izsvenezie.it)) (accessed on 4 October 2022). Information about treatments was also available for previous years, but not for all the regions considered. For this reason, and in keeping with the other analyzed variables, we considered the years from 2010 to 2011 onwards. The trend was not significant (*p*-value = 0.922). For each treatment against *V. destructor*, we calculated the percentage of apiaries undergoing treatments that year, determining the trend per year only for treatments with the highest percentages (Figure 2A–E).

![Graph A](image1.png)  
**Figure 2.** (A) Percentages of apiaries undergoing treatments based on drone brood removal. (B) Percentages of apiaries undergoing treatments using other biotechnical methods (e.g., trapping comb, complete brood removal, queen confinement). (C) Percentages of apiaries undergoing treatments with oxalic acid trickling. (D) Percentages of apiaries undergoing treatments with oxalic acid sublimation (evaporation). (E) Percentages of apiaries undergoing treatments with thymol (e.g., Apiguard, ApilifeVar).

Every year, the use of oxalic acid trickling stood in first place, with percentages of >70% and a peak of 85% in 2015–2016. In second place was oxalic acid sublimation (evaporation),
which was mostly used in autumn–wintertime, with percentages ranging from 28% to 53%. There appeared to be a significant decrease \( (p < 0.01) \) in the trend for this method of applying oxalic acid. In the same regions, the trend in the use of drone brood removal (23–31%) was found to slightly significantly increase \( (p = 0.040) \). In the seven regions considered, the trends in the use of other biotechnical methods (23–42%) (e.g., trapping comb, complete brood removal, queen confinement) and thymol-based drugs (17–34%; e.g., Apiguard\textsuperscript{®}, ApilifeVar\textsuperscript{®}) were not significant. The high percentage in 2020–2021 of formic acid use (long-term; e.g., MAQS\textsuperscript{®}) appeared unusual. We did not consider the periods 2010–2011 or 2011–2012 because of the incomplete data provided.

4. Discussion

The limited geographical participation in the Coloss questionnaire and the lack of continuity over the years placed several limitations on the relevance and reliability of the data collected, precluding any deeper analysis and substantial conclusions. Accordingly, we carried out data analysis only for the seven regions that sent data continuously from 2014 to 2020. Furthermore, we took account of the loss rates due to queen-related problems, natural disasters, and dead or empty colonies, since these questions remained unchanged over the years. In general, the percentage losses due to queen-related problems remained lower than 8%, which could be explained by the expertise of the beekeepers participating in the questionnaire. Furthermore, data collected in previous years through the Coloss questionnaire indicated queen-related losses of between 5 and 8.9% [59–63]. The percentage of lost colonies associated with natural disasters was very low (<2%) and could be attributed to the attention paid by beekeepers to the locations of their apiaries and to monitoring of weather-related natural events. Interestingly, the percentage losses arising from dead or empty colonies showed a decreasing trend. On average, this parameter ranged from 7 to 17% in the period considered, remaining around 15%, which is considered acceptable for Italian beekeepers. However, the acceptability range for winter colony losses differs among countries and regions and depends at least on operation size, management, geography, and climate [59], making comparisons difficult. If, for example, we consider the Coloss survey conducted in the winter of 2019–2020 [64] in countries recording overall winter loss rates similar to Italy (18.4%), the number of respondents was similar only for Ireland, being higher for Belgium and Slovakia and much higher for Germany. On the other hand, the number of colonies going into winter was much lower for Belgium and Ireland, higher for Slovakia, and extremely high for Germany. Furthermore, in the case of Italy, the collected data were not fully representative of the country, since geographical coverage was incomplete, based on the respondents’ origins.

