Journal of Apicultural Research 51(1): 100-114 (2012)
DOI 10.3896/IBRA.1.51.1.12

ORIGINAL RESEARCH ARTICLE

Managed honey bee colony losses in Canada, China, Europe, Israel and Turkey, for the winters of 2008-9 and 2009-10

Romée van der Zee[1]*, Lennard Pisa[1], Sreten Andonov[2], Robert Brodschneider[3], Jean-Daniel Charrière[4], Róbert Chlebo[5], Mary F Coffey[6], Karl Crailsheim[3], Bjørn Dahle[7], Anna Gajda[8], Alison Gray[9], Marica M Drazic[10], Mariano Higes[11], Lassi Kauko[12], Aykut Kence[13], Meral Kence[13], Nicola Kezic[14], Hrisula Kiprijanovska[2], Jasna Kralj[15], Preben Kristiansen[16], Raquel Martin Hernandez[11,17], Franco Mutinelli[18], Bach Kim Nguyen[19], Christoph Otten[20], Asli Özkırım[21], Stephen F Pernal[22], Magnus Peterson[9,23], Gavin Ramsay[23, 29], Violeta Santrac[24], Victoria Soroker[25], Grażyna Topolska[8], Aleksandar Uzunov[2], Flemming Vejsnæs[26], Shi Wei[27], Selwyn Wilkins[28]

[1]Netherlands Centre for Bee Research, Durk Dijkstrastr. 10, 9014 CC, Tersoal, Netherlands.
[2]Faculty for Agricultural Science and Food, bul. Aleksandar Makedonski b.b., 1000 Skopje, Republic of Macedonia.
[3]Department of Zoology, Karl-Franzens University Graz, Universitätsplatz 2, A-8010 Graz, Austria.
[4]Swiss Bee Research Centre, Agroscope Liebefeld-Posieux Research Station ALP, CH-3003 Bern, Switzerland.
[5]Slovak University of Agriculture, Department of Poultry Science and Small Husbandry, Tr. A. Hlinku 2, 94976 Nitra, Slovakia.
[6]University of Limerick, Department of Life Sciences, Limerick, Ireland.
[7]Norwegian Beekeepers Association, Dyrskuev. 20 NO-2040 Kløfta, Norway.
[8]Warsaw University of Life Sciences, Faculty of Veterinary Medicine, Ciszewskiego 8, 02-786 Warsaw, Poland.
[9]University of Strathclyde, Department of Mathematics and Statistics, Glasgow, G1 1XH, UK.
[10]Croatian Agricultural Agency, ILICA 101, 10 000 Zagreb, Croatia.
[11]Centro Apícola Regional, Camino de San Martín s/n, 19180 Marchamalo, Spain.
[12]Finnish Beekeepers Association, Kasarmikatu 26C34, 00130 Helsinki, Finland.
[13]Middle East Technical University, Department of Biology, 06800, Ankara, Turkey.
[14]University of Zagreb, Svetosimunska 25, 10000 Zagreb, Croatia.
[15]National Institute of Biology, Vecna pot 111, 1000 Ljubljana, Slovenia.
[16]Swedish Beekeepers Association, Trumpetarev 5, SE-59019 Mantorp, Sweden.
[17]Instituto de Recursos Humanos para la Ciencia y Tecnología, Parque Científico de Albacete, Spain.
[18]Istituto Zooprofilattico Sperimentale delle Venezie, National Reference Laboratory for Beekeeping, Viale dell'Universita' 10, 35020 Legnaro (PD), Italy.
[19]University of Liege, Gembloux Agro-Bio Tech, Department of Functional and Evolutionary Entomology, B-5030 Gembloux, Belgium.
[20]Dienstleistungszentrum Ländlicher Raum Westerwald-Osteifel, Fachzentrum Bienen und Imkerei, Im Bannen 38-54, 56727 Mayen, Germany.
[21]Hacettepe University, Biological Department, Elvankent A-34 D. 42, 06790 Etimesgut, Ankara, Turkey.
[22]Agriculture and Agri-Food Canada, P.O. Box 29, Beaverlodge, Alberta, T0H 0C0, Canada.
[23]Scottish Beekeepers Association, 20, Lennox Road, Edinburgh, EH5 3JW, UK.
[24]Veterinary Institute RS, Branka Radicevica 18, 78000 Banja Luka, Bosnia and Herzegovina.
[25]Agricultural Research Organization The Volcani Center, 50250 PO Box 6, Bet Dagan, Israel.
[26]Dänish Beekeepers Association, Fulbyvej, DK-4140 Sorø, Denmark.
[27]Bee Institute of the Chinese Academy of Agricultural Sciences, Xiang Shan, 100093 Beijing, China.
[28]National Bee Unit, Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ, UK.
[29]The James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK.

Received 4 August 2011, accepted subject to revision 26 September 2011, accepted for publication 2 December 2011.

*Corresponding author: Email: romee.van.der.zee@beemonitoring.org

Summary

In 2008 the COLOSS network was formed by honey bee experts from Europe and the USA. The primary objectives set by this scientific network were to explain and to prevent large scale losses of honey bee (*Apis mellifera)* colonies. In June 2008 COLOSS obtained four years support from the European Union from COST and was designated as COST Action FA0803 – COLOSS (Prevention of honey bee COlony

LOSSes). To enable the comparison of loss data between participating countries, a standardized COLOSS questionnaire was developed. Using this questionnaire information on honey bee losses has been collected over two years. Survey data presented in this study were gathered in 2009 from 12 countries and in 2010 from 24 countries. Mean honey bee losses in Europe varied widely, between 7-22% over the 2008-9 winter and between 7-30% over the 2009-10 winter. An important finding is that for all countries which participated in 2008-9, winter losses in 2009-10 were found to be substantially higher. In 2009-10, winter losses in South East Europe were at such a low level that the factors causing the losses in other parts of Europe were absent, or at a level which did not affect colony survival. The five provinces of China, which were included in 2009-10, showed very low mean (4%) *A. mellifera* winter losses. In six Canadian provinces, mean winter losses in 2010 varied between 16-25%, losses in Nova Scotia (40%) being exceptionally high. In most countries and in both monitoring years, hobbyist beekeepers (1-50 colonies) experienced higher losses than practitioners with intermediate beekeeping operations (51-500 colonies). This relationship between scale of beekeeping and extent of losses effect was also observed in 2009-10, but was less pronounced. In Belgium, Italy, the Netherlands and Poland, 2008-9 mean winter losses for beekeepers who reported 'disappeared' colonies were significantly higher compared to mean winter losses of beekeepers who did not report 'disappeared' colonies. Mean 2008-9 winter losses for those beekeepers in the Netherlands who reported symptoms similar to "Colony Collapse Disorder" (CCD), namely: 1. no dead bees in or surrounding the hive while; 2. capped brood was present, were significantly higher than mean winter losses for those beekeepers who reported 'disappeared' colonies without the presence of capped brood in the empty hives. In the winter of 2009-10 in the majority of participating countries, beekeepers who reported 'disappeared' colonies experienced higher winter losses compared with beekeepers, who experienced winter losses but did not report 'disappeared' colonies.

Pérdida de colonias manejadas de abejas en Canadá, China, Europa, Israel y Turquía, durante el invierno de los años 2008-9 y 2009-10

Resumen

En 2008, expertos de Europa y EEUU formaron una red, llamada COLOSS, con el objetivo de explicar y prevenir la pérdida de colonias de abejas a gran escala. Esta acción se designó en Junio del 2008 por la Unión Europea como acción COST FA0803. Se desarrolló un cuestionario estandarizado COLOSS que permitiera la comparación de los datos de pérdida entre los países participantes. Los datos presentados en este estudio fueron recogidos en 2009 en 12 países y en 2010 en 24. Las pérdidas invernales medias de colonias de abejas en Europa mostraron una gran variación del 7 al 22% en el invierno de 2008-9 y del 7 al 30% en 2009-10. Una observación importante es que para todos los países participantes en 2009, las pérdidas invernales observadas en 2010 fueron sustancialmente mayores que en 2009. En 2010 las perdidas invernales en el sureste de Europa fueron de un nivel tan bajo que parece que los factores causantes de las pérdidas en otras partes de Europa estuvieron ausentes o no a un nivel que afecta a la supervivencia de la colonia. Las cinco provincias de China, que fueron incluidas en 2010, mostraron una media muy baja (4%) de pérdidas invernales de *Apis mellifera*. Seis provincias de Canadá mostraron una variación de 16-25% de media de pérdidas invernales en 2010 con excepción de Nueva Escocia (40%). En la mayoría de los países y en ambos años de monitorización, los apicultores hobbistas (con 1-50 colonias) experimentaron mayores pérdidas en comparación con apicultores medianos (51-500 colonias). En 2010 se observó también este efecto, pero menos pronunciado. En Bélgica, Italia, Holanda y Polonia las pérdidas invernales medias en 2008-9 de apicultores que informaron de desaparición de colonias fue significativamente mayor en comparación con las pérdidas invernales medias de apicultores que no informaron de desaparición de colonias. Pérdidas invernales medias en 2008-9 en Holanda de apicultores que informaron de síntomas de "CCD" de: 1. ninguna abeja muerta en la colonia mientras; 2. había cría operculada presente fueron mayores que las pérdidas invernales medias de apicultores que informaron sobre desaparición de colonias sin cría operculada presente en colmenas vacías. En el invierno de 2009-10 apicultores que informaron de desaparición de colonias experimentaron mayores pérdidas invernales en comparación con apicultores con pérdidas invernales pero que no informaron de desaparición de colonias en la mayoría de los países.

