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ORIGINAL PAPER

Spatial clusters of *Varroa destructor* control strategies in Europe

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Abstract
Beekeepers have various options to control the parasitic mite *Varroa destructor* in honey bee colonies, but no empirical data are available on the methods they apply in practice. We surveyed 28,409 beekeepers maintaining 507,641 colonies in 30 European countries concerning *Varroa* control methods. The set of 19 different *Varroa* diagnosis and control measures was taken from the annual COLOSS questionnaire on honey bee colony losses. The most frequent activities were monitoring of *Varroa* infestations, drone brood removal, various oxalic acid applications and formic acid applications. Correspondence analysis and hierarchical clustering on principal components showed that six *Varroa* control options (not necessarily the most used ones) significantly contribute to defining three distinctive clusters of countries in terms of *Varroa* control in Europe. Cluster I (eight Western European countries) is characterized by use of amitraz strips. Cluster II comprises 15 countries from Scandinavia, the Baltics, and Central-Southern Europe. This cluster is characterized by long-term formic acid treatments. Cluster III is characterized by dominant usage of amitraz fumigation and formed by seven Eastern European countries. The median number of different treatments applied per beekeeper was lowest in cluster III. Based on estimation of colony numbers in included countries, we extrapolated the proportions of colonies treated with different methods in Europe. This suggests that circa 62% of colonies in Europe are treated with amitraz, followed by oxalic acid for the next largest percentage of colonies. We discuss possible factors determining the choice of *Varroa* control measures in the different clusters.

Keywords *Apis mellifera* · COLOSS · Beekeeping · Acaricide · *Varroa* control · Survey results

Introduction

After a shift from its original host, the Eastern honey bee *Apis cerana,* to the Western honey bee, *Apis mellifera*, the parasitic mite *Varroa destructor* (Anderson and Trueman 2000) became the main problem in beekeeping worldwide (Rosenkranz et al. 2010; Noël et al. 2020; Traynor et al. 2020; Vilarem et al. 2021; Reams and Rangel 2022). The mite, today found almost worldwide (with the exception of Australia), reached Europe in the 1960s and 1970s, and North America in the late 1980s (Rosenkranz et al. 2010; Traynor et al. 2020). The mite can only reproduce in sealed honey bee broods, though the reproductive success of mite variants is different in the two host species and in male or female bee brood (Lin et al. 2021). There are a number of studies indicating the major role of *Varroa* in colony losses of the economically important Western honey bee (Brodschneider et al. 2010; Genersch et al. 2010; Guzmán-Novoa et al. 2010; Beyer et al. 2018; Morawetz et al. 2019; Flores et al. 2021; Kulhanek et al. 2021; Hernandez et al. 2022). The mite primarily consumes fat body tissue (Ramsey et al. 2019) with effects, among others, on reduced weight, reserve protein levels and adult longevity of infested larvae (De Jong et al. 1982; Amdam et al. 2004). In addition to this, the mite

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transmits honey bee viruses (Ball and Allen 1988; Martin et al. 2012; Traynor et al. 2020; Flores et al. 2021).

Several different control methods have been developed. They can be roughly categorized into non-chemical or biotechnical treatments (drone brood removal, hyperthermia, complete brood removal or other methods), 'soft' acaricides (acaricides containing natural-based active ingredients), such as organic acids or essential oils, and 'hard' acaricides containing synthetic active ingredients from the groups of organophosphates, pyrethroids or formamidine (amitraz) (Rosenkranz et al. 2010; Roth et al. 2020; Jack and Ellis 2021). Unfortunately, a one-fits-all control method is not available, and each control method has its advantages and disadvantages. Most of all, they differ in efficacy, and this is often dependent on environmental conditions (Underwood and Currie 2003; Gregorc et al. 2018; Steube et al. 2021). Chemical acaricides could add stress to the known factors affecting honey bee health through sublethal or even lethal side effects on bees (Gregorc 2012; Berry et al. 2013; Gregorc et al. 2018; Colin et al. 2020; Alonso-Prados et al. 2021; Kast and Kilchenmann 2022; Ward et al. 2022), along with risk of contaminating hive products (Wallner 1999; Mullin et al. 2010; Kast et al. 2021). Therefore, new products with high varroacide efficacy and no side effects on bees are sought, with lithium salts being candidates that may meet these requirements (Ziegelmann et al. 2018). Lithium residues that reach bee products are considered irrelevant given the amount of lithium that consumers ingest through common food products (Szklarska and Rzymski 2019; Stanimirovic et al. 2021). Besides veterinary medicinal products authorized for the treatment of *Varroa* mite infestation (which differ between countries: Mutinelli 2016; Jack and Ellis 2021), unapproved products may be used, not to mention self-made products, off-label use, and products from the black market.

All participating countries in this study are represented in the colony loss monitoring group, which is a core project of the COLOSS research association (www.coloss.org), a facilitative network for honey bee research. The monitoring group undertakes annual surveys of beekeepers in spring via national survey coordinators, using a standardized questionnaire (van der Zee et al. 2013) designed to collect information on colony losses over winter as well as potential risk factors for winter loss (van der Zee et al. 2012, 2014; Brodschneider et al. 2016, 2018; Gray et al. 2019, 2020). The standardized design of the questionnaire enables the comparison of data collected in the participating countries. Questions concerning the monitoring and treatment of *Varroa* form an important part of the survey; however, these data have so far not been used to provide a description and comparison of *Varroa* monitoring and *Varroa* control in Europe. This paper provides such a descriptive study, using data returned from 30 European countries after completion of the national surveys carried out in spring 2020, and is the first study of its kind. Our study aims to contribute to better understanding of which *Varroa* control strategies beekeepers apply in Europe. So far, only a few investigations on this are available, for certain parts of Europe (Brodschneider et al. 2019; Sperandio et al. 2019; Tomljanović et al. 2020). Empirical multi-country studies using the same methods and definitions are largely missing, but would benefit our understanding of implemented beekeeping management practices. One exception is the study of Mezher et al. (2021) which surveyed more than 400 beekeepers globally, with Europe as the core area. Due to the small sample, they do not present differences or similarities of *Varroa* control methods at a country level, but in addition to *Varroa* control, their article also includes methods to manage bacterial honey bee brood diseases.

Not being able to properly manage the mite has been suggested as a reason for (small-scale) beekeepers in the USA to give up beekeeping (vanEngelsdorp and Meixner 2010). Our findings could support extension workers, improve hive management practices and hence reduce honey bee colony losses (Steinhauer et al. 2020; Kulhanek et al. 2021). The type of substance, mode of application and degree to which different substances are applied also have implications concerning residues present in hive products (Wallner 1999; Mullin et al. 2010; Kast et al. 2020, 2021; Abd El-Wahab et al. 2021; Stanimirovic et al. 2021) and/or mite resistance (Trouiller 1998; Stara et al. 2019; Rinkevich 2020; Jack and Ellis 2021). Such resistance has been proven for tau-fluvalinate (Milani 1995; Colin et al. 1997; Baxter et al. 1998; Johnson et al. 2013; Millán-Leiva et al. 2021), amitraz and flumethrin (Rodríguez-Dehaibes et al. 2005). We also provide an approximate picture of the market share of veterinary products used for *Varroa* control in Europe. So far, policy-makers, including the European Union, could only act based on the legislative status of varroacides, which gives no information on which *Varroa* control methods are effectively in use (Mutinelli 2016). Recently, possibilities to overcome this via online apiary management software were suggested, which are not yet fully effective (Scott et al. 2020). We aim here to elucidate this based on the world's largest voluntary beekeeper survey.

Materials and methods

Survey

All participating countries included the same compulsory questions in their own national honey bee colony loss monitoring questionnaires in local languages. We aimed in all countries to reach as many beekeepers as possible, for example by printing the questionnaire in beekeeping magazines, providing it on appropriate websites used by beekeepers, and in some cases distributing it at beekeepers' meetings. More than 20 of the coordinators collected data using the online survey software Limesurvey (Limesurvey version 3.22.19, Limesurvey GmbH., Hamburg, Germany) on a common platform; some countries used their own platform, other software, or other modes for the survey. As well as *Varroa* control methods, data on the number of colonies kept by the beekeepers before winter 2019/2020 was relevant for this investigation. Anonymous participation was possible for beekeepers. The survey was conducted in spring 2020, with all data being submitted to international coordinators by 1st July 2020. All the data were consistently quality-checked and coded by national coordinators for joint analysis.

Varroa control methods

Beekeepers were asked to indicate the month (April 2019 to March 2020) in which they monitored their bees for *Varroa* and/or started each activity related to *Varroa* control, irrespective of the legal status of the chemicals in the participating countries. All 19 *Varroa* diagnosis and control options and their abbreviations used in this article, as well as a short description, are listed in Table 1, and the survey questions on *Varroa* diagnosis and control are shown in Supplementary Figure S1.

Data

Only data from beekeepers providing essential information for this investigation were considered. In the raw data, not all beekeepers responding to the survey were managing any colonies at the start of winter, or this information was missing. Beekeepers who had no colonies at the start of winter or who did not state the number of their colonies going into winter 2019/2020 were therefore omitted from the analysis. There were 25 such beekeepers in the original set of 28,434 responses, leaving 28,409 beekeepers whose data were used in this work. On the other hand, beekeepers who did not give information on their *Varroa* control methods were not excluded, as those could constitute operations abstaining from *Varroa* control for many possible reasons.

Data analysis

The analysis was conducted with the software R version 4.0.4 (R Core Team 2021) and various packages (see Supplementary Table S1). The categorical binary survey data (where an entry of '1' indicates specific treatment started or monitoring carried out in the respective month, and a lack of data entry indicates treatment/monitoring was not used), was evaluated by creating country-conditional relative frequencies from contingency tables. For each of the two related datasets, (i) survey respondents and (ii) surveyed colonies,

Table 1 Abbreviations, examples and categories of the 19 different *Varroa* diagnosis and control methods surveyed

No.	Abbreviation	*Varroa* control method (example)	Category
1.	VarrMonit	Monitoring of varroa infestation level (e.g. counting mite-fall, sugar shake/roll)	Diagnosis
2.	DroneRemov	Drone brood removal	Biotechnical method
3.	Hypertherm	Hyperthermia (heat treatment of brood/bees)	Biotechnical method
4.	BiotechMeth	Other biotechnical methods (e.g. trapping comb, complete brood removal, queen confinement)	Biotechnical method
5.	FA_Short	Formic acid – short-term	Organic acid
6.	FA_Long	Formic acid – long-term (e.g. MAQS®)	Organic acid
7.	LacAcid	Lactic acid	Organic acid
8.	OA_Trickl	Oxalic acid—trickling	Organic acid
9.	OA_Sublim	Oxalic acid—sublimation (evaporation)	Organic acid
10.	OA_MixTr	Oxalic acid mixtures (e.g. Hiveclean®, Bienenwohl®, Varromed®)	Organic acid
11.	Thymol	Thymol (e.g.Apiguard®, ApilifeVar®, Thymovar®)	Essential oil
12.	TauFluv	Tau-fluvalinate (e.g. Apistan®)	Pyrethroid, synthetic acaricide
13.	Flumeth	Flumethrin (e.g. Bayvarol®, Polyvar®)	Pyrethroid, synthetic acaricide
14.	AmiStrip	Amitraz (strips, e.g. Apivar®, Apitraz®)	Formamidine, synthetic acaricide
15.	AmiFumig	Amitraz (fumigation, aerosol)	Formamidine, synthetic acaricide
16.	CoumTrick	Coumaphos (trickling, e.g. Perizin®)	Organophosphate, synthetic acaricide
17.	CoumStrip	Coumaphos (strips, e.g. Checkmite + ®)	Organophosphate, synthetic acaricide
18.	AnotChemPr	Another chemical product	Unspecified synthetic acaricide
19.	AnotMeth	Another method	Unspecified

In this investigation, beekeepers were asked to indicate in which months (April 2019 to March 2020) they applied any of these methods tables were created to show the extent of *Varroa* monitoring and level of usage of *Varroa* control methods in the participating countries, as well as tables for temporal application of treatments, categorizing apiary sizes and frequency of different treatment types. The percentage of beekeepers using *Varroa* monitoring or applying a certain *Varroa* control action was calculated based on the minimum indication of a respondent of application of a method in at least one month. Similarly, the percentage of colonies monitored or treated with a certain method was calculated for each country, where the number of colonies kept by respondents before winter 2019/2020 was considered.