Almost every beekeeper reported performing at least one type of treatment against *V. destructor*, in line with the annual national control program [66]. Each year, the use of oxalic acid trickling stood in first place, ranging from >70% to a maximum of 85% in 2015–2016, followed by the use of oxalic acid sublimation (evaporation), ranging from 28 to 53% and used mostly in autumn–wintertime. As observed also in other European countries, oxalic acid-based drugs appeared among the veterinary medicines used most during both the active season after queen caging and in natural broodless periods [67]. Varroa mites severely affect honeybee health throughout the year, and their part in honeybee colony losses should be considered [49]. *V. destructor* control strategies should be developed and spread among beekeepers, and compliance with recommended treatment regimens should be consolidated to improve the survival of honeybee colonies over winter [50,51]. Varroa mite infestation control should definitely become part of routine honeybee colony management and best beekeeping practices to improve its efficacy and reduce colony losses [50,51,66].

The trends for treatments against *V. destructor*, as presented in Figure 2, demonstrate beekeepers’ attention to controlling this severe parasitic infestation. The use of drone brood removal exhibited a steady trend of between 20 and 40%, and other biotechnical techniques have likewise shown an increase in the last two years. This is in line with the national
Varroa control program, beekeepers’ improved abilities to combine biotechnical tools with the application of veterinary medicinal products, and increased awareness about using less residue-producing drugs. This is related to the use of oxalic acid-based drugs administered by trickling in summertime and by evaporation mainly in autumn–wintertime. The use of thymol-based veterinary medicinal products was also reported to show a steady trend of between 20 and 30% during the last five years. Unexpectedly, in 2020–2021, the use of formic acid (long-term) reached an unusual high of 66%, considering that previously it had always remained below 10%. This could be partially explained by the fact that the characteristics of this veterinary medicinal product met beekeepers’ needs for an easier, safer application method for formic acid. An interesting topic to be considered in the framework of Varroa control strategies and surveys related to winter colony losses is the possible impact of weather on the winter mortality of honeybee colonies that has been recently investigated in Europe [50,52–55] and the USA [56].

All these interesting research topics could benefit from a tool like the Coloss questionnaire. It could be enriched each year with additional questions aimed at collecting information on specific fields, as has been done in the past for nutrition practices and queen replacement; in the future, questions could also potentially be included on weather conditions.

Despite favourable conditions in terms of the availability and proper application of veterinary medicines against *V. destructor* [66], there has been growing interest in *Varroa*-resistant colonies in Italy, too. This has not been limited to research groups but has also involved beekeepers’ associations. In Sicily, interest was directed at *Apis mellifera sicula*, which apparently requires less or no treatment compared to other subspecies. Of course, this condition needs closer investigation, but the national *V. destructor* control program gives the option to define a specific protocol which can, in agreement with local veterinary services, differ from the national one [66], thus taking into account the features of this *Apis* subspecies and local geographic and climatic conditions [68].

Extensive contacts with beekeepers’ associations at national and local levels have been stimulated since the implementation of the Coloss questionnaire began. Furthermore, the Coloss questionnaire was issued on behalf of the Directorate General of Animal Health and Veterinary Medicines of the Italian Ministry of Health. Italian beekeepers’ participation in the questionnaire has always been poor due to little interest in the survey, lack of or limited confidence in surveys managed by government institutions, the concentration of potential respondents on their own business in springtime, or participation in association questionnaires. Even the availability of an online version of the questionnaire in both Italian and German has not helped increase the number of participants. Nevertheless, the geographical spread of the respondents has considerably increased in recent years, with greater involvement of Central–Southern Italy than in the past [63]. In 2020–2021, the number of participants was not exceptional, despite the presence of many more colonies (Table 1), which could be explained by the participation of more professional beekeepers than previously. According to Gray et al. [64], the estimated percentages of beekeepers in the 2019–2020 survey ranged from <1 to 17, with Italy scoring <1, confirming the results recorded every year since the first questionnaire in 2008.