Keywords: *Apis mellifera*, colony loss presentation, CCD, CDS, COLOSS, colony losses, epidemiology, honey bee, operation size, survey mode

Introduction

In the last decade, elevated losses of western honey bee (*Apis mellifera*) colonies have been observed, mainly in Europe and North America, but their underlying causes still remain unclear (Aston, 2010; Brodschneider *et al.*, 2010; Charrière and Neumann, 2010; Currie *et al.*, 2010; Dahle, 2010; Ellis *et al.*, 2010; Gajger *et al.,* 2010; Giray et *al.*, 2010; Gray *et al.*, 2010; Hatjina *et al.*, 2010; Ivanova and Petrov, 2010; Mutinelli *et al.*, 2010; Neumann and Carreck, 2010; Topolska *et al.*, 2010; vanEngelsdorp *et al.*, 2009, 2010, 2011; Van der Zee, 2010; Vejsnæs *et al.*, 2010).

In 2008, European and USA honey bee experts formed a network, realizing that efforts by individual countries to identify the drivers of losses were unlikely to succeed, given the current consensus that causes are not only multi-factorial, but also interact with each other, further adding to the degree of their complexity (Potts *et al.*, 2010). This concerted action called "Prevention of honey bee COlony LOSSes" (COLOSS) was designated in June 2008 as COST action FA0803 by the European Union (European Cooperation in the field of Scientific and Technical Research – COST, 2008). The main objective of the action is to explain and to prevent large scale losses of honey bee colonies by the identification of the underlying causal factors and the development of emergency measures and sustainable management strategies. The COLOSS network currently comprises 268 experts and is no longer limited to Europe but has developed into a global network, with a growing number of countries from Asia, Oceania, North America and Africa adopting the objectives of COLOSS, and at July 2011 consisted of 55 countries.

The epidemiological Working Group 1 (WG1) of the COLOSS network aims to: 1. develop standardized questionnaires primarily to enable the comparison of representative annual colony loss data and possible causative factors between countries and over time; 2. organize a network which will implement the tools which are developed by the network; 3. provide a database for the collected data and; 4. enable analysis and dissemination of results. The protocols used to design and complete the questionnaires in 2009 and 2010 are presented here. A discussion of appropriate statistical methods to present colony losses is also described. To allow appropriate standardization, a case definition at colony level is given for losses with "Colony Depopulation Syndrome". Furthermore results of the analysis of the standardized questionnaire received in 2009 (9,881 beekeepers) and 2010 (14,958 beekeepers) are presented and discussed.

Materials and methods

Question design COLOSS Questionnaire 2009

The strategy implemented by the COLOSS network is based on the development of a detailed self-administered questionnaire standardised at the European level and beyond. The question design was discussed at international meetings of WG1. One of the main issues during development of the case definitions was the timeframe during which colony losses would be counted. Colony losses during winter can be objectively recorded with relative ease, but time, length and temperatures of winter vary often between and within countries. For the purpose of appropriate standardization, when designing the 2009 questionnaire, it was decided not to state a fixed timeframe for the winter, but to leave the definition of winter to the discretion of the beekeeper. In the introduction of the questionnaire, beekeepers were asked to consider their colonies to be 'wintered' once pre-winter preparations were finished. For many beekeepers this would be the completion of feeding. Some beekeepers winter small nuclei for the purpose of having young or reserve queens available or using the nuclei for merging with weak colonies in spring. Since the questionnaire was designed to look at production colonies, namely colonies which could be used for honey production or pollination services in 2009, the beekeeper was instructed not to include the numbers of small nuclei when responding to the questionnaire.

In the previous years, many lost colonies have been reported as having disappeared with no, or only a few, remaining living bees, a phenomenon referred to in the current study as "Colony Depopulation Syndrome" (CDS). In the USA, a proportion of dead and dying colonies was characterized by a more extensive set of symptoms including the presence of brood in hives of disappeared colonies, coupled with a noticeable lack of dead worker bees both within and surrounding the hive, indicating that the colony demise had occurred rapidly. This syndrome was termed "Colony Collapse Disorder" (CCD) (vanEngelsdorp *et al.*, 2009). Two questions were therefore added to the 2009 COLOSS questionnaire to obtain information about losses with CDS symptoms and CDS losses where brood was observed within the empty hive. The presence of brood could point to a sudden collapse, one of the main characteristics of CCD.

The final COLOSS 2009 questionnaire included the questions on winter losses shown in Box 1.

Box 1.

1. In the following question you are asked, among other things, to give the total number of colonies lost during last winter. Please include the number of colonies that were lost shortly after wintering. What is the total number of production colonies on all your apiaries that were:

   (a) wintered last year?

   (b) lost during last winter?

2. How many of the colonies that were lost during winter, <u>disappeared</u> with none or only a few living bees remaining, while enough food supply was present?

3. In how many hives of the <u>disappeared</u> colonies did you observe patches of capped brood?

Question design COLOSS Questionnaire 2010

Discussions during the development of the 2010 questionnaire revealed that the approach of leaving the beekeeper to define the timeframe of winter was not suitable for the USA, due to the large scale migration of colonies for almond pollination in California during winter. It was also inappropriate for countries such as Israel, Turkey and Spain, where there are areas in which winter is either short or absent. To tackle this problem a fixed timeframe was introduced into the 2010 questionnaire, with the aim of measuring the number of colonies on 1 October 2009 and 1 April 2010, and to ask for numbers of colony increases and decreases during this period. With these figures, losses were to be calculated for the total population at risk of being lost. This approach introduced a shift from the preceding 2009 questionnaire: the reported losses during winter 2008-9 included the number of colonies from the October cohort that had died. However, for the winter 2009-10 no question was included on colonies that died out, but questions on the operation size on 1 October 2009 and 1 April 2010, as well as colony decreases and increases during the chosen timeframe were the essential elements used to calculate total colony decrease at 1 April 2010. No distinction would be made between lost colonies caused by health problems or apparent losses resulting from uniting healthy colonies. The question on CDS losses included in the 2009 questionnaire was also included in the 2010 questionnaire. The essential questions of the 2010 questionnaire are shown in Box 2.

Box 2.

1. How many production colonies did you have at 1 October 2009?
2. How many production colonies did you have at 1 April 2010?
3. How many splits or increases did you make / buy between 1 October 2009 and 1 April 2010?
4. How many of your colonies / splits did you sell or remove from your operation in this period?
5. How many of your colonies that died between 1 October and 1 April, were lost without dead bees in the hive nor in the apiary?

Participants, survey modes and coverage

The 2009 COLOSS questionnaire was adapted and distributed in 12 countries. For the purposes of this study, the combined results for 2009 from Scotland, Northern Ireland, Wales and England are reported as the United Kingdom. In 2010, 24 countries participated. The German questionnaire was also answered by beekeepers from surrounding countries. Belgian (Flemish) beekeepers responded mainly to the Dutch questionnaire. The questionnaire data received were added to the international dataset according to the country of residence. For this reason, results of earlier national publications may show differences from the outcome of the present study.

National surveyors were asked to address the total beekeeper population by publishing the questionnaire in, or circulating it with, national beekeeping journals. This would give as many beekeepers as possible the opportunity to be included in the survey. Where addressing the total population would not be possible, mixed modes of data collection (telephone, meetings, internet, email) were advised in order to counterbalance the possible disadvantages of one method with the advantages of others (de Leeuw *et al.*, 2008). Circumstances such as national funding and opportunities such as the accessible infrastructure at the beekeeper level in countries dictated which mode(s) was / were appropriate and achievable in each case.

