Proceedings CSI WORKSHOP COPENHAGEN

25 - 27 January 2015
University of Copenhagen, Faculty of Life Sciences, Dyrlægevej, 1870 Frederiksberg C
J. van der Steen & Robert Brodschneider
Summary

The topics of the annual workshop of the CSI pollen taskforce were to report the preliminary 2014 results, to discuss the best practice for statistical analysis of the database, the publication policy of the results, both the overall results and the national results, to share the expertise of the national coordinators after one year of CSI pollen practice, to discuss new ideas and off-shoot projects and the second tier analyses of the CSI pollen project. In this one and a half day workshop we managed to discuss and agree upon these issues.

In 2014 21 countries participated, and the picture manual was translated into 15 languages. It was reported that the network of participants would increase for the second year of the study.

It was agreed that the CSI pollen group will write a scientific report after the 2-year study period 2014-2015 has ended. In order to prevent miscommunication/misinterpretation of the CSI pollen results, the national coordinators will not make scientific reports of their national data prior to the report of the CSI pollen group, although this could be done after publication of the main report. However to keep the participants involved and to increase participation, national descriptive outcomes of the 2014 study can be presented and published in beekeepers journals and (social) media. The issue of best practice for the statistical analysis of the database was very well prepared by Alison Gray and Elfie Kalcher-Sommersguter; the overall database will be analysed with the GLMM/negative binomial distribution suggested by Alison and Elfie. The national coordinators can do the same for the national databases. Elfie and Robert will in 2015 provide the national data more frequently in order to give the national coordinators data for providing ongoing information to the participants. Discussion on the requirement for three CSI colonies per apiary (whether this must be fixed colonies to be sampled for the entire period or just three colonies in the apiary) concluded that using the same colonies is preferred but is not mandatory. In order to establish results concerning any massive monofloral pollen flow, the category > 50% of the same colour was added to the picture manual. We can all learn from each other and benefit from sharing experience. Therefore
presentations and articles by national coordinators can be shared and and used for dissemination by the other national coordinators after written approval of the author(s).

Three associated projects were initiated that make use of data / material from the CSI pollen project and the existing network of citizen scientists: the viral load of pollen, pesticide load of pollen and nutrient value of pollen will be further investigated.

Organisation of palynological analysis by light microscopy for countries without the resources to perform these second tier analyses will be coordinated by Asli Ozkirim, on a limited basis per country.

Next meeting

In October 2015 a Coloss meeting is planned in Slovenia. On that occasion we will have a ½ day workshop before or after the Coloss meeting.

Upcoming international sampling dates for northern hemisphere in 2015 are:

- 2.-5. April
- 23.-26. April
- 14.-17 May
- 4.-7. June
- 25.-28. June
- 16.-19. July
- 6.-9. August
- 27.-30. August
- 17.-20. September

Acknowledgement

The CSI pollen workshop was perfectly organised. Asger Søgaard Jørgensen, Ole Kilpinen, Flemming Vejsnæs and Annette Bruun Jensen have looked after us both for mind and body. The workshop was financially supported by the Coloss, Ricol, Danmarks Biavlerorening, Jakobsens Honey, Promilleafgiftsfonden for Landbrug, DLF Trifolium, Ole Heyne’s Fond, Dansk Plantevaern and University of Copenhagen.

All participants thank the organisers for this workshop in Copenhagen.
Programme

Sunday 25. January
19 h  Dinner at Wagamama, Solbjergvej 3, Frederiksberg, for those arriving Sunday.

Monday 26 January
6.30  Breakfast at hotel
8 – 9 h Registration CSI workshop
9 – 10 h
   Opening CSI workshop (van der Steen & Brodschneider))
   Schedule workshop
   Introduction
10 – 12 h (with coffee break)
   Selected Presentations (chair Søgaard)
   - Van der Steen: Dive into year 2 of C.S.I. Pollen: What has been and will be
   - Bozic: Color difference and diversity of pollen loads with Image J
   - Brodschneider: Second level investigation of C.S.I. Pollen in Austria
   - Odoux: Citizen Scientist Investigation for Pollen in France
12 – 13 h Lunch
13 – 17 h Statistical analysis of data (chair Gray) with coffee break
   - Best practice statistics
   - Complete database
   - National databases
19 h  Dinner at WagaMama, Solbjergvej 3, Frederiksberg