Given the role and relevance of beekeeping in Italy, we will continue to share the results of the Coloss questionnaire at national level through dedicated articles published in beekeeping journals and magazines, reports at beekeepers’ conferences and associative meetings, and by promoting the questionnaire through our website. In addition, we will stimulate beekeepers and their associations, as well as government and research institutions, to support participation in the Coloss questionnaire, despite the very limited interest shown to date.

**Supplementary Materials:** The following supporting information can be downloaded at: [https://www.mdpi.com/article/10.3390/insects13111059/s1](https://www.mdpi.com/article/10.3390/insects13111059/s1), Table S1: Questions included in the study; Table S2: Coloss questionnaire; Table S3: Distribution of losses due to queen-related problems, natural disasters, and dead or empty colonies.
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Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy concerns.

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References

1. Decreto 4 Dicembre 2009 Ministero del Lavoro, della Salute e Delle Politiche Sociali. Disposizioni Per L’Anagrafe Apistica Nazionale [Provisions for the Italian National Beekeeping Registry]. GU n. 93 del 22-4-2010. Available online: https://www.gazzettaufficiale.it/atto/serie_generale/caricaDettaglioAtto?originario?atto.dataPubblicazioneGazzetta=2010-04-22&atto.codiceRedazionale=10A04712 (accessed on 22 August 2022).

2. Decreto 11 Agosto 2014 Ministero Della Salute. Approvazione del Manuale Operativo Per la Gestione Dell’Anagrafe Apistica Nazionale, in Attuazione Dell’Articolo 5 del Decreto 4 Dicembre 2009, Recante: «Disposizioni Per L’Anagrafe Apistica Nazionale» [Approval of the Operational Manual for the Italian National Beekeeping Registry Management, in Application of Article 5 of Decree 4 December 2009, Provisions for the Italian National Beekeeping Registry]. GU n. 291 del 16-12-2014. Available online: https://www.gazzettaufficiale.it/eli/id/2014/12/16/14A09510/sg (accessed on 22 August 2022).

3. Decreto Interdirezionale del 27 Novembre 2017. Il Direttore Generale Della Direzione Generale Della Sanità Animale e dei Farmaci Veterinari di Concerto con il Direttore Generale Della Direzione Generale Dello Sviluppo Rurale del Ministero Delle Politiche Agricole Alimentari e Forestali. Attuazione del Punto 12.3 del Manuale Operativo Per la Gestione Apistica Nazionale di Cui al D.M. 11 Agosto 2014 [Implementation of Point 12.3 of the Operational Manual for the National Beekeeping Management Referred to in the Ministerial Decree of 11 August 2014]. Prot. N. 0027115-27/11/2017-DGSAF-MDS-P. Available online: https://www.politicheagricole.it/flex/cm/pages.ServeBLOB.php/L/IT/IDPagina/12181 (accessed on 22 August 2022).

4. Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March 2016 on Transmissible Animal Diseases and Amending and Repealing Certain Acts in the Area of Animal Health (‘Animal Health Law’). OJ L 84, 31.3.2016, 1-208. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv%3AOJ.L_.2016.084.01.0001.01.ENG (accessed on 3 October 2022).

5. Commission Delegated Regulation (EU) 2019/2035 of 28 June 2019 Supplementing Regulation (EU) 2016/429, ai Sensi Dell’Articolo 14, Comma 2, Lettere a), b), g), h), j) e p), Della Legge 22 Aprile 2021, n. 53. GU n. 213 del 15-6-2021. Available online: https://www.gazzettaufficiale.it/eli/id/2019/06/22/19G00142/sg (accessed on 22 August 2022).

6. Commission Implementing Regulation (EU) 2022/1345 of 1 August 2022 Laying Down Rules for the Application of Regulation (EU) 2016/429 of the European Parliament and of the Council with Regard to Registration and Approval of Establishments Keeping Terrestrial Animals and Hatcheries, and the Traceability of Certain Kept Terrestrial Animals and Hatching Eggs. OJ L 314, 5.12.2019, 115–169. Available online: https://eur-lex.europa.eu/legal-content/IT/TXT/?uri=uriserv%3AOJ.L_.2019.314.01.0004.01.ENG (accessed on 3 October 2022).