A randomised sampling approach was considered, but for the purposes of reliable statistical analysis, it is necessary that all key segments are represented in the sample population. The variability in operation size, bee race, Varroa treatment, environmental conditions, and focus on pollination or honey production between operations within and between participating countries, is considerable and needed to be taken into account in a randomised approach to avoid coverage errors. This could have been obtained by a stratified multistage sampling design, but the information necessary for forming the strata and setting the selection probabilities was in general not available at the onset of the project, and the sample size would also have had to be prohibitively large. These considerations prevented COLOSS WG1 from adopting randomied sampling as a general guideline at present. The survey modes (Table 1) did not differ within individual countries in the two monitoring years reported here.

Calculations and statistical analysis

The mean colony loss rate was calculated as the mean number of dead colonies per beekeeper, divided by the mean number of colonies alive before winter. The resulting fraction was multiplied by 100 to give a percentage.

For both monitoring years, mean colony losses during winter were estimated with a generalized linear model using a negative binomial distribution with a log link function (SPSS 18). This model structure was chosen to limit the effect of overdispersion on standard errors and 95% confidence intervals (White and Bennetts, 1996; Brown *et al.*, 2002; Affleck, 2006). The number of colonies lost during winter was used as the dependent variable, and the number of colonies present before winter as the covariate. Estimated means of the dependent variable and the covariate, and the corresponding 95% confidence intervals were derived from the intercept-only (null) model. Confidence intervals for the dependent variable were scaled by the model covariate and multiplied by 100. As the distributional characteristics of the loss data could invalidate hypothesis testing based on a difference in means, conclusions relating to differences between groups were based on the estimation of the 95% confidence intervals (Gardner and Altmann, 1986).

Table 1. Survey modes used in the participating countries

	Data Collection							
Country	**Internet**	**Journal**	**Email**	**Meetings**	**Fax**	**Visit**	**Phone**	**Mail**
Austria		x	x	x	x			x
Belgium	x		x			x		x
Bosnia & Herzegovina		x		x				
Canada							x	x
P. R. China				x		x		
Croatia				x				
Denmark	x							
Finland							x	
Germany	x	x	x					x
Ireland			x	x				x
Israel			x	x			x	x
Italy				x			x	
Netherlands	x	x	x					x
Norway	x							
Poland	x	x	x	x				x
Rep. Macedonia				x				
Slovakia				x				
Slovenia				x				
Spain			x	x				
Sweden	x							
Switzerland	x	x	x					
Turkey		x		x		x		
UK (Scotland)								x
UK (England, Wales, N. Ireland)	x	x		x				x

For the 2009 dataset the number of colonies lost per operation during winter (Q1b) was set as the dependent variable, with the number of colonies alive in October as the covariate. For the 2010 dataset the number of colonies at risk of being lost per operation was calculated as: (the number of colonies at 1 October 2009 (Q1)) + (the number of colonies added between 1 October 2009 and 1 April 2010 (Q3)) - (the number of colonies removed between 1 October 2009 to 1 April 2010 (Q4)). This was set as covariate. The number of colonies lost per individual operation during the given period was calculated as: (the number of colonies at risk of being lost) – (the number of colonies present at 1 April 2010 (Q2)). This calculated number of colonies lost per individual operation was set as the dependent variable.

To compare possible differences in colony losses between different sizes of operation, operations were stratified into three groups, namely hobbyist beekeepers (1-50 colonies), intermediate beekeepers (51-500) and commercial beekeepers (>500), respectively. Mean colony losses during winter are reported per country, by operation size class per country (with a minimum of 10 operations in that size class) and for the total available dataset. For the determination of associations between the overwinter mortality and the observed presence or absence of CDS losses, or CDS losses with or without brood in the empty hive, the same GzLM was used. Presence or absence of CDS, CDS with brood or no brood on operations with colony losses, were added as categorical variables.

Results

Losses per country and by operation size 2008-9

In total 9,881 European beekeepers responded to the 2009 COLOSS questionnaire. Beekeepers who failed to provide the essential information for the mortality calculation (N = 407) or reported losses higher than 100% (N = 3) were excluded. The analysis was performed using data from 9,471 operations with a total of 172,252 colonies (Table 2).

The estimated 95% confidence intervals allowed for a classification of the countries into two groups: 1. those with a low (<15%) mean colony loss were Austria, Switzerland, Germany, Poland, Denmark, Norway, and Sweden; 2. those with a higher mean

Table 2. Mean winter losses per country in 2008-2009; N. op. = Number of operations, N. col. Oct. = number of colonies alive at 1 October 2008.

Country	N. op.	N. col. Oct. sum	N. col. Oct. median (interquartile range)	Mean winter loss % (95% CI)
Austria	575	18,141	15 (8-35)	9.3 (7.8-10.7)
Belgium	225	2,546	9 (5-15)	18.0 (13.4-22.6)
Denmark	419	9,056	10 (5-21)	7.5 (5.7-9.3)
Germany	3,715	49,696	9 (5-15)	10.4 (9.6-11.2)
Ireland	29	276	5 (3-14)	21.7 (15.1-28.3)
Italy	263	22,214	22 (10-50)	6.3 (6.9-25.8)
Netherlands	1,193	10,678	4 (3-8)	21.7 (18.5-24.9)
Norway	395	13,008	16 (8-34)	7.1 (5.6-8.7)
Poland	346	15,901	30 (15-60)	11.5 (8.3-14.7)
Sweden	564	7,354	6 (3-12)	14.6 (12.0-17.3)
Switzerland	342	5,301	12 (7-20)	9.1 (7.5-10.8)
UK	1,405	18,081	4 (2-8)	16.0 (13.4-18.6)
Total data set	9,471	172,252	8 (4-16)	12.3 (10.9-13.7)

colony loss were Belgium, the Netherlands, the UK and Ireland. It was difficult to place Italy in one of these groups because of the strong variation in colony loss within this country, which is reflected in the wide 95% confidence interval.

The difference in mean colony loss between the operation size classes and in overwinter mortality for the size classes 1-50 colonies and 51-500 colonies, based on the 95% confidence interval, was significant for Austria, the UK and for the total data set (Table 3). No significant effects were found for the remaining individual countries, although an overall trend can be observed of intermediate beekeepers reporting lower losses than hobbyist beekeepers. Only 13 beekeepers (with a total of 13,120 colonies) had more than 500 colonies and experienced a mean winter loss percentage of 13.8 (CI, 0.9-28.6). This number of commercial operations was too small for a comparison with the other two size classes.

Losses per country and by operation size 2009-10

In total 14,958 beekeepers responded to the 2010 COLOSS questionnaire. Responses from beekeepers who did not provide the essential information for the mortality calculation or who provided illogical loss data (for example, who reported no increases or decreases during winter, but had more colonies in April 2010 than October 2009) were considered as invalid (N = 448). 244 beekeepers (with a total of 19,010 colonies) reported increases in their numbers of colonies during winter, which was contradictory to the given numbers of colonies in October 2009 and April 2010, so these were also excluded from the analysis. A further 1,803 beekeepers (with a total of 153,264 colonies) reported decreases in their numbers of colonies during winter, but these beekeepers may have included lost, weak, split or merged colonies (the responses on Q3 and 4) after 1 April 2010, which would bias the outcome of the loss calculation. Because of this uncertainty, WG1 subsequently chose to report colony losses of this group separately (Table 4).

The concern about this group of respondents can best be illustrated with the Canadian survey results. All Canadian respondents who reported increases or decreases during the defined wintering period were contacted by the national surveyor or the provincial apiculturist, to verify whether such changes truly reflected the dynamics of the wintering population. Invariably, these changes reflected spring-time activities of the beekeepers (typically splitting colonies), where these activities could occur in warmer areas of the country prior to the defined end date of the wintering period. Moreover, these changes were not reflected in total colony counts at the end of the wintering period. As a result of this evaluation, increases and decreases during winter were ignored for this subset of Canadian beekeepers, and these producers were added to the larger valid dataset. The final valid dataset included 12,463 operations with a total of 464,815 colonies (Table 5).

Mean overwinter losses per European country can be divided into three groups: 1. low colony losses in the Republic Macedonia, Croatia, Bosnia and Herzegovina, Slovakia, and Norway; 2. moderate losses in Turkey, Austria, Germany, Poland, Denmark, Northern Ireland and; 3. high losses in Ireland, Belgium, Netherlands, Switzerland and Slovenia. The confidence interval for the mean losses in the remaining countries (Finland, England and Wales, Italy, Scotland, Spain and Sweden) was too wide for an appropriate classification (Table 5). The observed mean overwinter colony losses for all countries which participated in 2009 were substantially higher in 2010.