Tuesday 27 January
6.30  Breakfast at hotel
9 – 12 h (with coffee break)
   - Organisation of C.S.I. Pollen (chair Brodscheider & van der Steen)
   - Coordination by the national coordinators Number of participating beekeepers
     Experiences / improvements 2014
   - Communication National coordinator to beekeepers National coordinators to
     the press Exchange information between National coordinators Level 2
     experiments + preliminary data
   - 2015 activity
   - Time schedule 2015
   - National reports to the CSI ´ers
   - Termination of this 2-years study
   - Report
   - Publication strategy
   - What further needs to discuss
   - closure
12 – 13 h Lunch
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# Abstracts

The COLOSS Workshop on “CSI pollen”  
Copenhagen, 26. - 27 Januar 2015

## Abstract form

### Title (within 150 characters, Arial 12 pt. bold)

Second level investigation of C.S.I. Pollen in Austria: Palynological analysis

### Authors and Affiliations (Arial 12 pt. bold)

For the contact author only (typically the senior author), include email address, mail address and phone number. Denote contact author with an asterisk.

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### Text of Abstract (between 250 and 400 words)

In Austria, more than 50 beekeepers participated in the first level of C.S.I. Pollen. They collected pollen on 3 hives of one apiary up to 9 times from spring to autumn in the year 2014 and submitted the number of different pollen colors to the online database of the University of Graz. The aim of this second level investigation is 1. to obtain a detailed picture of pollen sources used by honeybees in different regions of Austria throughout the season, and 2. to contribute to the C.S.I. Pollen aim of matching beekeepers’ field estimation of colors to pollen richness in different habitats. In total, 38 of these beekeepers stored the pollen samples in the freezer and then sent a subsample of circa 20 g to the lab of the AGES, Department of Apiculture and Bee Protection, for detailed analysis of different pollen types by light microscopy. PONET, the AGES-pollen data base was used as pollen library: http://ponet.ages.at. In 2014, more than 750 samples of pollen pellets have been collected that will be classified in detail. Based on 500 analyzed pollen grains per sample the relative abundance of different pollen types was calculated as percentage. Preliminary results of 271 samples analyzed until the end of November 2014 indicate that there are differences in numbers of different pollen types collected by the bees between sampling dates, apiaries and the three sampled colonies of one apiary. Variation in the relative percentage of different pollen types between these factors was also observed. The analysis will be repeated during the 2015 season. The microscopic analysis of pollen origin was funded by the Austrian project “Zukunft Biene”. We greatly appreciate participating beekeepers for their great cooperation in this citizen science project.

Please send your abstract form to: Ole Kilpinen olek@biavl.dk (deadline is 10. December) 2014.
**Title** (within 150 characters, Arial 12 pt. bold)

Dive into year 2 of C.S.I. Pollen: What has been and will be.

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**Authors and Affiliations** (Arial 12 pt. bold)

For the contact author only (typically the senior author), include email address, mail address and phone number. Denote contact author with an asterisk.

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**Text of Abstract** (between 250 and 400 words)

For C.S.I. Pollen, beekeepers are invited to participate in this study by collecting pollen samples on a regular base. Citizen scientists establish the number of different colors of pollen pellets, and submit the results to a central online database in Graz. They are instructed with a picture manual in their preferred language. Each participating country has a national coordinator with language skills, assigned as their contact person for questions, troubleshooting, giving talks, writing articles in beekeeping journals and the internet. The study is performed throughout the active bee seasons of 2014 and 2015 with nine samplings per season at intervals of three weeks. Samplings are scheduled within a 4 day timeframe (Thursday to Sunday) from within citizen scientists can choose a day suitable for them to collect pollen samples. After pilot studies in 2013 in Austria and the Netherlands, we established the study 2014 in 21 European countries. Altogether, 466 beekeepers acted at least one time as a citizen scientist for C.S.I. Pollen in 2014. On average, 315 citizen scientists, spread across whole Europe, sampled up to three colonies per sampling. In total, more than 8000 pollen samples have been collected and analyzed by the beekeepers in 2014. Colonies were located (in descending order) in the following habitats: arable land, grassland, village, mixed forest, deciduous woodland, urban area, coniferous woodland, heathland/moorland, riparian forest, salt marsh. The present data allows us to evaluate the pollen diversity in the course of the bee season by identifying the habitats with high and low diversity of pollen available at different periods of the season. We suggest analyzing the data using either ANOVA or GLMM, which is to be discussed at the workshop. The extension of the study to other countries, and inclusion of more citizen scientists in 2015 is intended. C.S.I. Pollen is a COLOSS task force.