7. Decreto Legislativo 5 Agosto 2022, n. 134 Disposizioni in Materia di Sistema di Identificazione e Registrazione Degli Operatori, Degli Stabilimenti e Degli Animali Per L’Adeguamento Della Normativa Nazionale alle Disposizioni del Regolamento (UE) 2016/429, ai Sensi Dell’Articolo 14, Comma 2, Lettere a), b), g), h),), j) e p), Della Legge 22 Aprile 2021, n. 53. GU n. 213 del 12-9-2022. [Provisions on the Identification and Registration System of Operators, Establishments and Animals for the Adaptation of National Legislation to the Provisions of Regulation (EU) 2016/429]. Available online: https://www.gazzettaufficiale.it/eli/id/2022/09/12/22G00142/sg (accessed on 4 October 2022).

8. Mutinelli, F.; Costa, C.; Lodesani, M.; Baglio, A.; Formato, G.; Medrzycki, P.; Porrini, C. Honeybee colony losses in Italy. J. Apic. Res. 2010, 49, 119–120. [CrossRef]
10. Porrini, C.; Lodesani, M.; Libertà, A.; Bortolotti, L.; Gallina, A.; Colombo, R.; Sgolastra, F.; Medrzycki, P.; Bozza, M.A.; Mutinelli, F. BeeNet: Italian beekeeping monitoring network. In Proceedings of the XXXIII Apimondia, Kiev, Ukraine, 29 September–4 October 2013; Apimondia: Rome, Italy, 2013; pp. 179–180.

11. Porrini, C.; Mutinelli, F.; Bortolotti, L.; Granato, A.; Laurenson, L.; Roberts, K.; Gallina, A.; Silvester, N.; Medrzycki, P.; Renzi, T.; et al. The status of honey bee health in Italy: Results from the nationwide bee monitoring network. PLoS ONE 2016, 11, e0155411. [CrossRef] [PubMed]

12. Bellucci, V.; Lucci, S.; Campanelli, F.; Sannino, R.; Visicchio, F.; Altera, D.; Formato, G.; Felicioli, A.; Porrini, C.; Mutinelli, F. Nota sugli ultimi sviluppi relativi al monitoraggio dello spopolamento degli alveari [Note on the last development of honey bee colony depopulation monitoring]. APOides 2009, 6, 173–174.

13. Bellucci, V.; Lucci, S.; Bianco, P.; Ubaldi, A.; Felicioli, A.; Porrini, C.; Mutinelli, F.; Battisti, S.; Spallucci, V.; Cersini, A.; et al. Monitoring honey bee health in five nature protected areas in Italy. Vet. Ital. 2019, 55, 15–25.

14. Chauzat, M.-P.; Jacques, A.; Laurent, M.; Bougeard, S.; Hendriks, P.; Ribiére-Chabert, M.; EPILOBEE Consortium. Risk indicators affecting honey bee colony survival in Europe: One year of surveillance. Apidologie 2016, 47, 368–378. [CrossRef]

15. Jacques, A.; Laurent, M.; EPILOBEE Consortium; Ribiére-Chabert, M.; Saussac, M.; Bougeard, S.; Budge, G.E.; Hendriks, P.; Chauzat, M.-P. A pan-European epidemiological study reveals honey bee colony survival depends on beekeeper education and disease control. PLoS ONE 2017, 12, e0172991. [CrossRef]

16. Sabatini, A.G.; Astuti, M.; Mutinelli, F. Mortalità di api e spopolamento degli alveari nella primavera del 2008: Indagini in Lombardia e nel Triveneto [Honey bee mortality and colony depopulation in spring 2008: Investigations in Lombardy and northeastern Italy]. APOides 2008, 5, 88–90.