Changing the level of aggregation of colony losses at higher than country resolution (Fig. 1) provides more detailed information about the spatial distribution. Only information at regional level was available. The administrative regional boundaries that correspond with the collected information differ in scale between the participating countries, thus complicating regional comparisons between countries. The variation in regional losses is substantial within all countries with losses higher than 10%. Between the Canadian provinces (British Columbia, Manitoba, New Brunswick, Prince Edward Island and Québec) losses varied between 16–25%, with the exception of Nova Scotia (40%). The Chinese provinces of Sichuan, Zhejiang, Shanxi, Gansu and Jilin present in this study had very low losses (<10%). The relation between operation size and overwintering mortality for the hobbyist and intermediate size classes (1-50 colonies and 51-500 colonies respectively), based on the 95% confidence interval, was significantly different for Austria, Bosnia and Herzegovina, England and Wales, and for the total set. No significant effects were found for the remaining individual countries, but for some countries a trend, but

Table 3. Mean winter losses 2008-2009 per country, per size class, N. op. = number of operations. N. col. Oct. = number of colonies alive at 1 October 2008.

Country	1-50 col.				51-500 col.			
	N. op.	N col. Oct. sum	N. col. Oct. median (interquartile range)	Mean winter loss % (95% CI)	N. op.	N col. Oct. sum	N. col. Oct. median (interquartile range)	Mean winter loss % (95% CI)
Austria	494	8,506	13 (7-25)	13.4 (11.1- 15.7)	80	9,085	95 (60-130)	5.4 (3.9- 6.9)
Belgium	224	2,494	9 (5-15)	17.7 (13.2 -22.3)	1	52		
Denmark	385	4,898	9 (4-16)	8.6 (7.2- 10.0)	34	4,158	94 (72-142)	6.2 (3.1- 9.3)
Germany	3,618	41,087	9 (5-15)	10.7 (9.9- 11.4)	97	8,609	66 (56-100)	9.3 (6.5- 12.0)
Ireland	29	276	5 (3-14)	21.7 (15.1- 28.3)	0			
Italy	207	4,022	16 (8-28)	22.6 (18.6- 26.7)	48	9,012	146 (82-290)	14.5 (7.6- 21.4)
Netherlands	1,167	7,744	4 (3-8)	23.2 (21.1- 25.5)	26	2,934	100 (62-128)	17.6 (9.4- 25.7)
Norway	331	5,546	12 (8-24)	8.8 (7.4- 10.1)	63	6,912	90 (62-144)	5.6 (3.4- 7.7)
Poland	252	5,728	20 (12-32)	13.5 (10.5- 16.4)	94	10,173	80 (70-111)	10.4 (5.8- 14.9)
Sweden	541	4,615	6 (3-11)	16.7 (14.3- 19.2)	22	2,179	97 (64-124)	11.7 (7.7- 15.6)
Switzerland	338	4,987	12 (7-20)	8.9 (7.5- 10.3)	4	314		
UK	1,350	8,818	4 (2-7)	20.8 (18.8- 22.8)	53	6,983	102 (66-150)	11.9 (8.9- 14.9)
Total data set	8,936	98,721	12 (8-24)	13.7 (13.0- 14.2)	522	60,411	86 (64-134)	9.8 (8.3- 11.4)

Table 4. Mean winter loss 2009-2010 for operations with increases and decreases during winter. N. op. = number of operations, Pop. at risk = number of colonies alive at 1 October 2009 + number of colonies added between 1 October 2009 and 1 April 2010 – number of colonies removed between 1 October 2009 and 1 April 2010.

Country	N. op.	Pop. at risk. sum	Pop. at risk median (interquartile range)	Mean winter loss % (95% CI)	% Total response / country
Austria	92	3,035	22 (9-40)	17.3 (12.2-22.4)	29.1
Belgium	36	928	13 (8-18)	32.7 (9.7-55.7)	14.2
Denmark	38	1,296	9 (4-22)	30.1 (13.6-46.6)	5.6
Germany	573	11,127	14 (8-22)	37.8 (33.1-42.6)	12.1
Ireland	67	1,470	10 (6-25)	26.5 (17.8-35.2)	14.9
Israel	40	35,187	513 (121-975)	11.2 (3.7-18.7)	81.6
Italy	58	4,907	33 (13-56)	27.4 (14.8-40.0)	32.0
Macedonia	6	387	63 (58-74)	12.9 (3.8-22.1)	4.7
Netherlands	207	3,205	7 (4-15)	27.8 (18.5-37.2)	13.3
Norway	13	487	25 (10-38)	15.8 (1.1-30.5)	8.2
Poland	68	3,628	32 (16-59)	29.4 (12.2-46.6)	19.2
Slovakia	14	551	31 (16-69)	8.0 (4.3-11.7)	8.8
Spain	117	41,039	175 (50-384)	18.9 (12.0-25.7)	48.0
Sweden	138	4,172	9 (5-22)	28.5 (17.9-39.1)	18.3
Switzerland	1				0.1
Turkey	181	38,096	165 (90-274)	25.8 (20.8-30.8)	27.0
UK	154	3,749	5 (3-15)	36.5 (8.6-64.4)	14.4

Table 5. Mean winter losses per country in 2009-2010. N. op. = number of operations, N. col. Oct. = number of colonies alive at 1 October 2009.

Country	N. op.	N. col. Oct. sum	N. col. Oct. median interquartile range)	Mean winter loss % (95% CI)
Austria	224	4,920	12 (6-28)	14.7 (11.2-18.3)
Belgium	210	2,282	8 (5-14)	26.0 (19.2-32.7)
Bosn. & Herzegov.	268	15,286	50 (22-78)	8.6 (6.9-10.3)
Canada	392	106,093	38 (6-180)	23.8 (11.7-36.0)
China	127	13,439	72 (56-135)	4.2 (2.7-5.6)
Croatia	907	90,388	80 (50-120)	7.4 (6.5-8.3)
Denmark	618	11,433	8 (4-16)	15.1 (11.5-18.7)
England/Wales	564	14,580	4 (2-10)	17.5 (9.3-25.6)
Finland	40	4,069	45 (13-118)	19.6 (7.5-31.6)
Germany	4,032	55,560	9 (5-15)	18.3 (17.1-19.4)
Ireland	381	3,527	4 (2-10)	22.4 (17.0-27.8)
Italy	113	3,560	16 (8-30)	29.8 (12.7-47.0)
Netherlands	1,315	11,107	5 (3-8)	29.3 (22.8-35.7)
Northern Ireland	99	435	2 (1-7)	14.1 (8.9-19.4)
Norway	146	5,817	17 (9-38)	8.8 (6.5-11.1)
Poland	281	12,145	30 (15-56)	15.3 (12.0-18.7)
Rep. Macedonia	118	6,642	41 (29-72)	6.8 (4.9-8.6)
Scotland	111	4,233	3 (2-7)	25.5 (0.5-50.4)
Slovakia	146	4,643	20 (14-36)	7.4 (5.0-9.8)
Slovenia	505	15,158	21 (12-37)	21.1 (17.2-24.9)
Spain	119	25,935	84 (22-320)	19.2 (10.1-28.3)
Sweden	600	9,349	6 (3-12)	27.5 (14.9-40.1)
Switzerland	914	14,285	12 (7-20)	20.0 (17.7-22.2)
Turkey	233	29,929	97 (55 158)	17.4 (13.7-21.10)
Total data set	12,463	464,815	10 (5-28)	16.9 (14.0-19.8)