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Please send your abstract form to: Ole Kilpinen  olek@biavl.dk (deadline is 10. December) 2014.
We are experimenting with imaging software ImageJ to separate color values of pollen loads. The experiment included 72 samples from 2 locations each with 6 hives and 6 repetitions collected during summer 2011 in Karavanke region (Slovenia and Austria). During AT-SI Amc Promo BID project we obtained microscopical analysis of pollen samples from each location for separate days. Our process included right selection of imaging tools, selection of region of interest, background subtraction, color transformation and separation into CIaLab color space. Further threshold selection for binary processes was done using L*a*b*. After selected threshold watershed operation was used to separate grains of pollen. That enabled use of “Particle analysis” to get labeled particles and defined regions which is used to get separate L*, a* and b* values. This color space was used, because it can be better applied to calculate difference between colors. This method will be explained in detail and compared with other possibilities. Raw data was further analyzed to extract most similar grains of pollen loads as potential separate colors comparable to human eye separation. Calculated diversity index based on color was correlated with diversity index of microscopic determent pollen types. Other possibilities with this type of analysis will be discussed.
Spain sampling 2014 was composed by 43 hives and a total of 291 samples distributed in the middle north. During this first campaign, the South quadrant of the Iberian Peninsula has been sparsely sampled, the fact that we hope to correct it in the sampling of 2015. We have similar problem with both Spanish islands (Canary and Baleares islands). Under normal weather conditions, sampling in Iberian Peninsula should start in March, risking even when weather conditions are not the usual (as happened in 2014) and bees have not yet come to collect pollen. By contrast, the southern half of the Iberian Peninsula has a drier weather, especially in summer, which would end the season in August.

Considering only the homogeneous balls, we have identified 28 different taxa distributed in 41 colors (PANTONE- guide), what reveals the presence of different colors for the same taxon. These 41 colors founded correspond, highest to lowest abundance, with the following tones: orange, green, blue, brown (creams and toasted tonality included), violet, yellow and red. Also we have analyzed, in monospecific balls, nutritional components, such as protein, and healthy components, such as polyphenols. Kjeldahl method, in dry bee pollen (40 ° C / 4h), was applied to determine protein content. However, polyphenol values were assessed in fresh sample by Folin-Cicalteu method.

Mean protein values in monospecific balls range between 13-33.6%. Cistaceae present the lower values, however, Papaveraceae, Fabaceae and Boraginaceae the highest. Cistaceae family is one of the most abundant, and common, bee pollen in Mediterranean region, however is one of the poorest in protein content. The variability found in the protein content seems to depend specially of the botanical origin.

Polyphenols average content range between 1 and 2 g galic ac./100g pollen. Similarly to protein contents, polyphenols values are strongly connected with the botanical origin of samples.
Abstract form

**Title**  (within 150 characters, Arial 12 pt. bold)

Citizen Scientist Investigation for Pollen in France

**Authors and Affiliations**  (Arial 12 pt. bold)

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**Text of Abstract**  (between 250 and 400 words)

Biodiversity is threatened today in France as well as in other European countries and civil society needs a better understanding of environmental functioning. In addition, the honeybee could be endangered and less resistant to biotic and abiotic stressors when encounter a poor diversified pollen diet. In the framework of CSI Pollen, over 60 French beekeepers (commercial and amateurs, beekeeping organisations and education, pupils, scientists) shared their intention to belong to the pollen sampling net all over the country for 2014. The goal of this action is to assess the diversity of floral resources through the colour variation of the pollen loads brought by the worker bees at the colony. During the year 2014, 9 sampling periods were performed from April to September every 3 weeks, exactly at the same time that the European CSI net. In total almost 800 pollen samplings were carried out on beekeepers’ colonies. The maximum pollen colour diversity was reached during mid-May until mid-June, over 6 colours in average by colony, but the variation of this value was also highest at this time of year. The poorest diversity period set at the end of the season from the end of August, below 5 colours. At the apiary level, we could observe a higher number of pollen colours collected, reflecting a higher dispatching of the colonies on the different resources in a common environment, in particular in mid-August were found until supplementary 2 colours in average. Conversely, this difference between colony and apiary collect was low during July. Unfortunately many beekeepers met some difficulties to carry out their analyses all along the season, caused by rainy weather, swarming and queenless colonies, so that the samples number decreased after summer. CSI Pollen France was promoted at different level in French beekeeping net information (bee health magazine, ITSAP national bee institute website) as well as some Environmental event (Poitiers) and INRA institute media in particular a video promotion. We gratefully acknowledge all beekeepers who participated to this citizen investigation.
Abstract form