To estimate a low-dimensional representation of the frequency data comprising 30 countries and the usage of the 19 *Varroa* control methods (including monitoring), correspondence analysis (CA) (Sourial et al. 2010; Greenacre 2016) was utilized. Identification of the main components of the respective dimensions was undertaken by visual observation of the scree plot, the individual contributions to the dimensions and the qualities of representation.

For the identification of possible clusters, a subsequent hierarchical clustering on principal components (HCPC) with Euclidean distance and Ward's agglomeration rule was conducted on the most representative components from the CA. A symmetric two-dimensional visualization of the CA was then generated to display clusters and summarize the main associations and contrasts of the high-dimensional data. The validation of the generated clusters was managed by leave-one-out, two-out and three-out analyses. For this, CA, and subsequent clustering with a defined number of 3 clusters was automated, performing 1000 iterations per validation method, for both datasets. The assignment of countries to different clusters was recorded, expressed as a percentage of total number of assignments.

Further investigation of the number of *Varroa* control methods applied per beekeeper for each cluster was visualized via a violin plot, and statistical testing (Kruskal–Wallis test and post hoc Dunn test) was undertaken to identify significant differences.

Projection of number of colonies treated

To estimate the number of colonies in Europe on which the different *Varroa* control options are applied, the calculated usage percentages (in terms of colonies rather than beekeeepers) of each individual method were applied to the total number of colonies in the respective countries. Different forms of applications of the same active compound on the same colony were not pooled, as, e.g., the application of amitraz in strips and fumigation on the same colonies would positively bias our estimations. The results were summed up for all countries. As the basis for calculations, figures of numbers of colonies and beekeepers provided by authors for their respective countries were used (see Supplementary Table S2), which vary in their accuracy and means of estimation among countries.

Results

For the analysis, a sample of 28,409 valid responses from beekeepers from 30 countries collectively managing 507,641 colonies was available (Table 2). Most answers came from Germany (37.3%), followed by the Netherlands and the Czech Republic. The proportions of large beekeeping operations (more than 150 colonies) were highest in Bulgaria, Greece, and Spain (Table 2). In Belgium and the countries of the UK, no respondents from this category contributed to this survey.

The percentages of responding beekeepers applying any of the 19 *Varroa* monitoring and control options are shown in Table 3 for all 30 countries. The highest proportion of methods related to *Varroa* control over all countries was found for monitoring of *Varroa* infestation level (63.2% of beekeepers), followed by drone brood removal (50.2%) and oxalic acid trickling (46.0%). In Table 4, proportions of usage of the 19 *Varroa* monitoring/control actions based on the number of honey bee colonies maintained by respondents are shown. Similarly, as for the previous percentage of beekeepers in Table 3, the highest proportion of all methods applied over all countries was found for monitoring of *Varroa* infestation level (62.6% of colonies), followed by drone brood removal (44.1%) and oxalic acid trickling (42.6%).

The highest number of different *Varroa* monitoring/control actions applied in a country was 19 (i.e. all offered options were picked at least once by at least one respondent in the country), and this maximum was reached in Belgium, Latvia, Romania, Serbia and Ukraine. In Norway, only eight different *Varroa* monitoring/control actions were applied by all the respondents collectively (Table 3, last column). Six diagnosis or control measures were applied in all countries (monitoring of *Varroa* infestation level, drone brood removal, formic acid short-term and long-term evaporation, oxalic acid trickling and evaporation). Trickling of liquid coumaphos is applied in nine countries only (Table 3).

The number of different *Varroa* control options applied by beekeepers in the three identified spatial clusters of *Varroa* control actions is shown in Fig. 1. Beekeepers in cluster III (comprised of mainly Eastern European countries, see below) applied significantly fewer different control options compared to beekeepers in the two other clusters (Kruskal–Wallis test $p < 0.001$, and post hoc Dunn test $p < 0.001$). *Varroa* infestation monitoring was excluded for this analysis, so the maximum possible number of control options is 18. This analysis considers repeated applications of one control method in different months as one application.

Table 2 Description of sample of beekeepers

Country	Respondents (% of total)		Number of colonies (% of total)		Beekeepers with at most 50 colonies (% of the country)		Beekeepers with 51 to 150 colonies (% of the country)		Beekeepers with more than 150 colonies (% of the country)	
Austria	1453	(5.1%)	29,545	(5.8%)	1356	(93.3%)	78	(5.4%)	19	(1.3%)
Belgium	564	(2.0%)	4607	(0.9%)	559	(99.1%)	5	(0.9%)	0	(0.0%)
Bulgaria	51	(0.2%)	6897	(1.4%)	7	(13.7%)	28	(54.9%)	16	(31.4%)
Czech Republic	1729	(6.1%)	26,893	(5.3%)	1653	(95.6%)	66	(3.8%)	10	(0.6%)
Denmark	1087	(3.8%)	11,419	(2.2%)	1063	(97.8%)	17	(1.6%)	7	(0.6%)
England	1262	(4.4%)	6379	(1.3%)	1255	(99.4%)	7	(0.6%)	0	(0.0%)
Estonia	178	(0.6%)	6746	(1.3%)	148	(83.1%)	19	(10.7%)	11	(6.2%)
Finland	215	(0.8%)	8995	(1.8%)	175	(81.4%)	29	(13.5%)	11	(5.1%)
France	1030	(3.6%)	39,510	(7.8%)	929	(90.2%)	43	(4.2%)	58	(5.6%)
Germany	10,610	(37.3%)	123,496	(24.3%)	10,419	(98.2%)	174	(1.6%)	17	(0.2%)
Greece	170	(0.6%)	19,923	(3.9%)	60	(35.3%)	71	(41.8%)	39	(22.9%)
Ireland	375	(1.3%)	3505	(0.7%)	365	(97.3%)	9	(2.4%)	1	(0.3%)
Italy	364	(1.3%)	7963	(1.6%)	331	(90.9%)	27	(7.4%)	6	(1.6%)
Latvia	364	(1.3%)	12,210	(2.4%)	295	(81.0%)	56	(15.4%)	13	(3.6%)
Netherlands	1857	(6.5%)	14,169	(2.8%)	1840	(99.1%)	13	(0.7%)	4	(0.2%)
North Macedonia	217	(0.8%)	12,105	(2.4%)	126	(58.1%)	81	(37.3%)	10	(4.6%)
Northern Ireland	117	(0.4%)	593	(0.1%)	116	(99.1%)	1	(0.9%)	0	(0.0%)
Norway	765	(2.7%)	11,990	(2.4%)	719	(94.0%)	38	(5.0%)	8	(1.0%)
Poland	426	(1.5%)	16,281	(3.2%)	349	(81.9%)	66	(15.5%)	11	(2.6%)
Portugal	125	(0.4%)	11,691	(2.3%)	87	(69.6%)	16	(12.8%)	22	(17.6%)
Romania	121	(0.4%)	8298	(1.6%)	64	(52.9%)	47	(38.8%)	10	(8.3%)
Scotland	292	(1.0%)	1397	(0.3%)	292	(100.0%)	0	(0.0%)	0	(0.0%)
Serbia	125	(0.4%)	10,932	(2.2%)	59	(47.2%)	48	(38.4%)	18	(14.4%)
Slovakia	548	(1.9%)	9925	(2.0%)	515	(94.0%)	31	(5.7%)	2	(0.4%)
Slovenia	105	(0.4%)	3107	(0.6%)	97	(92.4%)	6	(5.7%)	2	(1.9%)
Spain	156	(0.5%)	19,669	(3.9%)	102	(65.4%)	23	(14.7%)	31	(19.9%)
Sweden	1646	(5.8%)	14,421	(2.8%)	1600	(97.2%)	38	(2.3%)	8	(0.5%)
Switzerland	1665	(5.9%)	21,934	(4.3%)	1642	(98.6%)	22	(1.3%)	1	(0.1%)
Ukraine	702	(2.5%)	42,518	(8.4%)	451	(64.2%)	195	(27.8%)	56	(8.0%)
Wales	90	(0.3%)	523	(0.1%)	89	(98.9%)	1	(1.1%)	0	(0.0%)
Total	28,409	(100%)	507,641	(100%)	26,763	(n.a.)	1255	(n.a.)	391	(n.a.)

Numbers of valid cases for respondents and colonies overall and by size of beekeeping operation (small-scale beekeepers with a maximum of 50 colonies; medium size operations with 51 to 150 colonies; and large operations with over 150 colonies)

The result zero here identifies beekeepers not applying any control measures or those who did not indicate in our survey any measures that they undertook. The distribution of the number of different methods applied by beekeepers at country level is shown in Supplementary Table S3. This table also reveals that in Wales, Greece, Norway, Netherlands and Ireland more than 20% of beekeepers did not use any of the control methods or did not indicate any treatment.

The correspondence analysis with subsequent agglomerative hierarchical clustering resulted in three distinct country clusters based on *Varroa* treatments and surveillance methods (Figs. 2, 3). This result was likewise obtained for both respondents' data (beekeeping operation level) and colony data, with only Wales being assigned to different clusters in the two datasets (see below).