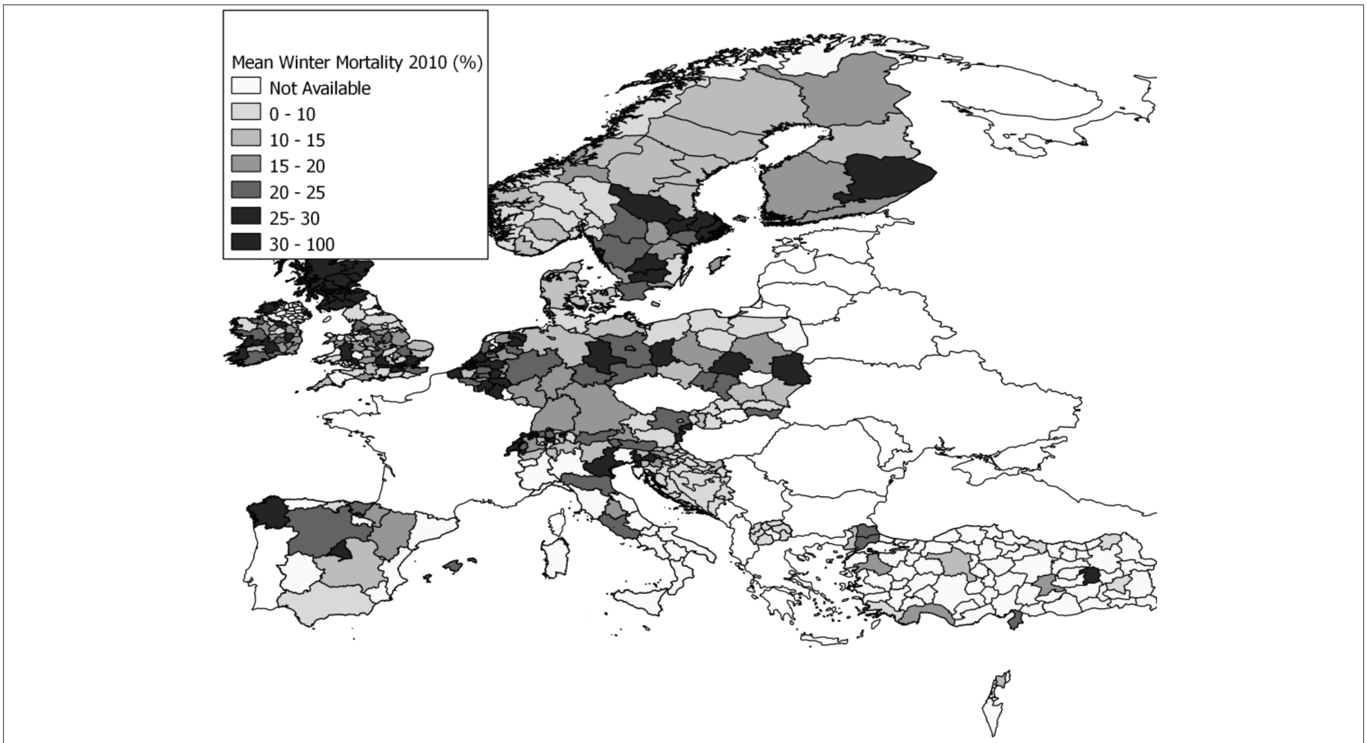

Fig. 1. Mean winter mortality 2009-10 in Europe, Turkey and Israel.

Table 6. Mean winter loss 2009-2010 per country, per size class. N. op. = number of operations, N. col. Oct. = number of colonies alive at 1 October 2009.

	1-50 col.				**51-500 col.**				**500 + col.**			
Country	**N. op.**	**N col. Oct. sum**	**N. col. Oct. median (inter-quartile range)**	**Mean winter loss % (95% CI)**	**N. op.**	**N col. Oct. sum**	**N. col. Oct. median (inter-quartile range)**	**Mean winter loss % (95% CI)**	**N. op.**	**N col. Oct. sum**	**N. col. Oct. median (inter-quartile range)**	**Mean winter loss % (95% CI)**
Austria	211	3,396	11 (6-23)	17.9 (14.3-21.4)	13	1,524	73 (65-139)	7.7 (2.7-12.6)	0			
Belgium	209	2,195	8 (5-14)	25.3 (19.3-31.3)	1	87			0			
Bos. & Herz.	137	3,409	22 (12-37)	12.4 (9.2-15.7)	131	11,877	78 (58-106)	7.5 (5.8-9.2)	0			
Canada	217	3,003	7 (3-20)	22.6 (17.6-27.7)	121	21,248	141 (80-225)	23.3 (18.7-31.8)	54	8,1842	853 (650-494)	23.5 (8.6-38.4)
P. R. China	27	910	32 (28-50)	6.8 (2.4-11.2)	99	11,929	80 (65-160)	4.1 (2.4-5.9)	1	600		
Croatia	235	9,214	40 (34-46)	7.8 (5.7-9.9)	667	77,170	96 (71-136)	7.6 (6.5-8.8)	5	4,004		
Denmark	574	5,981	7 (4-13)	16.0 (14.0-17.9)	44	5,452	96 (66-129)	14.1 (9.7-18.5)	0			
England/ Wales	513	3,475	3 (2-7)	22.7 (18.2-27.3)	48	7,259	110 (79-185)	13.8 (9.8-17.9)	3	3846		
Finland	21	430	14 (6-34)	22.3 (10.6-34.1)	17	2,264	112 (96-145)	17.4 (12.0-22.7)	2	1,375		
Germany	3,914	43,784	8 (5-15)	18.8 (18.0-19.7)	117	11,116	80 (61-101)	16.9 (13.9-19.9)	1	660		
Ireland	370	2,555	4 (2-9)	22.9 (19.5-26.2)	11	972	73 (59-120)	21.0 (11.1-30.9)	0			
Italy	103	1,790	14 (8-25)	27.8 (21.9-33.8)	10	1,770	96 (60-283)	31.8 (11.6-52.1)	1	600		
Nether-lands	1,298	8,602	5 (3-8)	24.9 (22.4-27.3)	16	1,980	82 (60-173)	48.5 (25.7-71.3)	1	525		
Northern Ireland	99	435	2 (1-7)	14.1 (8.9-19.4)	0				0			
Norway	118	1,976	14 (7-24)	12.5 (8.0-15.5)	28	3,841	104 (75-211)	7.2 (4.8-9.5)	0			
Poland	207	4,782	22 (11-33)	17.2 (13.6-20.7)	74	7,363	80 (69-100)	14.2 (9.5-18.9)	0			
R. Macedonia	73	2,189	32 (20-40)	8.9 (6.2-11.7)	45	4,453	83 (65-107)	5.7 (3.4-8.0)	0			
Scotland	104	541	3 (1-6)	31.2 (15.6-46.5)	5	990			2	2,702		
Slovakia	126	2,619	18 (12-26)	8.1 (6.6-9.6)	20	2,024	75 (60-98)	6.5 (1.4-11.6)	0			
Slovenia	439	9,028	20 (10-30)	21.8 (18.7-25.0)	66	6,130	75 (60-90)	19.9 (12.1-27.7)	0			
Spain	46	845	15 (7-30)	25.7 (14.8- 36.6)	56	11,648	182 (85-311)	22.0 (15.4-28.5)	17	13,442	650 (564-825)	16.4 (3.5 -29.3)
Sweden	563	4,623	5 (3-10)	24.9 (21.3-27.4)	35	3,506	80 (60-123)	22.7 (16.2-29.2)	2	1,220		
Switzer-land	891	12,728	12 (7-19)	20.3 (18.4-22.2)	23	1,557	64 (55-70)	17.1 (6.7-22.5)	0			
Turkey	54	1,933	39 (28-46)	20.9 (13.9-27.9)	177	26,646	120 (82-190)	16.2 (13.0-19.4)	2	1,350		
Total Set	10,549	130,443	9 (4-17)	18.4 (17.7-19.0)	1,824	222,806	91 (70-140)	12.6 (11.6-13.7)	90	111,566	780 (600-120)	21.9 (10.8-33.1)

Table 7. Operations with CDS losses compared with operations with losses but without CDS characteristics for the winters 2008-2009 and 2009-2010 per country and per size class. N op. = number of operations.