Title (within 150 characters, Arial 12 pt. bold)
The Contribution of Turkey to the CSI Pollen Project

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Text of Abstract (between 250 and 400 words)
Turkey is the active participant of CSI Pollen project. Total Pollen samples were collected by Turkish beekeepers from April to September during 3 days period in each month. The Turkish version of CSI Pollen Manual translated in Turkish by the national coordinator helped the beekeepers to follow the sampling method. After each sampling period, the beekeepers got Turkish mails from the system created by the cooperation of the international and national coordinators. The beekeepers entered their data according to mail directions. All steps of the project followed by the beekeepers and the national coordinator couldn’t checked the national data base. This is the main disadvantage of the project system. Although the beekeepers had some problems about the data, they contacted with the national coordinator and solved the problems.
At the end of the first year, pollen samples were collected from the six apiaries in Turkey. We have 52 beekeepers as the participants in the beginning in our mail list though, most of them couldn’t join the project actively due to several causes. One sample from Manisa province was sent to our Palinology laboratory for further pollen analyses.
The main aim of Turkish group is not only to collect more pollen in different regions of Turkey but also make pollen analyses I for the next year. By this way, we believe that all data from Turkey will reveal the pollen diversity in Turkey by real numbers observed by our citizien scientists.

Please send your abstract form to: Ole Kilpinen olek@biavl.dk (deadline is 10. December) 2014.
CSI-Pollen project in Sweden 2014

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Text of Abstract (between 250 and 400 words)

Around 70 beekeepers in Sweden participated in the project, they constitute around 15% of the total number of participants in Europe. At each collection occasion data from an average of 48 Swedish apiaries were submitted and 47 of the participating beekeepers collected pollen on six occasions or more. The average number of different colors (total for the three colonies in each apiary) was between 7 and 8 at most of the occasions, a little higher in early June, but lower in early April and at the two last collections. Results on differences between regions and habitats will be presented. The plan is to participate in the project even in 2015, and we hope that even more Swedish beekeepers will join.

Please send your abstract form to: Ole Kilipinen olek@biavl.dk (deadline is 10. December) 2014.
### Title (within 150 characters, Arial 12 pt. bold)

Results on CSI Pollen Study in Serbia and Montenegro

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### Text of Abstract (between 250 and 400 words)

During the season 2014 in Serbia and Montenegro took part in CSI pollen study with aims to evaluate pollen diversity per region, to identify seasonal changes in pollen diversity available to honey bees, to compare habitats and to enable beekeepers to simply compare pollen diversity of different apiaries. There are 13 beekeepers from bought countries that participated at least one time, 3 beekeepers from Montenegro and 10 from Serbia. Apiaries are located in central Serbia (north and south parts) and east Serbia and also in the costal and subcostal area of Montenegro. Pollen was sampled 8 times, from April to September, and pollen samples were analyzed for color diversity. Color which is represented with more than 20 pollen pellets in a sample of 20 gram is considered abundant, color which is represented between 3 and 20 pollen pellets in a sample of 20 gram was considered rare and color which is represented with 1 or 2 pollen pellets in a sample of 20 gram was considered very rare. All together 156 pollen samples were analyzed. More than 20 samples are taken only from one apiary, while between 10 and 20 samples were taken from 7 apiaries and less than 10 samples were taken from 5 apiaries. The highest color diversity of pollen is recorded in June when the average number of different colors was 14, and the lowest diversity of pollen is recorded in September when the average number of different colors was 4.7. Habitat types included were urban, village, grassland, heatland, decideus wood and riparian forest.
### Abstract form