17. Bortolotti, L.; Sabatini, A.G.; Mutinelli, F.; Astuti, M.; Lavazza, A.; Piro, R.; Tesoriero, D.; Medrzycki, P.; Sgolastra, F.; Porrini, C. Spring honeybee losses in Italy. Julius Kühn Archive 2009, 423, 148–152.

18. Mutinelli, F.; Sabatini, A.G.; Astuti, M.; Porrini, C. Neonicotinoids precautionary ban in Italy. Am. Bee J. 2009, 149, 269–270.

19. Neumann, P.; Carreck, N. Honey bee colony losses. J. Apic. Res. 2010, 49, 1–6. [CrossRef]

20. vanEngelsdorp, D.; Meixner, M.D. A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. J. Invert. Pathol. 2010, 103, 580–595. [CrossRef] [PubMed]

21. Potts, S.G.; Roberts, S.; Dean, R.; Marris, G.; Brown, M.A.; Jones, R.; Neumann, P.; Settele, J. Declines of managed honey bees and beekeepers in Europe. J. Apic. Res. 2010, 49, 15–22. [CrossRef]

22. Broschneider, R.; Moosbeckhofer, R.; Crailsheim, K. Surveys as a tool to record winter losses of honey bee colonies: A two year case study in Austria and South Tyrol. J. Apic. Res. 2010, 49, 23–30. [CrossRef]

23. Chauzat, M.-P.; Martel, A.-C.; Zeggane, S.; Drajnudel, P.; Clément, M.-C.; Ribiére-Chabert, M.; Aubert, M.; Faucon, J.-P. A case control study and a survey on mortalities of honey bee colonies (Apis mellifera) in France during the winter of 2005–6. J. Apic. Res. 2010, 49, 40–51. [CrossRef]

24. Nguyen, B.K.; Van der Zee, R.; Vejsnæs, F.; Wilkins, S.; Le Conte, Y.; Ritter, W. COLOSS Working Group 1: Monitoring and diagnosis. J. Apic. Res. 2010, 49, 97–99. [CrossRef]

25. Ivanova, E.N.; Petrov, P.P. Regional differences in honey bee winter losses in Bulgaria during the period 2006–9. J. Apic. Res. 2010, 49, 102–103. [CrossRef]

26. Tlak Gaiger, I.; Tomljanović, Z.; Petrinec, Z. Monitoring health status of Croatian honey bee colonies and possible reasons for winter losses. J. Apic. Res. 2010, 49, 107–108. [CrossRef]

27. Vejsnæs, F.; Lykke Nielsen, S.; Kryger, P. Factors involved in the recent increase in colony losses in Denmark. J. Apic. Res. 2010, 49, 109–110. [CrossRef]

28. Aston, D. Honey bee winter loss survey for England, 2007–2008. J. Apic. Res. 2010, 49, 111–112. [CrossRef]

29. Hatjina, F.; Bouga, M.; Karamatsou, A.; Kontothanasi, A.; Charistos, L.; Emmanouil, C.; Emmanouil, N.; Maistros, A.-D. Data on honey bee losses in Greece: A preliminary note. J. Apic. Res. 2010, 49, 116–118. [CrossRef]

30. van der Zee, R. Colony losses in the Netherlands. J. Apic. Res. 2010, 49, 121–123. [CrossRef]

31. Dahle, B. The role of Varroa destructor for honey bee colony losses in Norway. J. Apic. Res. 2010, 49, 124–125. [CrossRef]

32. Topolska, G.; Gajda, A.; Pohorecka, K.; Bober, A.; Kasprzak, S.; Skubida, M.; Semkiw, P. Winter colony losses in Poland. J. Apic. Res. 2010, 49, 126–128. [CrossRef]

33. Gray, A.; Peterson, M.; Teale, A. An update on recent colony losses in Scotland from a sample survey covering 2006–2008. J. Apic. Res. 2010, 49, 129–131. [CrossRef]