			2008-2009			2009-2010		
Country	**Size class**	**Loss type**	**N. op.**	**Mean winter loss % (95% CI)**	**P-value**	**N. op.**	**Mean winter loss % (95% CI)**	**P-value**
Austria	1-50	Non CDS				53	24.0 (14.8-33.2)	
	1-50	CDS				83	24.7 (17.2-32.3)	0.903
Belgium	1-50	Non CDS	37	24.9 (15.5-34.7)		47	20.8 (14.0-27.6)	
	1-50	CDS	44	39.2 (26.7-51.7)	0.071	64	52.7 (39.9-65.6)	<0.001
Canada	1-50	Non CDS				29	22.6 (12.3-32.9)	
	1-50	CDS				9	30.9 (13.4-38.4)	0,222
	51-500	Non CDS				46	20.3 (14.1-26.5)	
	51-500	CDS				23	47.1 (27.0-67.3)	0.020
	> 500	Non CDS				25	15.5 (5.5-26.5)	
	> 500	CDS				15	47.5 (8.1-86.9)	0.036
Switzerland	1-50	Non CDS				177	19.2 (16.1-22.3)	
	1-50	CDS				396	32.0 (28.9-35.3)	<0.001
Germany	1-50	Non CDS				991	18.7 (17.4-20.0)	
	1-50	CDS				1237	32.7 (30.8-34.5)	<0.001
	51-500	Non CDS				33	8.6 (5.9-11.4)	
	51-500	CDS				74	18.7 (14.9-22.6)	<0.001
Denmark	1-50	Non CDS				203	19.9 (16.9-22.9)	
	1-50	CDS				112	27.8 (22.6-33.0)	0.006
	51-500	Non CDS				23	8.4 (4.5-12.3)	
	51-500	CDS				19	17.7 (9.0-26.5)	0.031
Ireland	1-50	Non CDS				130	27.9 (22.3-33.4)	
	1-50	CDS				83	29.5 (22.2-36.6)	0.732
Italy	1-50	Non CDS	20	18.5 (10.1-26.9)				
	1-50	CDS	111	29.4 (24.0-34.7)	0.068			
Netherlands	1-50	Non CDS	199	23.4 (20.0-26.7)		293	31.6 (28.3-34.0)	
	1-50	CDS	435	39.5 (36.2-43.1)	<0.001	398	40.7 (36.2-45.2)	0.002
Norway	1-50	Non CDS				50	17.4 (12.0-22.7)	
	1-50	CDS				23	15.1 (8.1-22.2)	0.632
Poland	1-50	Non CDS	44	11.7 (7.5-16.0)		64	18.9 (13.4-24.3)	
	1-50	CDS	106	24.9 (19.7-30.2)	<0.001	81	25.3 (19.0-31.6)	0.134
	51-500	Non CDS	22	3.7 (1.1-6.2)		23	16.1 (8.2-24.0)	
	51-500	CDS	53	16.1 (9.5-22.7)	<0.001	34	18.4 (11.0-25.8)	0.684
Slovenia	1-50	Non CDS				115	17.0 (13.1-20.8)	
	1-50	CDS				137	30.7 (24.6-36.7)	<0.001
	51-500	Non CDS				13	8.0 (2.5-13.6)	
	51-500	CDS				33	31.2 (18.3-44.2)	0.016
Slovakia	1-50	Non CDS				59	10.5 (8.8-12.2)	
	1-50	CDS				25	10.9 (8.3-13.6)	0.773
Spain	51-500	Non CDS				10	12.1 (4.8-19.4)	
	51-500	CDS				36	28.1 (19.3-36.9)	0.016
Sweden	1-50	Non CDS				293	28.3 (25.0-31.5)	
	1-50	CDS				68	40.8 (31.6-49.8)	0.004

Table 8. Mean winter loss 2008-2009 compared between operations with CDS losses with brood observed in empty hives and operations with losses but without CDS characteristics, per country and size class.

Country	Size class	Loss type	N. op.	Mean winter loss % (95% CI)	P-value
Belgium	1-50 col	Non-CDS	37	25.9 (16.4-35.5)	
	1-50 col	CDS-brood	20	42.2 (23.0-63.4)	0.094
Nether-lands	1-50 col	Non-CDS	199	23.4 (20.0-26.7)	
	1-50 col	CDS-brood	153	46.2 (40.1-52.2)	<0.001
Poland	1-50 col	Non-CDS	44	12.0 (7.9-16.1)	
	1-50 col	CDS-brood	59	28.8 (21.4-36.3)	<0.001
	51-500 col	Non-CDS	22	3.7 (1.1-6.3)	
	51-500 col	CDS-brood	25	21.4 (9.1-33.7)	<0.001
Italy	1-50 col	Non-CDS	20	17.0 (9.5-24.5)	
	1-50 col	CDS-brood	78	29.9 (23.6-36.0)	0.024

Table 9. Mean colony losses 2008-2009 for beekeepers who reported CDS losses with brood present compared with operational losses with CDS characteristics but without brood present per country, per size class. N. op. = number of operations.

mean colony losses 2008-2009 CDS with brood present and CDS with no brood present					
Country	**N. col**	**Loss type**	**N. op.**	**Mean winter loss % (95 % CI)**	**P-value**
Belgium	1-50 col	CDS-brood	20	37.7 (19.9-55.6)	
	1-50 col	CDS-no brood	24	33.5 (18.8-48.1)	0.713
Nether-lands	1-50 col	CDS-brood	153	51.5 (44.0-59.0)	
	1-50 col	CDS-no brood	256	34.7 (30.4-38.9)	<0.001
Poland	1-50 col	CDS-brood	59	28.7 (21.3-36.2)	
	1-50 col	CDS-no brood	39	20.7 (13.4-27.9)	0.115
	51-500 col	CDS-brood	25	20.5 (13.7-27.3)	
	51-500 col	CDS-no brood	18	11.6 (3.1-20.9)	0.339
Italy	1-50 col	CDS-brood	78	32.4 (25.6-39.2)	
	1-50 col	CDS-no brood	33	22.2 (14.7-29.7)	0.069

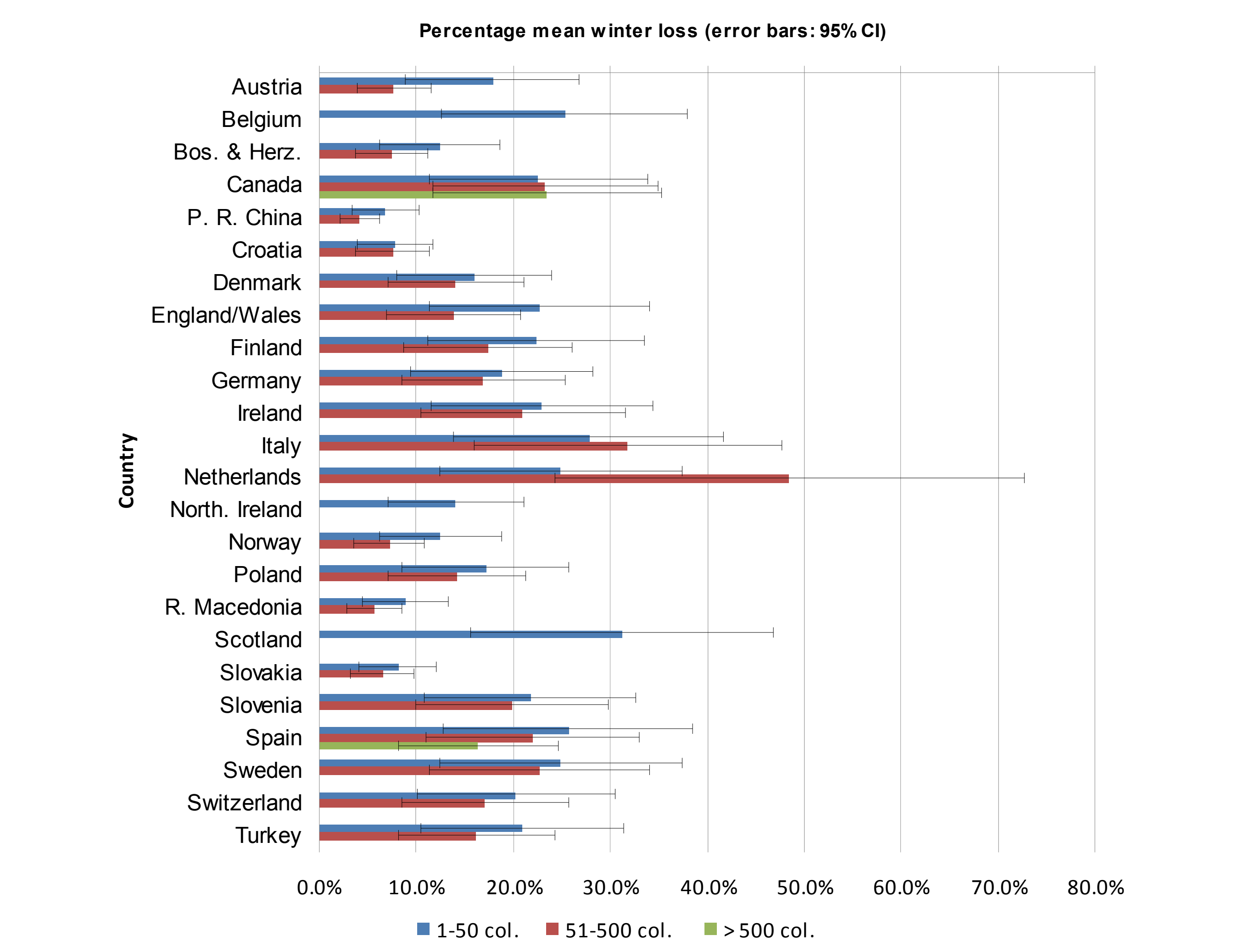

Fig. 2. Mean winter loss 2009-2010 per country per size class

less pronounced compared to losses in 2009, can be observed for intermediate beekeepers reporting lower losses than hobbyist beekeepers (Table 6; Fig 2.).