**Title** (within 150 characters, Arial 12 pt. bold)

**Extending participation in CSI Pollen in the UK and Ireland**

**Authors and Affiliations** (Arial 12 pt. bold)

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**Text of Abstract** (between 250 and 400 words)

Participation in the CSI Pollen project currently involves 19 volunteers in England, 2 in Wales and 1 in Ireland, who have provided their own pollen traps, and 11 volunteers in Scotland. A couple of the Scottish volunteers provided their own traps, a couple were provided with traps by local beekeeping associations and the majority were provided by Science and Advice for Scottish Agriculture (SASA) for the 2 year duration of the project. The CSI Pollen project has not yet taken off in Ireland, however further volunteers will be sought through the running during winter of workshops by MC, national co-ordinator for CSI Pollen in Ireland, to demonstrate pollen analysis and encourage participation in CSI Pollen. Recruitment in Scotland, England and Wales was more successful than in Ireland, but there was felt to be room for expansion of the project as a whole and for better coverage of the countries.

Experience indicated that providing pollen traps was likely to encourage greater participation and to ease further recruitment of CS volunteers. Funding was sought to make this possible. We are very grateful to the C.B. Dennis British Beekeepers’ Research Trust for funding a large number of extra pollen traps to enable us to extend the project and to cover postal costs for level 2 of the analysis, as well as for assistance with costs at the publishing stage. The level 2 palynological analysis has been made possible by an extremely generous offer of help from Dr Asli Özkirım of the University of Hacettepe, Ankara, Turkey, to whom we are...
also very grateful.

As a result we expect to be able to extend participation in the project to involve a further 20 volunteers in England and Wales, 12 more in Scotland, and a further 20 volunteers in Ireland and Northern Ireland combined for year 2 of the project. Recruitment will aim to involve beekeepers who are well distributed across each country.

We hope that the improved geographical coverage of the British Isles and Ireland will enable us to obtain a good picture of the foraging opportunities available to honey bees across the spring/summer season and to highlight any clear geographical differences that may exist in forage availability within the UK and Ireland. There is already evidence of geographical variation in diversity. Involvement of more CS volunteers will also enable wider dissemination of the results to inform beekeepers.

Please send your abstract form to: Ole Kilpinen olek@biavl.dk (deadline is 10. December) 2014.
CSI Pollen is the only COLOSS Task Force which gives common Citizens (i.e. willing beekeepers) the opportunity to become Scientists. In fact, CS (Citizen Scientists) are actually included and involved in the research project. This is the most innovative characteristic of the Task Force, together with the significant topic studied: the importance of pollen quality and diversity for honey bee health.

CSI Pollen has been carried out in Italy in 2014 and the data collected by the Italian beekeepers (CSs) will be presented. Furthermore, both positive (i.e. high amount of data collected, dissemination of specific/technical knowledge among beekeepers, …) and negative (i.e. data missing or biased due to the non-scientific background of some CSs, relatively low participation, …) aspects of the project recorded during the first year will be discussed. Ideas and suggestions regarding the CSI Pollen project in 2015 will be presented.

In addition, recent studies on the importance of pollen quality on honey bee health, collected within the Italian National monitoring project “BeeNet”, will be shown. The health of around 3000 honey bee colonies during the last years have been recorded and the results presented show the influence of pollen quality (i.e. protein content, ..) on honey bee colonies survival. Information on the land use of the area surrounding the apiaries are studied too.

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Abundance and diversity of pollen collected by honey bees; a case study in Romania, based on the preliminary data within Coloss CSI Pollen

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This paper presents the preliminary data obtained in Romania within Coloss CSI Pollen, during 2014, concerning abundance and diversity of pollen collected by honey bees. The data were obtained from 5 different habitats, where 21 CSI beekeepers have collected, at least once, pollen samples from their hives. As biological material was used *Apis mellifera carpatica* breed.

Data on pollen collected by honey bees (average over three beehives) have shown that the pollen was abundant (as quantity) and much diversified as number of colours in April (14 colours), and little abundant and diversified in September (1-2 colours). Despite the high diversity of pollen colour from April (14 colours) compared to May-June (6-8 colours), there are less melliferous plant species (as blooming period) in April than in May-June period when, according to the Romanian beekeeping literature, there are a "blooming explosion" of botanical species very attractive for honey bees, due to their nectar and pollen richness. We could conclude that the honey bees were focused on the nectar gathering instead of collecting pollen, during periods rich in botanical species. Therefore, the lack of botanical species rich in nectar promotes to honey bees an intense activity of pollen harvest.