34. Charrière, J.-D.; Neumann, P. Surveys to estimate winter losses in Switzerland. J. Apic. Res. 2010, 49, 132–133. [CrossRef]

35. Hristov, P.; Shumkova, R.; Palova, N.; Neov, B. Factors associated with honey bee colony losses: A mini-review. Vet. Sci. 2020, 7, 166. [CrossRef]

36. Genersch, E.; von der Ohe, W.; Kaatz, H.; Schroeder, A.; Otten, C.; Büchler, R.; Berg, S.; Ritter, W.; Mühlen, W.; Gisder, S.; et al. The German bee monitoring project: A long term study to understand periodically high winter losses of honey bee colonies. Apidologie 2010, 41, 332–352. [CrossRef]

37. Broschneider, R.; Brus, J.; Danilikh, J. Comparison of apiculture and winter mortality of honey bee colonies (Apis mellifera) in Austria and Czechia. Agric. Ecosyst. Environ. 2019, 274, 24–32. [CrossRef]

38. Köglberger, H.; Moosbeckhofer, R.; Derakhshifar, I.; Etter, K.; Mayr, J.; Schwarz, M. Verluste von Bienenvölkern während der Überwinterung oder bei Verdacht auf Bienenfrevel—Was waren die Ursachen? Bienen Aktuell 2014, 74, 28–31.
39. Morawetz, L.; Köglberger, H.; Griesbacher, A.; Derakhshifar, I.; Crailsheim, K.; Brodschneider, R.; Moosbeckhofer, R. Health status of honey bee colonies (Apis mellifera) and disease-related risk factors for colony losses in Austria. *PLoS ONE* **2019**, *14*, e0219293. [CrossRef] [PubMed]

40. van der Zee, R.; Gray, A.; Pisa, L.; de Rijk, T. An observational study of honey bee colony winter losses and their association with Varroa destructor, neonicotinoids and other risk factors. *PLoS ONE* **2015**, *10*, e0131611. [CrossRef] [PubMed]

41. Brown, P.; Newsom-Lloyd, L.E.; Foster, B.J.; Badger, P.H.; McLean, J.A. Winter 2016 honey bee colony losses in New Zealand. *J. Apic. Res.* **2018**, *57*, 278–291. [CrossRef]

42. Currie, R.W.; Pernal, S.F.; Guzmán-Novoa, E. Honey bee colony losses in Canada. *J. Apic. Res.* **2010**, *49*, 104–106. [CrossRef]

43. vanEngelsdorp, D.; Underwood, R.; Caron, D.; Hayes, J., Jr. An estimate of managed colony losses in the winter of 2006–2007: A report commissioned by the apiary inspectors of America. *Am. Bee J.* **2007**, *147*, 599–603.

44. vanEngelsdorp, D.; Underwood, R.M.; Pettis, J. A Survey of Honey Bee Colony Losses in the U.S., Fall 2007 to Spring 2008. *PLoS ONE* **2008**, *3*, e4071. [CrossRef] [PubMed]

45. Hernandez, J.; Hattendorf, J.; Aebi, A.; Dietemann, V. Compliance with recommended Varroa destructor treatment regimens and the role of weather conditions and the weight of regulatory conditions. Results from a survey among beekeepers. *Res. Vet. Sci.* **2018**, *118*, 52–60. [CrossRef] [PubMed]

46. Kulhanek, K.; Steinhauer, N.; Rennich, K.; Caron, D.M.; Sagili, R.R.; Pettis, J.S.; Ellis, J.D.; Wilson, M.E.; Wilkes, J.T.; et al. A national survey of managed honey bee 2015–2016 annual colony losses in the USA. *J. Apic. Res.* **2017**, *56*, 328–340. [CrossRef]

47. Tang, J.; Ma, C.; Shi, W.; Chen, X.; Liu, Z.; Wang, H.; Chen, C. A National survey of managed honey bee colony winter losses, Varroa destructor control strategies, and the role of weather conditions: Results from a survey among beekeepers. *Sci. Total Environ.* **2017**, *579*, 1581–1587. [CrossRef] [PubMed]