For respondents' data, the clustering calculation was conducted on the first four dimensions of the CA, describing 24.6% and 20.8% for the first two (Fig. 4a), 11.7% for the third and 11.3% of explained variances for the fourth dimension. Together this accumulates to 68.4%. The Kaiser criterion (Kaiser 1960) indicates that 6 dimensions of the CA should be retained for further calculations, while the subjective scree test (Cattell 1966) resulted in 2 dimensions. Hierarchical clustering on principal components

Table 3 Percentages of beekeepers' usage of 19 different *Varroa* control methods including pest surveillance in the period April 2019 to March 2020 in 30 European countries

Cluster	Country	Respondents	VarrMonit	DroneRemov	Hypertherm	BiotechMeth	FA_Short	FA_Long	LacAcid	OA_Trickl	OA_Sublim	OA_MixTr	Thymol	TauFluv	Flumeth	AmiStrip	AmiFumig	CoumTrick	CoumStrip	AnotChemPr	AnotMeth	Used methods per country
II	Austria	1453	87.1	53.5	4.5	25.3	37.0	48.4	3.4	35.4	51.1	26.6	7.6	0.2	0.5	0.6	0.6	0.0	0.0	0.6	1.5	17
II	Belgium	564	57.4	41.8	1.6	12.8	17.6	13.1	1.2	42.2	20.9	13.1	17.2	1.6	5.9	8.3	0.2	0.2	0.5	1.6	12.4	19
II	Bulgaria	51	31.4	23.5	0.0	0.0	15.7	2.0	2.0	56.9	13.7	5.9	5.9	5.9	9.8	7.8	11.8	0.0	2.0	7.8	11.8	16
III	Czech Republic	1729	80.6	34.9	1.3	4.8	55.2	18.7	1.6	21.4	3.1	0.0	6.8	36.4	0.1	0.9	72.0	0.0	0.0	0.6	0.0	15
II	Denmark	1087	43.8	74.3	1.1	1.4	33.1	38.0	2.2	85.2	2.9	2.6	13.0	0.0	2.4	0.4	0.0	0.0	0.0	0.2	2.0	15
I	England	1262	74.3	16.4	0.6	3.0	1.5	15.7	0.1	23.1	15.7	6.1	38.9	3.3	0.3	10.6	0.1	0.0	0.0	1.8	6.0	17
II	Estonia	178	58.4	52.8	2.2	17.4	10.1	20.8	0.0	52.2	51.7	16.9	15.2	6.7	2.2	6.7	2.2	0.0	0.0	5.1	5.6	16
II	Finland	215	52.1	60.5	0.0	1.9	9.3	31.2	0.0	77.7	7.4	4.7	45.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	10
I	France	1030	54.7	8.9	0.0	1.7	3.1	1.9	0.0	8.7	3.3	10.7	3.8	2.3	0.5	67.9	0.9	0.0	0.0	1.6	3.3	15
II	Germany	10,610	60.0	64.2	0.8	11.8	37.1	50.0	16.6	59.8	13.0	3.6	5.9	0.1	1.3	0.8	0.0	0.1	0.0	0.0	0.0	15
II	Greece	170	62.9	11.8	0.0	4.7	2.4	1.2	0.0	28.2	8.8	8.8	9.4	0.0	6.5	12.9	3.5	0.6	0.0	1.8	7.6	15
I	Ireland	375	65.1	12.3	0.8	2.1	0.8	3.5	0.3	7.2	21.6	0.3	44.5	0.3	0.5	22.1	0.3	0.0	0.3	0.8	3.7	18
II	Italy	364	81.3	28.3	2.2	37.9	8.5	7.4	0.8	66.5	35.4	6.3	28.3	6.6	1.1	18.4	0.0	0.0	0.0	1.1	3.8	16
II	Latvia	364	31.3	51.4	1.4	5.8	8.8	3.0	0.5	27.2	11.3	48.4	2.5	2.2	31.6	5.2	1.1	0.5	0.5	4.1	6.6	19
II	Netherlands	1857	46.6	40.9	1.1	2.7	23.7	16.0	1.0	43.7	8.0	12.9	22.0	0.6	0.9	1.1	0.4	0.1	0.0	1.0	2.7	18
III	North Macedonia	217	30.4	28.1	0.9	6.9	9.2	2.8	0.0	56.7	30.0	0.0	12.4	8.8	4.1	15.2	39.2	27.2	15.7	0.9	5.5	17
I	Northern Ireland	117	71.8	10.3	0.0	1.7	2.6	12.8	0.0	34.2	25.6	1.7	54.7	3.4	0.0	24.8	0.0	0.0	0.0	0.9	5.1	13
II	Norway	765	68.9	50.2	0.0	0.0	3.9	1.0	12.3	62.7	2.4	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
III	Poland	426	77.5	62.9	1.6	18.5	12.2	8.9	0.0	25.4	7.7	0.0	10.3	0.0	8.7	32.9	75.8	0.0	0.0	7.7	13.6	14
I	Portugal	125	68.8	24.0	4.0	3.2	8.8	2.4	1.6	8.8	8.0	8.0	14.4	5.6	2.4	66.4	3.2	0.0	3.2	1.6	3.2	18
III	Romania	121	79.3	38.8	7.4	25.6	8.3	5.0	3.3	16.5	9.9	9.9	9.9	35.5	10.7	24.8	67.8	2.5	2.5	10.7	9.1	19
I	Scotland	292	72.6	24.7	0.7	1.0	1.4	15.1	0.7	22.3	30.1	3.4	17.8	2.7	1.4	34.9	0.7	0.0	0.0	1.7	4.8	17
III	Serbia	125	86.4	32.0	4.0	12.8	16.0	9.6	4.0	66.4	6.4	7.2	13.6	8.8	3.2	31.2	53.6	4.8	14.4	9.6	12.8	19
III	Slovakia	548	67.9	48.4	2.0	3.3	27.9	9.9	1.1	16.2	4.2	10.8	54.0	18.4	3.8	3.6	70.6	0.0	0.0	3.1	3.5	17
II	Slovenia	105	79.0	73.3	1.9	14.3	36.2	30.5	5.7	49.5	41.0	5.7	5.7	0.0	8.6	3.8	42.9	0.0	4.8	5.7	1.0	17
I	Spain	156	79.5	25.0	5.1	4.5	2.6	1.9	0.0	15.4	14.7	6.4	14.1	10.3	0.6	78.8	3.8	0.0	1.3	3.2	3.8	17
II	Sweden	1646	69.0	49.6	0.8	0.6	27.9	6.9	2.5	57.6	16.6	3.2	15.0	3.4	0.4	0.7	0.0	0.0	0.0	0.2	1.7	16
II	Switzerland	1665	71.2	63.5	0.5	12.7	13.7	80.0	0.0	39.9	60.2	0.0	5.0	0.0	0.6	0.0	0.0	0.0	0.1	0.0	1.7	12
III	Ukraine	702	40.6	26.2	6.3	4.1	8.0	3.8	2.4	8.8	8.0	3.4	9.1	10.8	18.1	14.2	46.0	0.6	1.9	7.4	3.3	19
I	Wales	90	75.6	16.7	0.0	3.3	3.3	18.9	0.0	12.2	14.4	2.2	25.6	5.6	0.0	3.3	0.0	0.0	0.0	2.2	6.7	13

Table 3 (continued)

Clus-ter	Country	Respond-ents	Varr-Monit	Dro-neR-emov	Hyper-therm	Bio-tech-Meth	FA_Short	FA_Long	LacAcid	OA_Trickl	OA_Sub-lim	OA_MixTr	Thy-mol	Tau-Fluv	Flu-meth	AmiS-trip	Ami-Fumig	CoumTrick	Coum-Strip	AnotCh-emPr	Anot-Meth	Used meth-ods per country
	Cluster I	3447	67.3	14.9	0.7	2.4	2.3	9.1	0.2	16.2	13.8	6.4	25.4	3.1	0.6	36.4	0.7	0.0	0.2	1.7	4.6	
	Cluster II	21,094	61.5	58.2	1.1	10.4	29.6	39.9	9.5	55.3	19.2	6.8	9.4	0.6	1.8	1.5	0.4	0.1	0.1	0.4	1.4	
	Cluster III	3868	68.5	38.0	2.6	7.0	32.7	12.0	1.5	22.1	6.5	2.7	14.9	22.7	5.5	9.7	64.9	1.9	1.8	3.6	3.6	
	Total	28,409	63.2	50.2	1.3	9.0	26.7	32.4	7.3	46.0	16.8	6.2	12.1	3.9	2.2	6.8	9.2	0.3	0.3	1.0	2.1	

Additionally, percentages for the total dataset and the three clusters identified in the correspondence analysis (Figs. 2, 3) are presented. For abbreviations of *Varroa* control options, see Table 1

was undertaken with different numbers spanning from 2 to 6 dimensions, resulting in the chosen number of 4, as adding additional dimensions does not change the clustering outcome. The quality of representation of applied *Varroa* control methods in dimensions 1 to 4 was highest for amitraz (fumigation), trickling of liquid coumaphos, amitraz strips and coumaphos strips, and lowest for the items "another method", lactic acid and biotechnical methods (Fig. 4c). Six *Varroa* control methods have an above average contribution to dimensions 1 and 4, with values highest for amitraz strips, amitraz (fumigation), trickling of liquid coumaphos, oxalic acid mixtures, flumethrin and treatments with formic acid (long-term) (Fig. 4b). Quality of representation of countries for dimensions 1 and 2 was highest for Spain, Portugal, and France, whereas it was lowest for Latvia, Bulgaria, and Italy.

Validation of the correspondence analysis (Fig. 5) demonstrated how countries would have been assigned to the three clusters if a smaller dataset including fewer countries had been available. In such cases some countries could switch to other clusters. Based on respondents' (not colony) data, the leave-one-out approach showed 11 countries to be consistently assigned in 100% of all iterations to one particular cluster, e.g. Austria, Belgium, Denmark and Estonia to cluster II. Expanding the validation to leave-three-out, these countries show very low probabilities of being assigned to another cluster. The classification to clusters was less unambiguous for Italy and England, for example, resulting in a probability of up to 30% in the leave-one-out validation for being assigned to another cluster than shown in Figs. 2 and 3. However, up to the level of leave-three-out validation, the assigned cluster with the highest probability does not change for all 30 countries. The validation based on the colony data in general showed fewer clear classifications, but mostly the same picture regarding cluster assignment, except for Wales, which is assigned to cluster I according to respondents' data or cluster II based on colonies (Fig. 5). Noteworthy is the discrepancy between countries in clusters I and II, compared to those of cluster III. Countries in cluster III show high assignment probabilities in all leave-out validations using colony data.

Figure 6 displays the seasonal pattern of applications of the six important *Varroa* control options for the three different clusters. Based on extrapolations including estimates of the total number of colonies kept in the different countries, we found that most colonies in Europe are treated with amitraz (in strips or fumigation, 34.0% and 28.0%, but see "Materials and methods" section for the disclaimer that some colonies could be included in both categories) followed by oxalic acid trickling (34.0%, Fig. 7, Supplementary Table S2). The estimations of colony numbers and calculated extrapolations for each treatment per country are shown in Table S2.