Disappeared colonies

The optional question 2 (Q2) regarding disappeared colonies was included in the 2009 questionnaires used in Belgium, Poland, Italy and the Netherlands. Beekeepers who answered Q2, but not question 3 on the presence of brood (for the Netherlands 26 beekeepers, Poland 18) were excluded. Colony losses in operations reporting CDS symptoms were higher compared with losses in operations where colonies were lost with no symptoms of CDS. For Poland and the Netherlands this effect was significant (Table 7). A limited number of beekeepers (N = 335) in Belgium, Poland, Italy and the Netherlands reported the presence of brood in the empty hives of disappeared colonies. Where brood was present, losses for this group of beekeepers were higher compared with operations where colonies were lost without CDS symptoms and with no brood present in the hives. For Poland, Italy and the Netherlands this effect was significant (Table 8).

We found no significant difference between beekeepers reporting CDS losses with no brood present in the empty hive and operations reporting CDS losses with brood present in Italy, Poland and Belgium. This is not unexpected, given the small datasets for these countries. In the Netherlands, where a more substantial dataset was available, the difference was significant. Losses in operations with CDS and brood present were at a significantly higher level (Table 9). In 2010, in 12 out of 15 countries, significantly higher losses in operations with CDS losses were observed compared with operations with losses but no CDS symptoms. The exceptions were Austria, Ireland, Norway and Poland (Table 7).

Discussion

The development and analysis of the 2009 and 2010 COLOSS questionnaires demonstrate not only the complexity of questionnaire design, but also the complications associated with collating comparative multinational and multicultural data for the purposes of research. This was apparent, for example, in the difficulties experienced when defining the timeframe and concept of winter, and in the exploration of robust statistics to be used for the presentation of colony losses. In recent studies, Total (colony) Losses (TL) are presented and used to determine average operational losses (COLOSS, 2009; Hendrikx *et al.*, 2009; Kluser *et al.*, 2010; Nguyen *et al.*, 2010; vanEngelsdorp *et al.,* 2008, 2011). Comparison of TL was achieved using the Chi-square test with a Bonferroni correction for multiple comparisons (Nguyen *et al.*, 2010). In these studies, TL was calculated as the summarized number of colonies lost, divided by the summarized number of colonies present in October for a defined group of operations. The TL does not account for the influences of the distributional characteristics of the loss data on the standard error and 95% confidence intervals. The magnitude of TL can be heavily influenced by large operations if variation in operation size is large. Larger operations may be subject to other factors which influence losses compared to smaller operations, e.g. migration of bees or different management practices, and also for these a greater range of loss rates is possible.

The point estimate of the mean colony losses as calculated in the present study is equal to the result of a TL calculation. Mean colony loss, calculated with a GzLM accounts, however, for the distributional characteristics of losses through the confidence intervals. Calculation of average losses was not used in this study because of its specific disadvantages. Every individual operation would have the same contribution to the overall mean, regardless of the size of the operation. In addition, smaller operations can only have a limited number of loss outcomes and larger operations have a decreased chance on zero loss.

A drawback in the collection of valid data on the prevalence of honey bee mortality is the circumstance that in general for the majority of countries there is only limited, or no funding available, which results in non-participation, or the use of the most economical survey modes, with the trade-off that the sample population might not be representative of national situations because of coverage errors. A Standardized Mortality Rate (SMR) at country level to correct for over or under representation of model factors cannot be reported, as for most countries reliable reference material is not available. Against this background one of the main results of the COLOSS Questionnaire development and implementation is that a global expert network has been organized, which is conscious of the fragility of the outcome presented in this study and is addressing the crucial issues to obtain a valid research frame.

In countries with a high response rate, there is no information on reasons for non-response, which is not unusual in large scale surveys. Non-response seldom occurs at random, and introduces error, which should be minimized. An estimation of the non-response is difficult, because national beekeeping statistics that are necessary for the evaluation of the survey frame are not available in countries where there is no beekeeper registration. Even where registration is compulsory not all of the beekeepers may be compliant (Nguyen *et al.*, 2010). The consequence is that generalization of the results must be limited to trends, which can be observed in a majority of countries, to avoid the risk of artefacts due to the sampling methods used.

The population of interest for the 2009 and 2010 COLOSS surveys was the general beekeeper population. Questions that would only be suitable for an experienced target group of beekeepers had to be avoided. In this study, CDS was defined as the disappearance of a colony with no or only a few dead bees remaining in the hive or the apiary. The case definition for CCD (vanEngelsdorp *et al.*, 2009) includes: 1. rapid loss of adult worker bees evidenced by the presence of brood in affected colonies; 2. a noticeable lack of dead worker bees both within and surrounding the affected hives and; 3. delayed invasion of hive pests and kleptoparasitism from neighbouring honey bee colonies. The first two CCD characterisations were used in the 2009 questionnaire to allow for comparisons between operations where these symptoms were present or absent. The presence of brood in the hive of a disappeared colony does not, however, necessarily point to a rapid collapse. The presence of a limited amount of brood may also point to a longer lasting decline. As this study establishes, mean winter losses on operations with CDS affected colonies differed in most countries in both winters from losses on operations where CDS symptoms were absent. This suggests that different risk factors might be involved for both conditions. For the Netherlands, mean winter losses of CDS affected colonies differed significantly depending on the presence of brood. Different risk factors might be involved for these conditions too.

High winter losses in 2009-10 observed in the Netherlands and Belgium can be partly explained by the distribution of a toxic inverted sugar solution to some beekeeping shops, which was then used to winter colonies (van der Zee and Pisa, 2010). In the Netherlands the mean mortality is decreased from 29 to 23%, if the users of this feed are considered as a confounder and excluded from the analysis (van der Zee and Pisa, 2011).

In summary, the present study establishes that mean honey bee winter losses across Europe showed a large variation from 7-22% in the winter of 2008-9 and 7- 30% in the winter of 2009-10. An important finding is that for all countries which participated in the 2009 survey, the observed overwinter losses in 2010 were substantially higher.

In 2010, colony losses in south east Europe were at such a low level that it seems that factors causing losses in other parts of Europe were either absent or not at levels affecting colony survival. The five provinces of China, which were included in 2010, showed very low mean (4%) *A. mellifera* losses. Six Canadian provinces showed a variation from 16-25% of mean overwintering losses in 2010 with the exception of Nova Scotia (40%). The distribution of colony losses in 2010 at regional level showed a large variation within countries, which supports the notion that a complex combination of factors is causing colony losses (Potts *et al.*, 2010). In most countries and in both monitoring years, hobbyist beekeepers (1 -50 colonies) experienced higher losses compared with intermediate beekeepers (51 – 500 colonies). A similar relationship, but less pronounced, between scale of practice and losses was observed in 2010. The outcome of the 2011 COLOSS monitoring will indicate whether losses are continuing to rise and if so where, and if rising levels are associated with less difference between the two operation size classes.

Operational losses of colonies overwintering in 2008-9 in the Netherlands displaying the CCD symptoms of: 1. no dead bees in the hive while; 2. capped brood was observed, were significantly higher than operational losses where colonies disappeared (CDS) when no capped brood was seen in the empty hives. More research is necessary to determine whether this points to different risk factors. In Belgium, Italy, the Netherlands and Poland in 2008-2009, overwinter losses where CDS symptoms were observed were higher compared with operations where losses were experienced, but without these symptoms.

In the winter of 2009-10 operational losses with CDS symptoms were higher compared with operational losses without CDS symptoms in most countries. In Sweden, Norway and as far as observed in Canada, relatively few operations had CDS losses, which may be due to fewer opportunities for individual bees to leave the hives because of long winters. A spatial temporal analysis, including climatic variables, may better explain possible associations between loss symptoms and the spatial distribution of losses.

Acknowledgements

We thank all beekeepers and surveyors who took the effort to provide information. We recognize the efforts of all participating Canadian provincial apiculturists for the collection and provision of standardized data as part of their regional surveys. These included: Chris Jordan; Joanne Moran; Chris Maund; Claude Boucher; Rhéal Lafrenière and Paul van Westendorp. Financial support for WG1 workshops was granted by COST via the Action FA0803 COLOSS. The Dutch Ministry of Economic Affairs, Agriculture and Innovation supported a WG1 workshop in Amsterdam and supported funding for the analysis of the data in the present study within the BIJ-1 framework.

References

AFFLECK , D L R (2006) Poisson mixture models for regression analysis of stand-level mortality. *Canadian Journal of Forestry Research* 36: 2994-3006. DOI: 10.1139/X06-189.