48. Steinhauser, N.A.; Rennich, K.; Wilson, M.E.; Caron, D.M.; Lengerich, E.J.; Pettis, J.S.; Rose, R.; Skinner, J.A.; Tarpy, D.R.; Wilkes, J.T.; et al. A national survey of managed honey bee 2012–2013 annual colony losses in the USA: Results from the Bee Informed Partnership. *J. Apic. Res.* **2014**, *53*, 1–18. [CrossRef]

49. Le Conte, Y.; Ellis, M.; Ritter, W. Varroa mites and honey bee health: Can Varroa explain part of the colony losses? *Apidologie* **2010**, *41*, 353–363. [CrossRef]

50. Beyer, M.; Junk, J.; Eickermann, M.; Clermont, A.; Kraus, F.; Georges, C.; Reichart, A.; Hoffmann, L. Winter honey bee colony losses, Varroa destructor control treatment regimens and the role of weather conditions. Results from a survey among beekeepers. *Res. Vet. Sci.* **2021**, *118*, 52–60. [CrossRef] [PubMed]

51. Hernandez, J.; Hattendorf, J.; Aebi, A.; Dietemann, V. Compliance with recommended Varroa destructor treatment regimens improves the survival of honey bee colonies over winter. *Res. Vet. Sci.* **2022**, *144*, 1–10. [CrossRef] [PubMed]

52. Becsi, B.; Formayer, H.; Brodschneider, R. A biophysical approach to assess weather impacts on honey bee colony winter mortality. *R. Soc. Open Sci.* **2021**, *8*, 201618. [CrossRef]

53. Smolinski, S.; Langowska, A.; Glazaczow, A. Raised seasonal temperatures reinforce autumn Varroa destructor infestation in honey bee colonies. *Sci. Rep.* **2021**, *11*, 22256. [CrossRef]

54. Switanek, M.; Crailsheim, K.; Trehetz, H.; Brodschneider, R. Modelling seasonal effects of temperature and precipitation on honey bee winter mortality in a temperate climate. *Sci. Total Environ.* **2017**, *579*, 1581–1587. [CrossRef] [PubMed]

55. Johannessen, J.; Wöhl, S.; Berg, S.; Otten, C. Annual Fluctuations in Winter Colony Losses of Apis mellifera L. Are Predicted by Honey Flow Dynamics of the Preceding Year. *Insects* **2022**, *13*, 829. [CrossRef] [PubMed]

56. Calovi, M.; Grozinger, C.M.; Miller, D.A.; Goslee, S.C. Summer weather conditions influence winter survival of honey bees (Apis mellifera) in the northeastern United States. *Sci. Rep.* **2021**, *11*, 1553. [CrossRef]

57. Lodesani, M.; Costa, C.; Franceschetti, S. Rilevamenti e ipotesi sulla mortalità degli alveari: Dati di un monitoraggio in alcune province dell’Emilia-Romagna [Surveys and hypotheses on honey bee colony mortality: Monitoring data from some provinces of Emilia Romagna region]. *Apidote* **2008**, *5*, 117–124.

58. Mutinelli, F.; Golastra, F.; Gallina, A.; Medrzycki, P.; Bertolotti, L.; Lodesani, M.; Porrini, C. A network for monitoring honeybee mortality and colony losses in Italy as a part of the APENET research project. *Am. Bee J.* **2010**, *150*, 389–390.