Table 4 Percentages of honey bee colonies treated with 19 different *Varroa* control methods including pest surveillance in the period April 2019 to March 2020 in 30 European countries

Cluster	Country	Colonies	VarrMonit	DroneRemov	Hypertherm	BiotechMeth	FA_Short	FA_Long	LacAcid	OA_Trickl	OA_Sublim	OA_MixTr	Thymol	TauFluv	Flumeth	AmiStrip	AmiFumig	CoumTrick	CoumStrip	AnotChemPr	AnotMeth
II	Austria	29,545	86.1	54.5	5.1	37.2	40.9	44.3	3.0	35.7	55.5	27.2	5.4	0.1	0.3	0.4	0.8	0.0	0.0	0.2	1.7
II	Belgium	4607	59.2	44.5	1.4	19.1	16.6	15.0	1.1	45.3	26.3	11.5	13.5	1.2	6.2	13.0	0.0	0.3	0.7	1.5	15.0
II	Bulgaria	6897	30.7	16.3	0.0	0.0	16.2	4.3	0.6	65.4	11.2	7.2	6.1	6.4	6.7	9.2	11.1	0.0	3.4	10.3	13.4
III	Czech Republic	26,893	84.1	40.2	1.2	5.4	53.8	18.5	1.8	25.0	3.3	0.0	5.5	42.9	0.1	0.5	76.1	0.0	0.0	0.5	0.0
II	Denmark	11,419	38.8	53.4	0.9	1.0	25.0	40.4	3.3	87.2	5.2	3.3	13.6	0.0	10.5	0.7	0.0	0.0	0.0	0.1	1.6
I	England	6379	71.5	20.0	0.5	3.7	1.6	19.0	0.1	25.8	21.1	7.6	38.1	3.7	0.5	19.3	0.1	0.0	0.0	1.5	4.3
II	Estonia	6746	59.5	53.4	0.6	23.2	10.2	17.9	0.0	64.6	60.7	12.0	12.7	13.0	7.6	12.3	6.1	0.0	0.0	2.5	3.9
II	Finland	8995	80.2	47.2	0.0	0.2	6.8	35.1	0.0	67.2	31.2	2.7	42.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
I	France	39,510	48.6	13.3	0.0	3.4	4.8	5.7	0.0	23.9	9.0	9.2	3.9	6.1	0.3	78.1	0.4	0.0	0.0	0.6	3.3
II	Germany	123,496	58.6	67.8	0.9	16.9	37.1	50.3	16.9	62.0	14.7	3.8	6.0	0.1	1.6	1.4	0.0	0.0	0.0	0.0	0.0
II	Greece	19,923	66.6	13.5	0.0	5.2	0.5	0.7	0.0	35.0	7.7	7.1	7.3	0.0	5.5	17.0	3.5	1.5	0.0	1.1	6.7
I	Ireland	3505	66.8	10.5	0.3	1.3	0.4	7.3	0.2	8.8	35.0	0.1	48.2	0.1	0.5	30.6	0.1	0.0	0.3	0.1	2.8
II	Italy	7963	80.6	24.6	1.5	36.8	10.7	8.1	1.0	67.4	39.9	8.4	23.2	6.0	3.2	34.5	0.0	0.0	0.0	1.1	2.2
II	Latvia	12,210	28.1	59.2	0.7	5.9	7.9	6.5	0.9	45.4	19.6	38.6	2.5	3.0	28.5	8.1	2.8	0.1	0.1	10.2	3.8
II	Netherlands	14,169	47.4	42.4	0.7	6.6	25.3	19.3	1.1	44.6	14.6	11.8	16.6	0.5	7.0	7.4	0.6	0.0	0.0	1.9	2.8
III	North Macedonia	12,105	38.7	32.0	0.6	7.5	10.9	3.5	0.0	56.0	32.5	0.0	17.1	10.9	7.6	14.6	35.5	34.4	13.7	0.3	5.1
I	Northern Ireland	593	76.1	18.5	0.0	1.2	1.0	22.8	0.0	32.5	30.2	1.5	44.7	1.9	0.0	33.7	0.0	0.0	0.0	0.5	4.9
II	Norway	11,990	68.7	47.8	0.0	0.0	4.1	0.7	9.7	71.1	2.8	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
III	Poland	16,281	81.6	58.8	1.8	28.0	8.3	10.7	0.0	31.3	11.7	0.0	7.1	0.0	12.9	31.8	71.9	0.0	0.0	18.3	19.2
I	Portugal	11,691	54.6	18.5	1.9	5.0	17.9	0.7	0.6	17.2	15.5	13.5	30.7	4.6	1.6	65.7	1.0	0.0	2.9	0.6	0.8
III	Romania	8298	88.1	41.4	7.4	31.0	9.3	6.9	5.3	17.2	14.7	10.7	8.4	42.1	8.0	21.2	66.0	1.6	1.6	8.6	9.9
I	Scotland	1397	69.3	30.8	0.1	1.1	1.0	12.5	1.0	27.0	32.1	2.5	15.3	2.9	1.4	40.2	0.4	0.0	0.0	1.4	4.0
III	Serbia	10,932	89.3	24.9	3.3	18.9	14.7	8.7	2.5	61.5	7.0	11.5	9.6	9.7	5.4	30.4	56.2	4.9	13.2	10.6	16.0
III	Slovakia	9925	68.7	51.1	1.8	5.2	29.1	8.7	1.7	19.2	4.5	9.6	56.7	24.0	5.7	4.3	71.3	0.0	0.0	4.0	6.5
II	Slovenia	3107	76.5	80.7	1.8	16.6	34.1	17.9	1.8	60.3	51.7	2.7	7.1	0.0	7.4	16.7	39.9	0.0	12.7	3.8	0.6
I	Spain	19,669	94.6	24.9	4.4	7.3	6.4	1.2	0.0	24.6	30.3	3.3	7.6	2.4	2.8	86.9	1.9	0.0	0.5	14.0	2.5
II	Sweden	14,421	68.9	44.8	0.3	0.4	32.1	4.3	2.4	63.2	15.9	5.1	21.0	4.3	0.2	4.9	0.0	0.0	0.0	0.1	1.7
II	Switzerland	21,934	69.1	65.4	0.6	15.9	12.8	82.2	0.0	37.7	65.9	0.0	4.4	0.0	0.6	0.0	0.0	0.0	0.1	0.0	1.7
III	Ukraine	42,518	38.7	23.6	5.5	4.7	9.3	2.6	1.8	5.9	7.8	3.7	10.7	11.8	19.8	12.5	47.2	0.4	1.4	6.0	5.2
I	Wales	523	71.1	18.4	0.0	10.3	2.7	27.7	0.0	15.9	24.1	1.1	16.8	6.1	0.0	2.7	0.0	0.0	0.0	1.0	5.5

Table 4 (continued)

Cluster	Country	Colonies	VarrMonit	DroneRemov	Hypertherm	BiotechMeth	FA_Short	FA_Long	LacAcid	OA_Trickl	OA_Sublim	OA_MixTr	Thymol	TauFluv	Flumeth	AmiStrip	AmiFumig	CoumTrick	CoumStrip	AnotChemPr	AnotMeth
	Cluster I	83,267	63.5	17.5	1.3	4.5	6.5	5.4	0.1	22.7	17.6	7.7	13.6	4.5	1.1	70.5	0.8	0.0	0.5	3.8	2.9
	Cluster II	297,422	61.8	55.1	1.1	14.8	26.4	36.6	8.1	55.8	24.2	8.3	8.9	1.0	3.6	4.5	1.3	0.1	0.2	1.0	1.9
	Cluster III	126,952	63.8	35.9	3.3	11.1	20.8	8.4	1.7	24.5	9.8	3.7	13.1	19.5	10.5	14.1	59.2	3.9	3.0	6.3	7.2
	total	507,641	62.6	44.1	1.7	12.2	21.7	24.4	5.2	42.6	19.5	7.0	10.7	6.2	4.9	17.7	15.7	1.1	1.0	2.8	3.4

Additionally, percentages for the total dataset and the three clusters identified in the correspondence analysis (Figs. 2, 3) are presented. For abbreviations of *Varroa* control options, see Table 1

Discussion

Varroa control is crucial for honey bee colony survival (Rosenkranz et al. 2010; Jacques et al. 2017; Noël et al. 2020; Traynor et al. 2020; Roth et al. 2020). Effective control is reached by the choice of adequate methods in relation to colony and environmental conditions, and the combination of methods (Gregorc and Curk 2000; Jack and Ellis 2021). The most often applied activity in relation to *Varroa* control by the 28,409 European beekeepers in our survey was surveillance of *Varroa* mite infestation levels. This suggests that 63% of the participating beekeepers are applying control methods based on informed decisions, indicating good pest control management. Overall, the most frequent chemical application for *Varroa* control was oxalic acid trickling, applied by 46.0% of all participating beekeepers, whereas another 6.2% could be added to this group as they apply ready-to-use oxalic acid (mix) formulations that are also trickled. Oxalic acid in the form of crystals is evaporated by another 16.8% of beekeepers. The next most common chemical treatments are formic acid applications. Drone brood removal was the most common non-chemical treatment, practised by about half of the European beekeepers (Table 3). In Luxembourg, from which no data for our study were available, similar voluntary beekeeper survey data suggest drone brood removal and use of organic acids, as well as essential oils, to be the most widespread control methods (Beyer et al. 2018). For comparison, in the USA, organic acids and essential oils are most frequently applied in small-scale beekeeping operations managing fewer than 50 colonies (Haber et al. 2019). More than 20% of beekeepers from Wales, Greece, Norway, Netherlands and Ireland did not indicate any use of any mite control method (Table S3). Though it was not the aim of this study to identify any trends in "treatment-free" beekeeping, we can at least speculate on whether these beekeepers keep resistant bees, as reported from some of those countries (Oddie et al. 2017; Panziera et al. 2017; McMullan 2018).

The rank and magnitude of the *Varroa* control options are very similar, whether they are derived from the number of respondents (percentage of beekeepers) that applied a method (Table 3) or the percentage of colonies treated (Table 4). We also present the latter in this article, because it is of course the number of colonies that is relevant for the acaricide market. Further, projections on the total amounts of chemicals applied are of interest to understand chemical exposure of hives and hive products to acaricides, and even emerging acaricide resistances. For such a projection, the variations in application of methods in different countries, as well as the very different numbers of colonies kept in different countries, need to be considered.