In terms of pollen colours per hive belonging to the same beekeeper, significant differences between the three honeybee families were recorded. Preliminary analysis of apportionment of pollen source has showed an intraspecific variability picking. Honey bee families have not preferentially collected from the same botanical source. Some bees families have adopted the strategy to collect pollen from one botanical species, while others families from a large number of botanical species.

In terms of pollen collected per habitat, the colour number of pollen showed that habitats with meadow vegetation are the most valuable habitats. Honey bees from such habitats have collected very abundant and diversified pollen, throughout the 9 samples of pollen harvest. Grasslands have provided abundant quantity of pollen by various colours only during spring-summer period, while land with intensive agriculture have provided a smaller amount of pollen, with a few colours, but the pollen presence was fairly stable throughout the 9 pollen samples.
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<td>CSI (Pollen) Switzerland</td>
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<td>For the contact author only (typically the senior author), include email address, mail address and phone number. Denote contact author with an asterisk.</td>
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<td>Geoff Williams1,2*, Gina Retschnig1,2, Vincent Dietemann2</td>
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<td>1 Institute of Bee Health, University of Bern, Bern Switzerland</td>
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<td>2 Agroscope, Swiss Bee Research Centre, Bern, Switzerland</td>
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<td>*<a href="mailto:geoffrey.williams@vetsuisse.unibe.ch">geoffrey.williams@vetsuisse.unibe.ch</a></td>
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<td>Nineteen beekeepers from Switzerland participated in the international COLOSS CSI pollen study in 2014 to assess pollen forage diversity for honey bees. Corbicular pollen was collected using front hive-mounted pollen traps approximately every three weeks between April and September, for a total of 9 sampling periods. Beekeepers defined diversity as number of different colour shades of collected pollen. Diversity was highly temporally and spatially variable. Highest and lowest diversities were observed in June and September, respectively; diversity ranged from an average of 4 to 11 different colour shades during the entire sampling period depending on the region. Further recruitment of citizen scientists is expected for 2015 sampling.</td>
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### Abstract form

**Title** (within 150 characters, Arial 12 pt. bold)

A short report on the CSI Pollen project in Scotland

**Authors and Affiliations** (Arial 12 pt. bold)

For the contact author only (typically the senior author), include email address, mail address and phone number. Denote contact author with an asterisk.

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**Text of Abstract** (between 250 and 400 words)

We recruited beekeepers in Scotland to participate in CSI Pollen through direct local contacts, by approaching local beekeepers’ associations for support in funding pollen traps, and emailing those respondents to our series of winter loss monitoring surveys who had already provided their email contact details, in order to generate interest and to seek participants directly. We placed a short advertisement for the project on the Scottish Beekeepers’ Association (SBA) website, directing members to a dedicated webpage set up locally by AG for the Scottish participants. MP spoke briefly about the project at the Annual General Meeting of the SBA. AG also arranged for an online discussion forum for participants. MP acts as national co-ordinator for CSI Pollen in Scotland, as well as being a CS volunteer himself.

Three local beekeepers’ associations were prepared to fund pollen traps for one or two volunteers but were only able to recruit 2 volunteers in total from their members. Two other volunteers provided their own traps. However what enabled more than this small number of beekeepers to participate was the fact that after we approached Science and Advice for Scottish Agriculture (SASA) with details of the project, they generously agreed to supply 24 pollen traps to enable a further 8 beekeepers to become CS volunteers. We are grateful to SASA for the use of these traps for the 2 year duration of the project, and to Dr Fiona Higlett for arranging this and also for assisting with distribution of these traps to beekeepers through the national bee inspector network in Scotland.
Of the beekeepers who initially volunteered, 3 dropped out early on and 11 have participated on an ongoing basis. The recruitment of CS volunteers did result in a reasonably wide geographical scatter of beekeepers, including one in the far north of Scotland where environmental conditions differ from those in the more populated areas further south.

We plan to extend the participation in CSI Pollen in year 2, in a collaborative funded project with the national co-ordinators in England and Wales and Ireland, reported on separately.

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