59. van der Zee, R.; Gray, A.; Holzmann, C.; Pisa, L.; Brodschneider, R.; Chelebo, R.; Coffey, M.F.; Kence, A.; Kristiansen, P.; Mutinelli, F.; et al. Standard survey methods for estimating colony losses and explanatory risk factors in Apis mellifera. *J. Apic. Res.* **2013**, *52*, 1–36. [CrossRef]

60. Brodschneider, R.; Gray, A.; van der Zee, R.; Adljane, N.; Brusbrardis, V; Charrière, J.-D.; Chelebo, R.; Coffey, M.F.; Crailsheim, C.; Dahle, B.; et al. Preliminary analysis of loss rates of honey bee colonies during winter 2015/16 from the COLOSS survey. *J. Apic. Res.* **2016**, *55*, 375–378. [CrossRef]

61. Brodschneider, R.; Gray, A.; Adljane, N.; Ballis, A.; Brusbrardis, V; Charrière, J.-D.; Chelebo, R.; Coffey, M.F.; Dahle, B.; de Graaf, D.C.; et al. Multi-country loss rates of honey bee colonies during winter 2016/2017 from the COLOSS survey. *J. Apic. Res.* **2018**, *57*, 452–457. [CrossRef]

62. Gray, A.; Brodschneider, R.; Adljane, N.; Ballis, A.; Brusbrardis, V; Charrière, J.-D.; Chelebo, R.; Coffey, M.F.; Cornelissen, B.; Amaro da Costa, C.; et al. Loss rates of honey bee colonies during winter 2017/18 in 36 countries participating in the COLOSS survey, including effects of forage sources. *J. Apic. Res.* **2019**, *58*, 479–485. [CrossRef]
63. Gray, A.; Adjlane, N.; Arab, A.; Ballis, A.; Brusbardis, V.; Charrière, J.-D.; Chlebo, R.; Coffey, M.F.; Cornelissen, B.; Amaro da Costa, C.; et al. Honey bee colony winter loss rates for 35 countries participating in the COLOSS survey for winter 2018-2019, and the effects of a new queen on the risk of colony winter loss. *J. Apic. Res.* **2020**, *59*, 744–751. [CrossRef]

64. Gray, A.; Adjlane, N.; Arab, A.; Ballis, A.; Brusbardis, V.; Bugeja Douglas, A.; Cadahia, L.; Charrière, J.-D.; Chlebo, R.; Coffey, M.F.; et al. Comparative honey bee colony loss rates for 37 countries participating in the COLOSS survey for winter 2019–2020 and some associated risk factors for winter loss. *J. Apic. Res.* **2022**, *61*. [CrossRef]

65. Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the Protection of Natural Persons with Regard to the Processing of Personal Data and on the Free Movement of Such Data, and Repealing Directive 95/46/EC (General Data Protection Regulation). OJ L 119, 4.5.2016, 1–88. Available online: https://eur-lex.europa.eu/eli/reg/2016/679/oj (accessed on 3 October 2022).

66. Italian Ministry of Health. *Aethina Tumida e Varroa-Piano di Sorveglianza Nazionale e di Gestione—Anno 2022*. [Aethina Tumida and Varroa-Italian National Plan of Surveillance and Management-Year 2022]. Prot. N. 0004352-21/02/2022-DGSAF-MDS-P. Available online: https://www.izsvenezie.it/documenti/temi/api/normativa/ministero-salute/2022-02-21-nota-4352.pdf (accessed on 21 September 2022).

67. Brodschneider, R.; Arakelyan, I.; Ballis, A.; Brus, J.; Brusbardis, V.; Cadahia, L.; Charrière, J.-D.; Chlebo, R.; Coffey, M.F.; Cornelissen, B.; et al. Spatial clusters of Varroa destructor control strategies in Europe. *J. Pest Sci.* **2022**. [CrossRef]

68. Büchler, R.; Costa, C.; Hatjina, F.; Andonov, S.; Meixner, M.D.; Le Conte, Y.; Uzunov, A.; Berg, S.; Bienkowska, M.; Bouga, M.; et al. The influence of genetic origin and its interaction with environmental effects on the survival of *Apis mellifera* L. colonies in Europe. *J. Apic. Res.* **2014**, *53*, 205–214. [CrossRef]