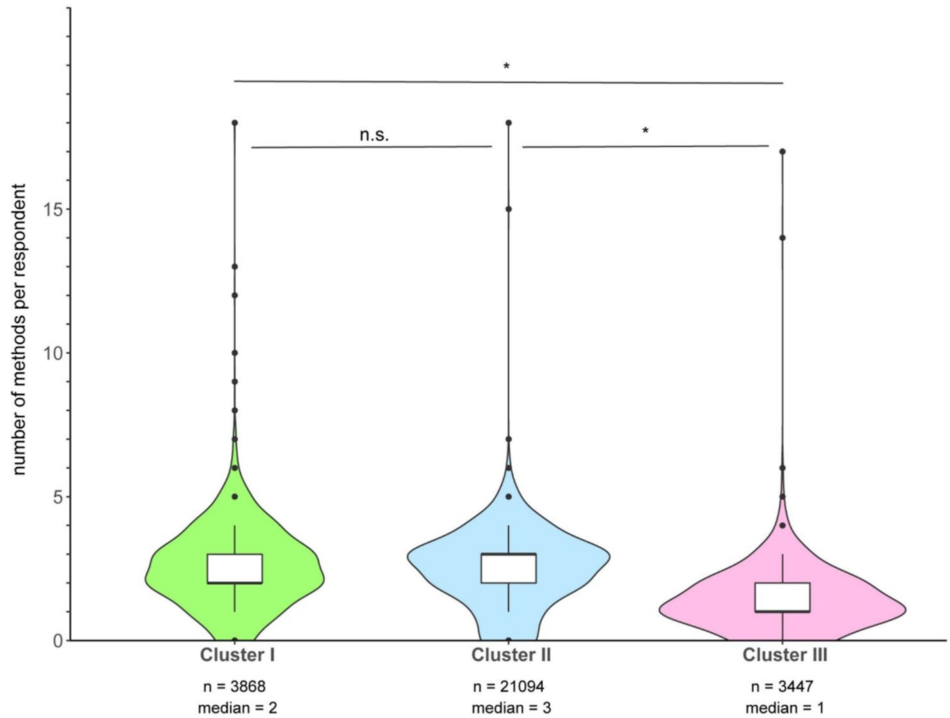

Fig. 1 Number of *Varroa* control methods applied per beekeeper in the three identified clusters. Violin plots showing the number of applied control methods (excluding *Varroa* diagnosis) per respondent; Kruskal–Wallis test, $p < 0.001$, $\eta^2 = 0.0855$ (moderate effect) and post hoc Dunn test (*$p < 0.05$; n.s. not significant)

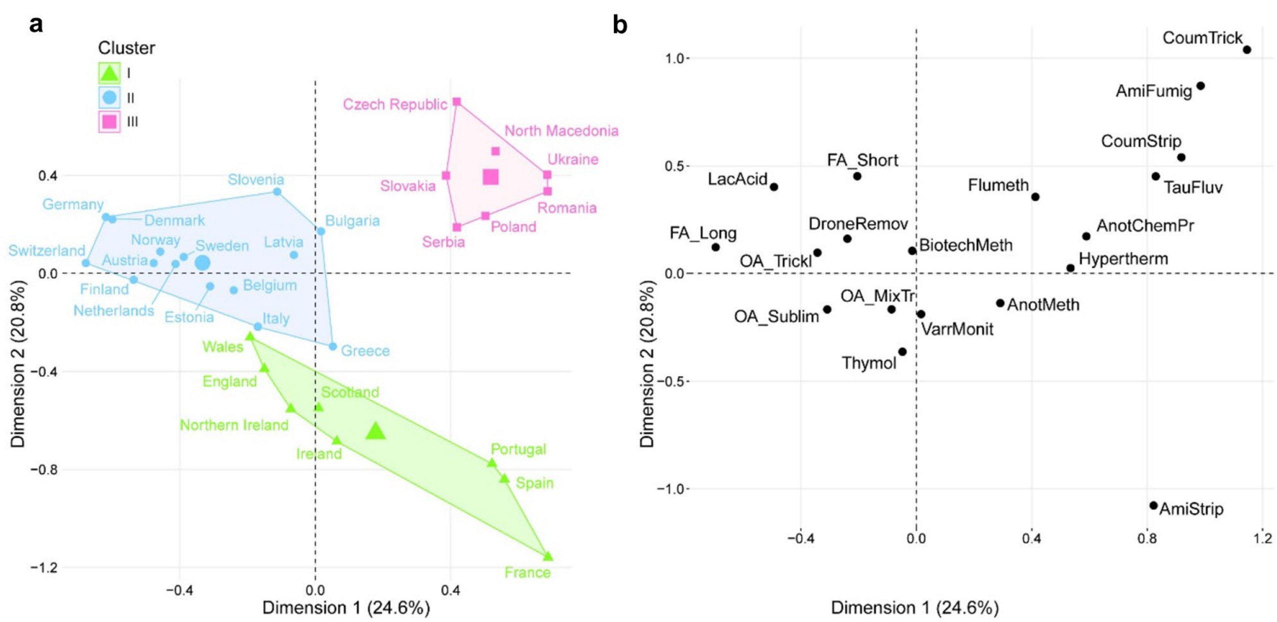

Fig. 2 Correspondence analysis of *Varroa* diagnosis and control methods in Europe based on respondent data. **a** Factor map of 30 countries and the three clusters they form. **b** All 19 factors. For abbreviations of *Varroa* control options, see Table 1

Based on the percentages of colonies treated in different countries (Table 4) and estimations of the total numbers of colonies managed in the countries involved in this study (Table S2), we found that most colonies in Europe are treated with amitraz (Fig. 7). Roughly, it can be estimated that 6 out of 10 colonies in Europe are treated with amitraz, with much higher frequencies in certain countries in clusters I and III. This estimation does not consider whether single colonies were treated with both types of amitraz application (strips and fumigation), so lower numbers are possible. Knowledge about the total application is crucial for the understanding of mite resistance (Floris

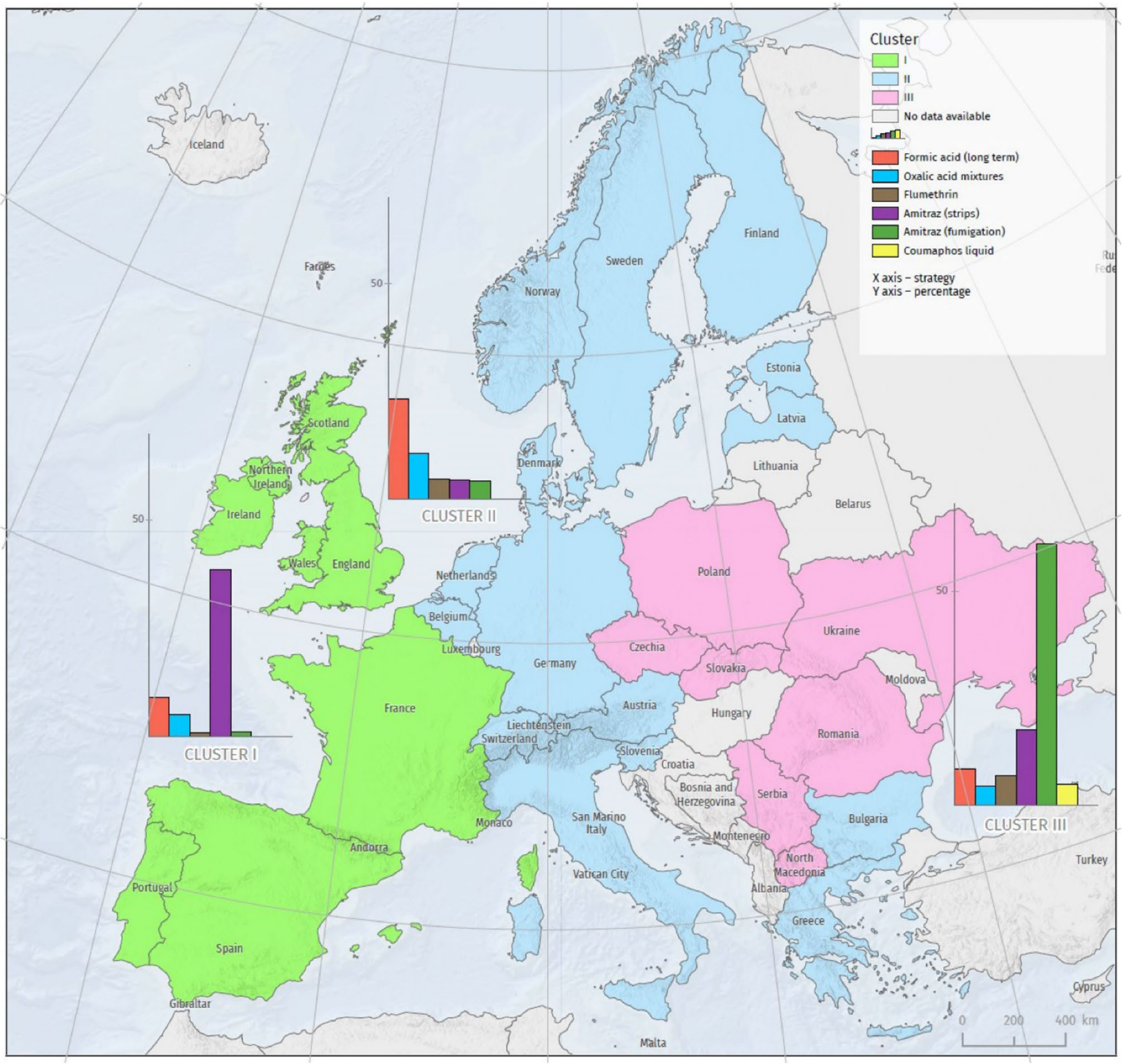

Fig. 3 Spatial representation of the three *Varroa* control clusters identified in Europe. Inserts show the magnitude of application of the six *Varroa* control methods identified to be significant for cluster forming and are shown on the same scale (data from Table 3, averages of countries in clusters)

et al. 2001; Rodríguez-Dehaibes et al. 2005; Maggi et al. 2010; Almecija et al. 2020; Rinkevich 2020; Hernández-Rodríguez et al. 2021), though the load of substances collectively applied is not easily inferable from our data. For this, estimates of the amount of active ingredient per application (including the amount of active ingredient per strip, the number of strips applied per hive or repetitions of applications) are required.

Our set of surveyed management techniques also included '*Varroa* monitoring' (counting mites by applying various methods, such as on sticky bottom boards, on brood, as well as on bodies of captured workers) (Roth et al. 2020). Though this is not a control method per se, it is an important aspect of integrated *Varroa* mite management (Gregorc and Sampson 2019). We found that circa 70% of hives kept in Europe are monitored for the mite (Fig. 7). This study does not allow establishing the precision or efficiency of *Varroa* surveillance, as this monitoring includes practices greatly differing in precision (Branco et al. 2006; Gregorc and Sampson 2019). However, we demonstrated that in some countries a large majority of beekeepers perform this surveillance during the season (e.g. 87% in Austria, Table 3).