ASTON, D (2010) Honey bee winter loss survey for England, 2007-8. *Journal of Apicultural Research* 49(1): 111-112. DOI: 10.3896/IBRA.1.49.1.21

BRODSCHNEIDER, R; MOOSBECKERHOFER, R; CRAILSHEIM, K (2010) Surveys as a tool to record winter losses of honey bee colonies: a two year case study in Austria and South Tyrol. *Journal of Apicultural Research* 49(1):23-30. DOI: 10.3896/IBRA.1.49.1.04.

BROWN, L D; CAI, T; DASGUPTA, A (2002) Confidence intervals for a binomial proportion and Edgeworth expansions. *Annals of Statistics* 30: 160–201.

CHARRIÈRE, J-D; NEUMANN, P (2010) Surveys to estimate winter losses in Switzerland. *Journal of Apicultural Research* 49(1):132-133. DOI: 10.3896/IBRA.1.49.1.29.

COLOSS (2009) *Proceedings of the 4th COLOSS Conference, Zagreb, Croatia.* http://www.coloss.org/publications/Zagreb%20Proceedings

CURRIE, R W; PERNAL, S F; GUZMÁN-NOVOA, E (2010) Honey bee colony losses in Canada. *Journal of Apicultural Research* 49(1): 104-106. DOI: 10.3896/IBRA.1.49.1.18.

DAHLE, B (2010) The role of *Varroa destructor* for honey bee colony losses in Norway. *Journal of Apicultural Research* 49(1): 124-125. DOI: 10.3896/IBRA.1.49.1.26.

DE LEEUW, E; HOX, J J; DILLMAN, D A (2008) *International Handbook of Survey Methodology.* European Association of Methodology / Lawrence Erlbaum Associates; New York, NY, USA. 549 pp. ISBN 978-0-8058-5753-5.

ELLIS, J D; EVANS, J D; PETTIS, J (2010) Colony losses, managed colony population decline, and Colony Collapse Disorder in the United States. *Journal of Apicultural Research* 49(1): 134-136. DOI: 10.3896/IBRA.1.49.1.30.

EUROPEAN COOPERATION IN THE FIELD OF SCIENTIFIC AND TECHNICAL RESEARCH (COST) (2008) Memorandum of Understanding for the implementation of a European Concerted Research Action designated as COST Action FA0803: Prevention of honey bee COlony LOSSes (COLOSS).

GAJGER, I T; TOMLJANOVIC, Z; PETRINEC, Z (2010) Monitoring health status of Croatian honey bee colonies and possible reasons for winter losses. *Journal of Apicultural Research* 49(1):107-108. DOI: 10.3896/IBRA.1.49.1.19.

GARDNER, M J; ALTMAN, D G (1986) Confidence intervals rather than p-values: estimation rather than hypothesis testing. *British Medical Journal* 283: 600–602.

GIRAY, T; KENCE, M; OSKAY, D; DOKE, M A; KENCE, A (2010) Colony losses survey in Turkey and causes of bee deaths. *Apidologie* 41 (4):451-453. DOI: 10.1051/apido/2009077

GRAY, A; PETERSON, M; TEALE, A (2010) An update on recent colony losses in Scotland from a sample survey covering 2006-2008. *Journal of Apicultural Research* 49(1): 129-131. DOI: 10.3896/IBRA.1.49.1.28.

HATJINA, F; BOUGA, M; KARATASOU, A; KONTOTHANASI, A; CHARISTOS, L; EMMANOUIL, N; MAISTROS, A D (2010) Data on honey bee losses in Greece; a preliminary note. *Journal of Apicultural Research* 49(1): 116-118. DOI: 10.3896/IBRA.1.49.1.23

HENDRIKX, P; CHAUZAT, M-P; DEBIN, M; NEUMANN, P; FRIES, I; RITTER, W ; BROWN, M ; MUTINELLI, F ; LECONTE, Y; GREGORC, A (2009) EFSA Bee mortality and bee surveillance in Europe. CFP/EFSA/AMU/2008/02. http://www.efsa.europa.eu/it/scdocs/doc/27e.pdf.

IVANOVA, E N; PETROV, P P R (2010) Regional differences in honey bee winter losses in Bulgaria during the period 2006-9. *Journal of Apicultural Research* 49(1): 102-103. DOI: 10.3896/IBRA.1.49.1.17

KLUSER, S; NEUMANN, P; CHAUZAT M-P; PETTIS, J S (2010). Global honey bee colony disorders and other threats to insect pollinators. *UNEP Emerging Issues.* http://www.unep.org/dewa/Portals/67/pdf/Global_Bee_Colony_Disorder_and_Threats_insect_pollinators.pdf

MUTINELLI, F; COSTA, C; LODESANI, M; BAGGIO, A; MEDRZYCKI, P; FORMATO, G; PORRINI, C (2010) Honey bee colony losses in Italy. *Journal of Apicultural Research* 49(1): 119-120. DOI: 10.3896/IBRA.1.49.1.24

NEUMANN, P; CARRECK, N L (2010). Honey bee colony losses. *Journal of Apicultural Research* 49(1): 1-6. DOI: 10.3896/IBRA.1.49.1.01

NGUYEN, B K; MIGNON, J; LAGET, J; DE GRAAF, D C; JACOBS, F J; VANENGELSDORP, D; BROSTAUX, Y; SAEGERMAN, C; HAUBRUGE, E (2010) Honey bee colony losses in Belgium during the 2008-2009 winter. *Journal of Apicultural Research* 49(4): 333-339. DOI: 10.3896/IBRA.1.49.4.07

POTTS, S G; ROBERTS, S P M; DEAN, R; MARRIS, G; BROWN, M A; JONES, R; NEUMANN, P; SETTELE, J (2010). Declines of managed honey bees and beekeepers in Europe. *Journal of Apicultural Research* 49(1): 15-22.DOI: 10.3896/IBRA.1.49.1.02

TOPOLSKA, G; GAJDA, A; POHORECKA, K; BOBER, A; KASPRZAK, S; SKUBIDA, M; SEMKIW, P (2010) Winter colony losses in Poland. *Journal of Apicultural Research* 49(1): 126-128. DOI: 10.3896/IBRA.1.49.1.27

VAN DER ZEE, R (2010) Colony losses in the Netherlands. *Journal of Apicultural Research* 49(1): 121-123. DOI: 10.3896/IBRA.1.49.1.25

VAN DER ZEE, R; PISA, L (2010) Wintersterfte 2009-10 en toxische invertsuikersiroop. *Netherlands Centre for Bee Research Report 02/2010*. http://www.beemonitoring.org/Downloads/Bijensterfte%202009-10_en%20toxische_%20invertsuikersiroop.pdf

VAN DER ZEE, R; PISA, L (2011) Monitor Bijensterfte Nederland 2009-10. *Netherlands Centre for Bee Research Report 01/2011*. http://www.beemonitoring.org/Downloads/Monitor_Bijensterfte_2009-2010.pdf

VANENGELSDORP, D; EVANS, J D; SAEGERMAN, C; MULLIN, C; HAUBRUGE, E; NGUYEN, B K; FRAZIER, M; FRAZIER, J ; COX-FOSTER, D; CHEN, Y; UNDERWOOD, R; TARPY, D R; PETTIS, J S (2009). Colony Collapse Disorder: A descriptive study. *PloS ONE* 4:e6481. DOI: 10.1371/journal.pone.0006481

VANENGELSDORP D; HAYES, J Jr; UNDERWOOD, R M; PETTIS, J (2008) A survey of honey bee colony losses in the US, fall 2007 to spring 2008. *PLoS ONE* 3: e4071. DOI: 10.1371/journal.pone.0004071

VANENGELSDORP, D; HAYES, J Jr; UNDERWOOD, R M; PETTIS, J S (2010) A survey of honey bee colony losses in the United States, fall 2008 to spring 2009. *Journal of Apicultural Research* 49(1): 7-14. DOI: 10.3896/IBRA.1.49.1.03

VANENGELSDORP, D; HAYES, J Jr; UNDERWOOD, R M; CARON, D; PETTIS, J S (2011) A survey of managed honey bee colony losses in the USA, fall 2009 to spring 2010. *Journal of Apicultural Research* 50(1): 1-10. DOI: 10.3896/IBRA.1.50.1.01

VEJSNÆS, F; NIELSEN, S L; KRYGER, P (2010) Factors involved in the recent increase in colony losses in Denmark. *Journal of Apicultural Research* 49(1): 109-110. DOI: 10.3896/IBRA.1.49.1.20

WHITE, G C; BENNETTS, R E (1996) Analysis of frequency count data using the negative binomial distribution. *Ecology* 77: 2549–2557. DOI: 10.2307/2265753