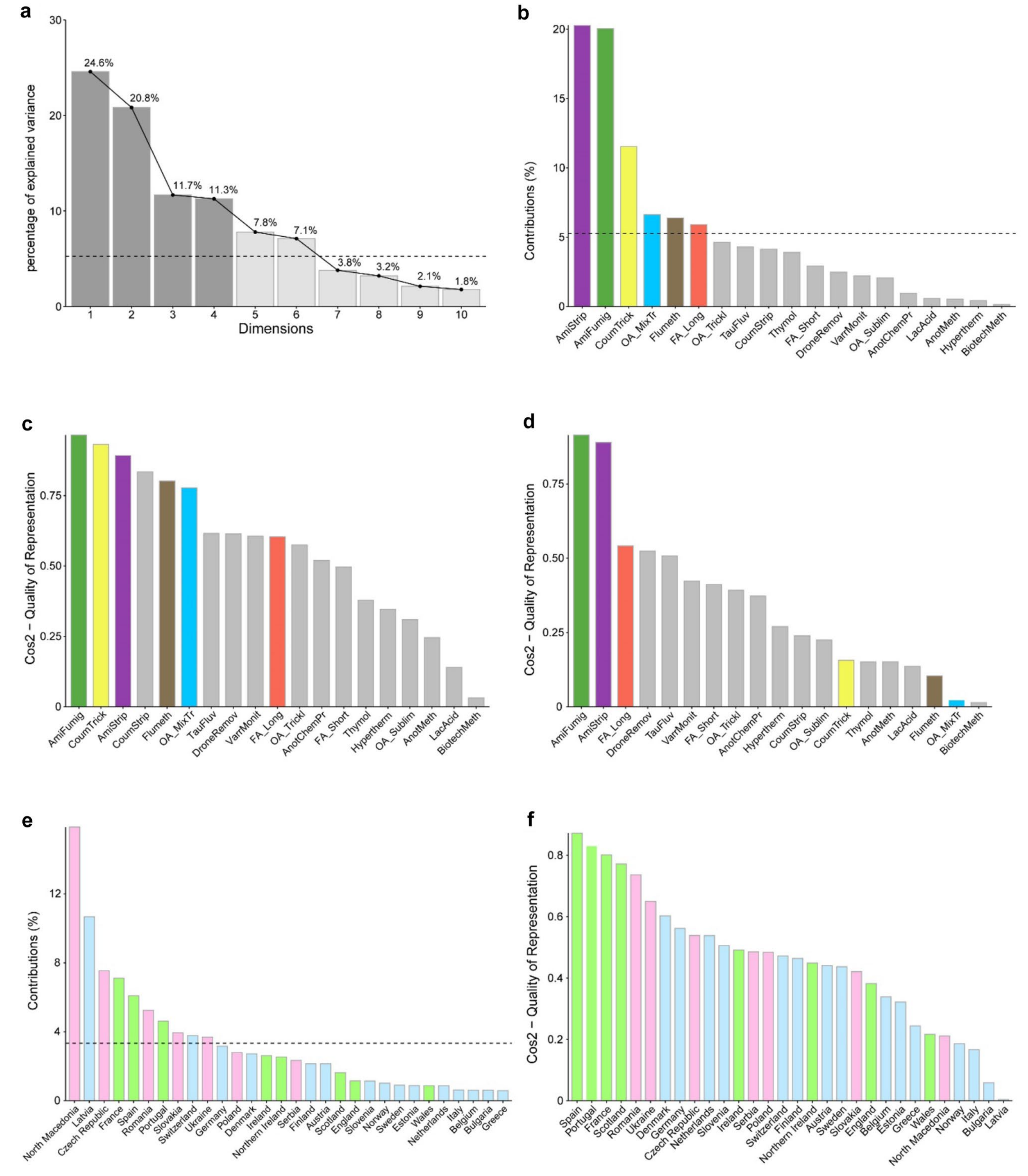

Fig. 4 Details of correspondence analysis (CA). **a** Scree plot showing percentage of explained variances for different dimensions for respondent data; darker bars were selected for further analysis. **b** Contribution of applied *Varroa* control methods to dimensions 1–4. **c** Quality of representation (Cos2) of applied *Varroa* control methods for dimensions 1–4. **d** Cos2 for dimensions 1–2. **e** Contribution of countries to dimensions 1–4. **f** Cos2 of countries for dimensions 1–2. Coloured bars in **b–d** indicate the 6 main *Varroa* control methods contributing the most to CA dimensions 1–4, coloured bars in **e** and **f** indicate the 3 clusters; dashed lines show the expected averages. For abbreviations of *Varroa* control options, see Table 1

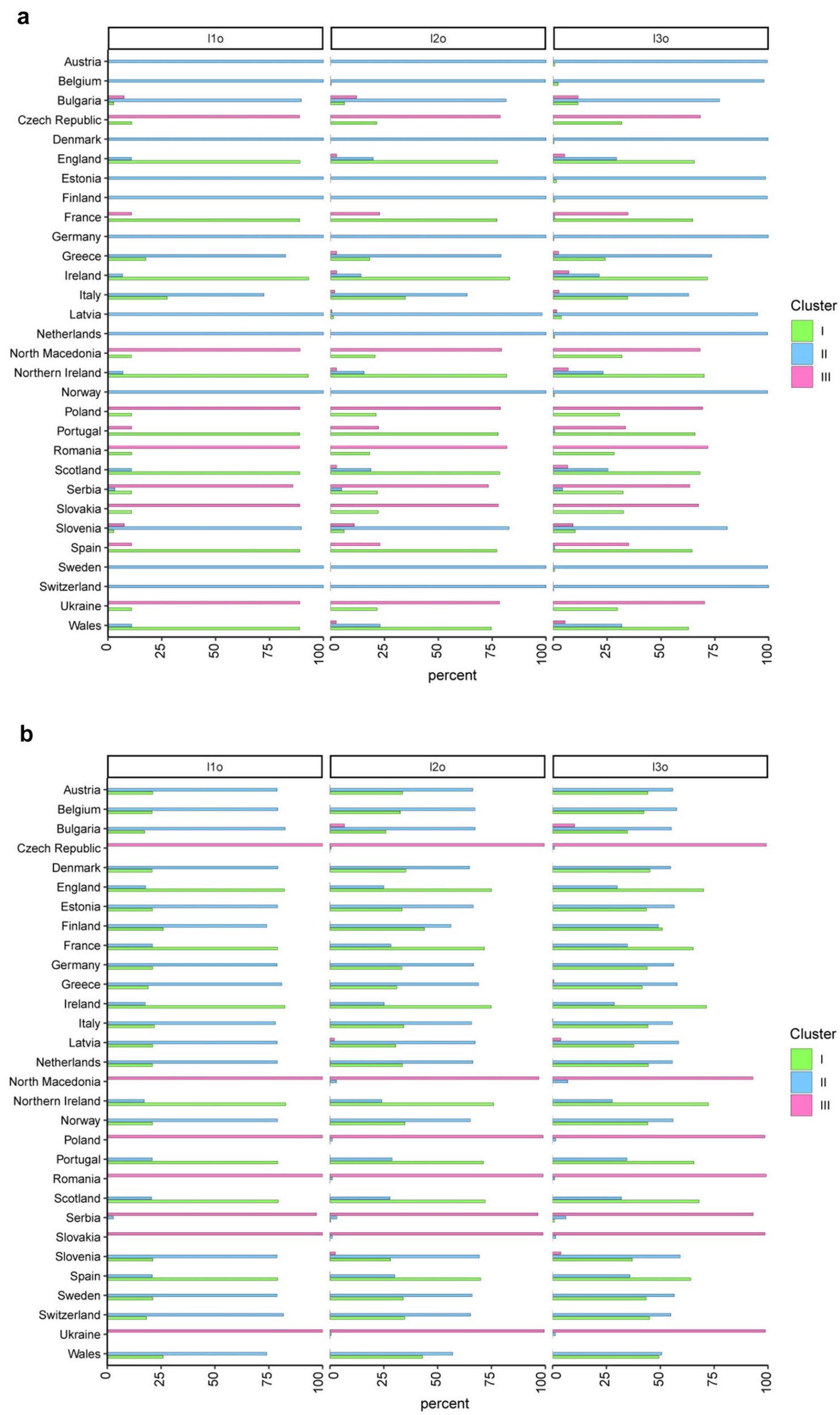

Fig. 5 Assignment probability and validation of clustering from correspondence analysis. **a** Assignment to clusters based on respondent (beekeeping operation) data leaving-one-out (l1o), leaving-two-out (l2o) and leaving-three-out (l3o) in percent. **b** Assignment to clusters based on colony data leaving-one-out (l1o), leaving-two-out (l2o) and leaving-three-out (l3o) in percent. Calculations with 1000 repeats for each leave-out validation

Other countries, like North Macedonia, Latvia and Bulgaria (where around 30% of beekeepers perform this monitoring), require more training, technical assistance and provision of information to beekeepers to explain the importance of *Varroa* surveillance.

We found a clear spatial variability and segregation of *Varroa* control methods applied in European countries.

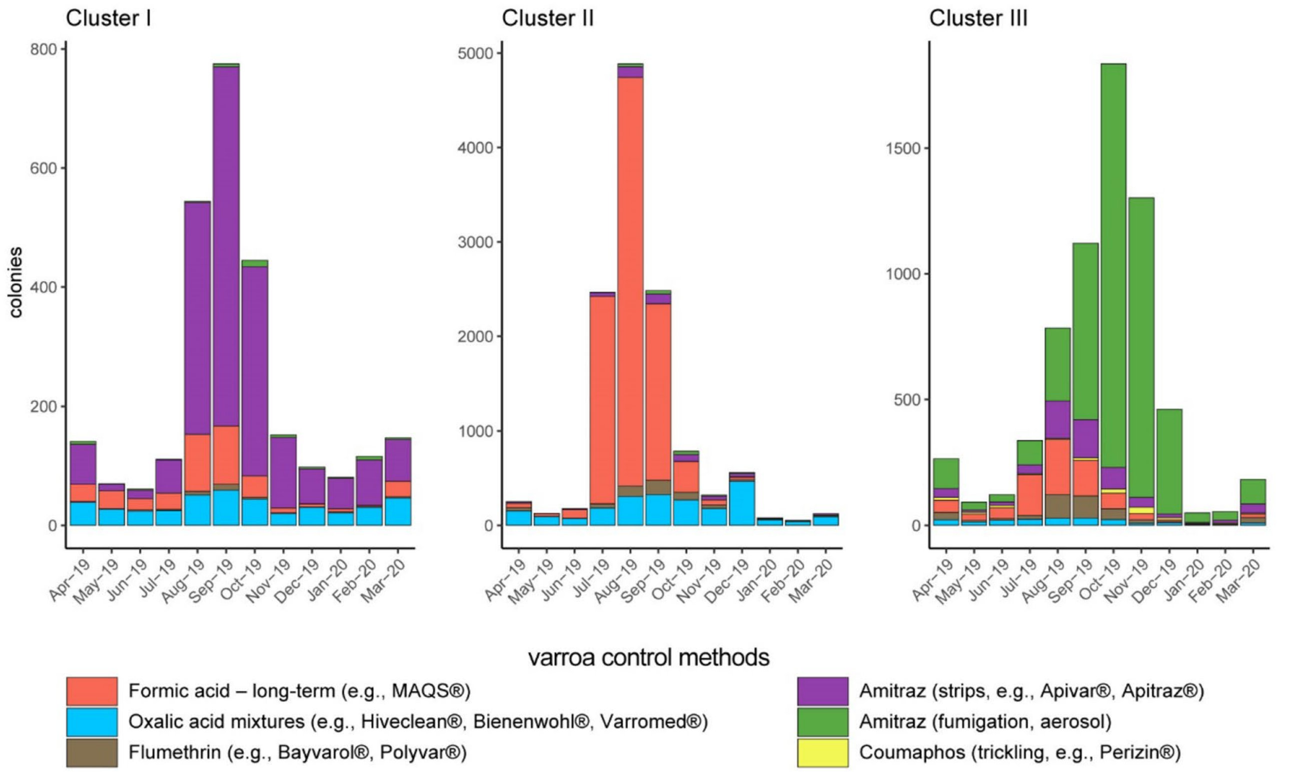

Fig. 6 Temporal dynamics of application of the six *Varroa* control methods contributing to cluster forming. Count of beekeepers applying a control option in a certain month for the three different clusters are shown

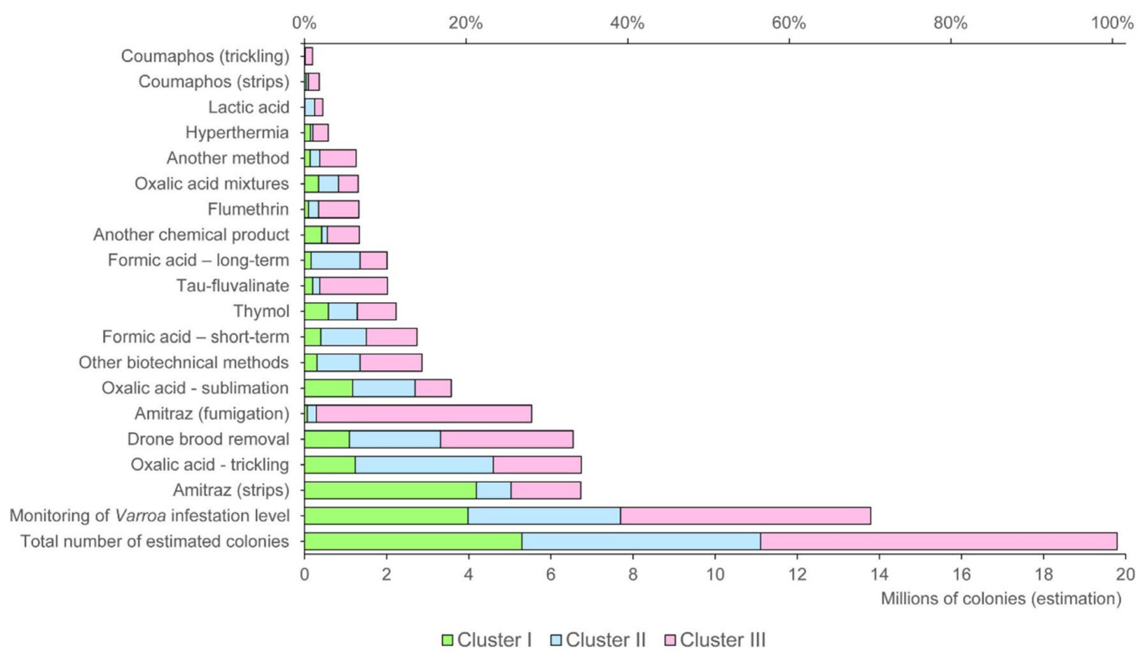

Fig. 7 Extrapolations of different *Varroa* control options on honey bee colonies in Europe. Extrapolations are made based on different numbers of colonies managed in countries shown in Table S2, adding up to almost 20 million hives (= 100%, secondary axis). Colours show how countries from the three clusters contribute to different control options. The contribution of countries in detail can be seen in Table S2

Haber et al. (2019) compared *Varroa* treatments used by small-scale beekeepers in Northern and Southern climate regions of the USA, but no significant differences are reported. They demonstrate, however, a clear preference of large-scale beekeeping enterprises for chemicals, compared to small-scale operations. In Europe, for example, formic acid treatments are widely applied in Austria, Denmark, Germany, Switzerland and Slovenia, whereas they are not frequently used in geographically distant countries like France, Greece and Norway. Striking differences can also be found for synthetic acaricides, which are rarely used in many countries, but certain substances are heavily applied in individual countries, like the Czech Republic and Romania (fluvalinate), Latvia (flumethrin), France and Spain (amitraz in strips), or several Eastern European countries (amitraz fumigation, see Tables 3 and 4). The application of synthetic acaricides probably is greatly influenced by history, the legal status and beekeeper attitude (Mutinelli 2016; Thoms et al. 2019; Jack and Ellis 2021). Biotechnical methods, like complete brood removal or brood interruption, are most often applied in Italy, by 38% of beekeepers. In Scandinavia and the British islands, as well as some other countries, these biotechnical methods (excluding drone brood removal and hyperthermia, which are surveyed and reported separately) are applied by less than 3% of beekeepers. Such methods, often combined with certain chemical treatments, have proved to be efficient for different regions (Büchler et al. 2020; Jack et al. 2020; Mancuso et al. 2020). Other *Varroa* control methods are largely dependent on environmental conditions like temperature (Ostermann and Currie 2004; Underwood and Currie 2003; Steube et al. 2021). Thus, one could expect a North–South zonation of control methods (Haber et al. 2019). Contrary to this, we found a probably historically or culturally conditioned East–West segregation of *Varroa* control methods in Europe. Even though the study was only conducted for the year 2020, the results can be considered representative for a longer period, as practices to control *Varroa* change only slowly from year to year. In some cases, methods may be new or unknown in some countries, but this can change over time. For example, an increase in organic acids and a decrease in essential oils were shown over a four-year period for small-scale beekeepers in the USA (Haber et al. 2019).

Sample sizes of our study varied among countries. Previous investigations showed that the representation rate (estimated % of beekeepers represented) in COLOSS surveys ranges from below 1% to circa 20% in different countries (Brodschneider et al. 2018; Gray et al. 2019, 2020). The representation rate here is similar, reaching more than 25% in Northern Ireland and Norway (Figure S6). An advantage of the analysis presented here is that these large differences in the number of beekeepers and responses were balanced out by standardization, keeping its influence for the clustering process low (Kassambara 2017; Greenacre 2016). Still, one could argue that the precision of estimates for a country is lower for countries with a low number of responses, but that is true for any survey-based study (van der Zee et al. 2013). In our study, the influence of one beekeeper's response on the representation of *Varroa* control methods ranged from circa 0.01% in Germany to 2% in Bulgaria, which had the lowest sample size. Consequently, care must be taken in interpretation of rare *Varroa* control options. These may not be precisely displayed in our study, for several reasons such as low sample size, biased sampling, or aversion of beekeepers to admit usage of non-authorized products.

Correspondence analysis and hierarchical clustering on principal components revealed three different *Varroa* control clusters in Europe. The six most important *Varroa* control methods to differentiate these clusters are based on the following active ingredients of veterinary medicinal products (and their means of administration): amitraz (strips), amitraz (fumigation), formic acid (long-term evaporation), fluvalinate, oxalic acid (trickling) and coumaphos (liquid) (Figs. 2, 3, 4). Alone, these six treatments are sufficient to generate the three clusters. Cluster I is characterized by a frequent application of amitraz strips, thymol and oxalic acid-based products and formed by eight Western European countries. Amitraz strips are applied most during August, September and October (Fig. 6). The formamidine amitraz has been in use in the USA and many European countries for more than two decades (Floris et al. 2001; Rinkevich 2020). For the USA, large variability of amitraz resistance of mites was reported, and resistance monitoring was suggested to ensure effective *Varroa* control. Cluster II is formed by 15 countries from Scandinavia, the Baltics, and Central-Southern Europe. Italy also joins this cluster, though we need to emphasize that we received most Italian responses from the northern part of the country. Luxembourg is not included in our survey, but would probably also join this cluster, according to Beyer et al. (2018). Most beekeepers in this cluster apply oxalic acid and formic acid. Formic acid is typically applied after honey harvest, in July, August and September (Fig. 6). No peak of usage of oxalic acid is visible in Fig. 6, as less used preformulated oxalic acid mixtures were found to be important for clustering and shown there. The more common (Table 3) trickling of oxalic acid shows a clear peak in winter, though sublimation is also used in summer (Supplementary Figure S5). Cluster III is formed by seven Eastern European countries characterized by dominant usage of amitraz-based products applied mainly via fumigation, with two seasonal peaks (late summer and a smaller one at the end of winter, Fig. 6). All countries in this cluster had registered national amitraz fumigation products shortly after the arrival of *Varroa* on their territory, around the mid-1960s (Rosenkranz et al. 2010).

The border between clusters II and III largely follows the Iron Curtain (the physical separation of communist Eastern Europe from the West) in Europe, except for Estonia, Latvia and Bulgaria. The pronounced differences in *Varroa* control across the border of two neighbouring countries with common history, but separated for decades by the Iron Curtain, have already been discussed for Austria and the Czech Republic (Brodschneider et al. 2019). We suggest that the two Baltic countries presented here, with no national amitraz fumigation products, abandoned Russian products after their independence. Since there was no influence from domestic acaricide producers, those former socialist countries were more open to accepting treatment models from abroad (EMA 2019). Bulgaria represents the smallest dataset of our analysis. A significant proportion of the surveyed beekeepers there are professionals engaged in breeding activities of the local honey bee *A. m. rodopica* (other authors see that as *A. m. macedonica*), and engaged in organic beekeeping, following rules compliant and coinciding with the measures widely used in cluster II. The Bulgarian honey bee was also shown to have good survival without *Varroa* treatment (Büchler et al. 2014). Greece is also part of cluster II, but is, of all countries forming this cluster, closest to cluster I according to the first 2 dimensions of the correspondence analysis (Fig. 2). In this country, usage of amitraz is probably under-represented, as a suspected off-label use and self-made application of liquid amitraz was poorly reported and often probably categorized as 'another method'.

Clusters I and III are both characterized by frequent amitraz usage; the difference is in the mode of application (use of amitraz strips in cluster I, in contrast to the fumigation of amitraz applied in cluster III). Long-term amitraz strips are considered a less labour-intensive modern approach applied mainly by commercial beekeepers, like multi-state large-scale beekeepers in the USA (Haber et al. 2019). Sample size and representation in southern countries in cluster I are rather low, but no unusually high proportion of large beekeeping operations responded in those countries. We do not consider this result as biased due to large-scale beekeeping in cluster I countries, as there were high proportions of large beekeeping operations responding in other clusters too (Table 2). In fact, several countries in cluster I have their own domestic producers of amitraz strips. The domestic origins, the trust in them by beekeepers and veterinarians, and the easy availability of amitraz strips on the market, probably contributed to wide use in those countries. Amitraz fumigation in cluster III, on the other hand, seems to be a historic left-over from the Russian-influenced era. The application of amitraz there goes back to the arrival of the mite and was coordinated by veterinary services. Intriguingly, beekeepers in cluster III are using the lowest number of different *Varroa* control methods (Fig. 1), which might be linked to the frequent use of synthetic acaricides (not only amitraz fumigation; see Tables 3, 4 and Fig. 3), a sufficiently high efficacy, or again is resulting from tradition in *Varroa* control.

Two countries show a remarkable usage of synthetic acaricides other than amitraz: in Latvia (cluster II) more than 30% of beekeepers use products based on flumethrin. In North Macedonia, more than 25% of beekeepers reported usage of liquid products containing coumaphos. These deviations from the other countries (see Tables 3, 4) are visible in dimensions 3 and 4 of the correspondence analysis (see Supplementary Figure S2) and would result in a separation of two single country clusters when the quantity of clusters during analysis is raised (see the dendrogram in Supplementary Figure S4). The report of the coumaphos usage, at least, in North Macedonia in the year used for analysis is questionable and needs to be treated with caution. In previous years, and the year following our investigation, never more than 4.6% of beekeepers in North Macedonia reported coumaphos usage (Table S4). The peak in reports here was reviewed and at the moment cannot be explained but will be further investigated.

We mainly present the countries' assignments to clusters based on respondent (beekeeper) data, not based on colony data. The only different assignment between beekeeper and colony data would be for the small dataset of Wales, which according to colony data would join cluster II, instead of cluster I. The assignment according to both datasets, and the robustness of the clustering, is seen in Fig. 5. We performed a leave-one-/two-/three-out analysis to understand whether the cluster formation would be different in a dataset composed of fewer countries than we present here. This showed that all countries would be consistently assigned to the same cluster, even if up to three datasets from any three countries had not been available. Swing candidates can be identified by the assignment to one cluster in considerably less than 100% of iterations in Fig. 5, and include, for example, Czech Republic, England, France, Greece, Italy, North Macedonia, Romania, Scotland, Serbia and Spain.

We can now only speculate about the drivers that lead to the different usage of *Varroa* control methods in the three identified clusters, but attribute several of the factors mentioned below to be responsible for this. An example of the decision-making process in *Varroa* control based on various factors, such as season, infestation level and cost, was published by Gregorc and Curk (2000) for Slovenia. Studies from the USA suggest that, rather than the geographical differences we found for Europe, the beekeeper's attitude may more strongly determine their choice of control method (Thoms et al. 2019; Underwood et al. 2019). Our results show that in some of the countries with historical Russian influence satisfaction with amitraz fumigation (including minimal residue and resistance problems) persists. Treatment strategies there were created by the authorities, e.g. fumigation with amitraz was obligatory, cheap or even free. In the South European region with a higher share of commercial beekeepers, preference for easy and fast (but less sustainable) solutions is visible, including use of long-lasting amitraz strips. Beekeepers in Central and Northern Europe are historically focused on local consumers, so they look for more sustainable and ecological solutions, lowering risks of residues in bee products.

There are probably many reasons that govern the spatial clusters of *Varroa* control strategies identified in this article. Only some factors shaping *Varroa* control have been scientifically studied so far. In Table 5, we summarize several factors possibly determining the application of different control measures, and references, if available. We suggest that different factors at work in different countries may be responsible for the usage of the respective *Varroa* control strategies. This field needs more research, to better understand and shape *Varroa* control in different countries. Mezher et al. (2021) found that the main sources of assistance concerning the control of honey bee diseases were beekeepers' associations and cooperatives, expert beekeepers and veterinarians, while one quarter to one third of beekeepers declared that they did not receive any kind of assistance.

Suggestions for further research

Monitoring and analysis should be extended to more European and non-European countries. Further research on the provenance of beekeeping knowledge and decision-making of beekeepers in *Varroa* control is needed (Mezher et al. 2021). Stratification for operation size should be made to understand the different subpopulations of beekeepers (hobbyists, sideliners, professionals). We know very well that large and small-scale beekeeping operations differ in many traits of their hive management, which probably also affects the overwintering survival of colonies (Seitz et al. 2015; Chauzat et al. 2016; Brodschneider et al. 2016; Haber et al. 2019; Oberreiter and Brodschneider 2020). Previous investigations have tried to identify best practice in *Varroa* control (van der Zee et al. 2014; Haber et al. 2019; Kulhanek et al. 2021). Based on our findings, it may be more suitable to discuss differences in mortality rates among countries by considering the *Varroa* control clusters identified. Multi-year honey bee colony loss rates could be aggregated for these clusters rather than geographically based on latitude, for example.

One *Varroa* control method is often not sufficient. Extension of our analysis will enable us to learn about which different control methods are commonly combined by beekeepers and which ones are repeatedly applied in a season (Beyer et al. 2018; Haber et al. 2019; Oberreiter and Brodschneider 2020). Historically, the first comparable data on *Varroa* control as presented in this article from COLOSS surveys have been available since 2014, which would further allow us to follow the historic formation and stability of the clusters identified in this article. Continued monitoring could reveal emerging trends in *Varroa* control, for example towards uniformity or further differentiation among European countries. Additionally, our improved understanding of weather effects on honey bees and *Varroa*, as well as climate change or the emergence of resistance, will modify treatments (Smoliński et al. 2021).

Suggestions for action by institutions

Beekeepers' associations and cooperatives and veterinary services should reinforce technical assistance and training of beekeepers for effective *Varroa* control following an integrated pest management approach. Concepts of integrated pest management applied to beekeeping parasites and pathogens should also be included in the training and education of veterinarians. Finally, an evaluation of the efficacy of the veterinary products on the market for *Varroa* control, considering the conditions and history of their application in each country, should be undertaken.

Conclusion

Varroacide product legislation, advisory services and effective *Varroa* control are important leverages in the reduction of honey bee colony losses (van der Zee et al. 2014). Policy-makers so far could only gauge the *Varroa* control measures applied in Europe through legal status in different countries, but no information on the usage was available (Mutinelli 2016; Jack and Ellis 2021). In this article, we present data of more than 28,000 beekeepers from 30 countries, surveyed about their *Varroa* control strategies using a standardized questionnaire. We found heterogeneous patterns among countries in the use of *Varroa* control methods, and for the first time provide empirical estimation of the proportions of beekeeping operations applying these. We identified three distinct *Varroa* control regimes applied over three large European regions, which probably derive from different reasons, including (beekeeping) culture, education and history, recent legislations and recommendations from authorities.

Table 5 Factors possibly determining the application of *Varroa* control measures

Factor(s)	Comment	Evidence
Culture and history	Culture and history could affect the prevailing control methods in a country	This study
Habit	Habit, i.e. when beekeepers do something often and regularly, they may be less inclined to change their behaviour	The effect of habit on *Varroa* control has not been studied
Economic costs	In beekeeping for profit, expenses for varroa control should be minimized	Gregorc and Curk (2000), Mancuso et al. (2020)
Marketing	Marketing could influence beekeepers' choice of a certain product	The effect of marketing on *Varroa* control has not been studied
Subsidies	Certain acaricides could be subsidised from the EU, national bodies or at local level	Regulation (EU) N. 1308/2013
Prescriptions	Differences in prescription policies of veterinary medicinal products at the national level do exist	Directive 2001/82/EC, Directive 2004/28/EC, Regulation (EU) 2019/6 (that shall apply from 28 January 2022)
Philosophy and cultural values	Beekeepers may for various reasons be more inclined or disinclined to use certain *Varroa* control options. Examples are applications of synthetic acaricides	Thoms et al. (2019), Underwood et al. (2019)
Efficacy	Efficacy is the portion of mites that are killed by a certain treatment. Efficacies of different methods are not the same in all countries due to different climatic or resistance conditions	Underwood and Currie (2003), Gregorc et al. (2018), Jack et al. (2020), Steube et al. (2021), Smodiš Škerl et al. (2021), Jack and Ellis (2021)
Method of action (with or without brood)	Strictly dependent on the characteristics of the active ingredient(s) and its mode of action (via evaporation, contact, sublimation)	Mutinelli (2016), Maggi et al. (2016), Smodiš Škerl et al. (2021)
Time of season where they can be applied	Treatments should be adjusted to the life cycle of the colony (broodright or broodless). Usage of oxalic acid in artificially broodless colonies is also possible	Smodiš Škerl et al. (2021)
Colony conditions (strength, infestation)	Strength and level of infestation of the colony should be checked before treatment	Pietropaoli et al. (2021)
Climatic conditions	The evaporation of some acaricides is dependent on temperature or humidity	Ostermann and Currie (2004), Underwood and Currie (2003), Steube et al. (2021)
Recommendations from beekeeping associations, extension workers, veterinarians, magazines, universities, teachers, influencers/ best management practices	In many countries, *Varroa* fighting strategies based on specific chemicals (organic or synthetic) are promoted by different bodies	B-Practices Consortium (2020), Kulhanek et al. (2021)
Legal status (regulatory framework)	There are large differences among countries in the number of registered products and in the frequency of checks on compliance with legal provisions	Directive 2001/82/EC, Directive 2004/28, European Medicines Agency, 2019, Regulation 2019/6/EU (that shall apply from 28 January 2022)
Availability of products	In countries with a small beekeeping sector, e.g. Belgium, operators from the supply chain of veterinary pharmaceutical products find no economic interest in the import of certain products for the treatment of varroa	Jack and Ellis (2021)

Table 5 (continued)

Factor(s)	Comment	Evidence
Resistances	Pyrethroids (tau-fluvalinate, flumethrin), formamidine (amitraz), and organophosphates (coumaphos) have been shown to produce mites that become resistant to the active ingredient	Faucon et al. (1995, 1996), Lodesani et al. (1995), Colin et al. (1997), Baxter et al. (1998), Fernandez and Garcia. (1998), Elzen et al. (1998a, b, 1999, 2000), Elzen and Westervelt (2002), Macedo et al. (2002), Trouiller (1998), Milani (1999), Spreafico et al. (2001), Thompson et al. (2002), Pettis (2004), Rodríguez-Dehaibes et al. (2005), Maggi et al. (2009, 2010), Johnson et al. (2013), Stara et al. (2019), Rinkevich (2020), Hernández-Rodríguez et al. (2021), Higes et al. (2020), Almecija et al. (2020), Millán-Leiva et al. (2021), Jack and Ellis (2021)
Residues	Synthetic substances, like pyrethroids (tau-fluvalinate, flumethrin), formamidine (amitraz), or organophosphates (coumaphos), result in residues in hive products	Wallner (1999), Korta et al. (2001), Tsigouri et al. (2004), Bogdanov (2006), Martel et al. (2007), Mullin et al. (2010), Pettis (2013), Kast et al. (2020), Kast et al. (2021), Abd El-Wahab et al. (2021), Murcia-Morales et al. (2021)
Side effects on bees	All control options can have effects on queen bees, drone spermatozoa, worker bees, intoxication, or worker bee background mortality	Pettis et al. (1991), Currie (1999), Higes et al. (1999), Rinderer et al. (1999), Sylvester et al. (1999), Haarmann et al. (2002), Collins et al. (2004), Pettis et al. (2004), Burley et al. (2008), Gregorc (2012), Berry et al. (2013), Charpentier et al. (2014), Tihelka (2018), Colin et al. (2020), EFSA et al. (2020), Smodiš Škerl et al. (2021)
Level of difficulty and equipment needed	Organic acids and essential oils often require technical and professional competencies (dosage, mode of administration, management of the colony during the treatment, possible side effects, …)	There are currently no studies evaluating the difficulty of use of different *Varroa* control methods
Time and number of applications needed	Strips are the easiest, fastest and most preferred mode of application. This method is also the most subject to bad practice (strips forgotten in the hive and left for longer than recommended). Organic treatments (organic acids and essential oils) usually require a longer time of application and multiple (repeated) administrations	Jacques et al. (2017), Sperandio et al. (2019), National varroa control programs
User safety	Some treatments require protective equipment for the user that is expensive and uncomfortable to wear	Gumpp (2004)

Author's contribution

RB and all authors conceived and designed the research. All authors organized and undertook data acquisition. RB and JSch analysed the data. RB wrote the manuscript. All authors read, had the opportunity to edit, and approved the manuscript.

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Declarations

Conflict of interest The authors declare no potential conflict of interest.

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