



Agricultural institute of Slovenia

The COLOSS Workshop on “Varroa control strategies”

Bled, Slovenia, May 22nd - 23rd 2014



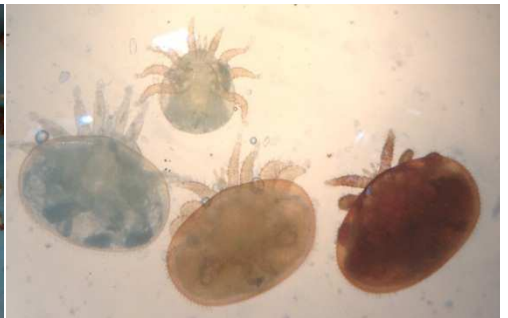
Univerza v Ljubljani
Veterinarska fakulteta



Ricola Foundation
Nature & Culture

Organized by the «Varroa Control Taskforce»

Editor: Aleš Gregorc



Agenda

| TIME | PROGRAM |
|---------------------------------|--|
| 21st May 2014 | |
| 19:00 – 21:00 | Welcome dinner |
| 22nd May 2014 | |
| 08:30 – 08:45 | Registration |
| 08:45 – 09:00 | Welcome and plenary session: organizational matters Aleš Gregorc |
| | 1. session: Organic and chemical treatments: Chair: Aleksandar Uzunov |
| 09:00 – 09:10 | Ana Sofia Lima: Volatile phytochemicals from Portuguese aromatic flora as a potential alternative for <i>Varroa destructor</i> control |
| 09:10 – 09:20 | Noureddine Adjlane: Field experiment to determine the efficacy of some acaricides used to control <i>Varroa destructor</i> on <i>Apis mellifera intermissa</i> in Algeria |
| 09:20 – 09:30 | Aleš Gregorc: Varroa mites control in honey bee colonies using organic and chemical substances |
| 09:30 – 10:40 | Mary Frances Coffey: Efficacy and tolerability of authorized Varroa treatments in Ireland |
| 09:40 – 09:50 | Marco Pietropaoli: Homeopathic approach to the treatment of <i>Varroa destructor</i> : results of <i>Phosphorus 30CH</i> and <i>Nosode</i> field trials |
| 09:50 – 10:00 | Stefan Berg: Results from a field test with the “Varroa-Gate” |
| 10:00 – 10:50 | Workshop: organic and chemical treatments: three groups |
| 10:50 – 11:20 | Reporting on the Working Theme and discussion |
| 11:20 – 11:40 | Coffee break |
| | 2. session: biotechnical & IPM control: Chair: Antonio Nanetti |
| 11:40 – 11:50 | Raffaele Dall'Olio: “Early brood removal” as alternative drug free strategy for Varroa control |
| 11:50 – 12:00 | Benjamin Dainat: Dispenser robustness under different temperature and humidity conditions for Formic acid treatment against <i>Varroa destructor</i> |
| 12:00 – 12:10 | Tjeerd Blacquièr: Cookbook based Varroa control for fewer colony winter losses |
| 12:10 – 12:20 | Antonio Nanetti: Removing heavy summer Varroa infestations with queen caging and oxalic acid |
| 12:20 – 12:30 | Aleksandar Uzunov: Potential of brood removal method for sustainable Varroa control |
| 12:30 – 12:40 | Giovanni Formato: Comparison of two different methods to estimate the amount of <i>Varroa destructor</i> in honey bees |
| 12:40 – 13:30 | LUNCH |
| 13:30 – 14:20 | Workshop: Biotechnical & IPM control: three groups |
| 14:20 – 14:50 | Reporting on the Working Theme and discussion |
| | 3. session: national Varroa control strategies Chair: Aleš Gregorc |
| 14:50 – 15:00 | Per Kryger: Improving the current strategy for Varroa control in Denmark |
| 15:00 - 15:10 | Ivana Tlak Gajger: Varroa disease control and eradication procedures in Croatia |
| 15:10 - 15:20 | Patricia Aldea Sánchez: Factors affecting sanitary control of <i>Varroa destructor</i> in Chile |
| 15:20 - 15:30 | Vincent Dietemann: Varroa control experiments and experiences |
| 15:30 - 15:40 | José Antonio Ruiz: Strategy to control Varroa in Tenerife island |
| 15:40 - 15:50 | Alessandra Giacomelli: Investigation on honey bee colony losses in Italy: the role |

COLOSS Workshop “**Varroa control strategies**”, 22. – 23. 5. 2014

| | |
|--|--|
| | of Varroaosis |
| 15:50 – 16:10 | Coffee break |
| | |
| 16:10 – 17:00 | Workshop: national Varroa control strategies: three groups |
| 17:00 – 17:30 | Reporting on the Working Theme and discussion |
| 17:30 – 18:30 | Final discussion, common experiments |
| 20:00 – 22:00 | Dinner |
| 23rd May 2014 | |
| | 4. session: breeding for resistance Chair: Ralph Büchler |
| 09:00 - 09:10 | Cecilia Costa: Is the trait “Varroa Sensitive Hygiene” present in European colonies? |
| 09:10 - 09:20 | Ralph Büchler: Selection on Varroa sensitive hygiene (VSH) in European honey bees |
| 09:20 - 10:20 | Workshop: breeding for resistance: three groups |
| 10:20 - 10:50 | Reporting on the Working Theme and discussion |
| 10:50 - 11:10 | Coffee break |
| 11:10 - 12:30 | Plenary conclusion discussion: <ul style="list-style-type: none"> - common experiments Varroa control - common project Varroa tolerant colonies and breeding experimental set up and their participants; |
| 12:30 - 14:00 | LUNCH |
| Option 14:00 - approx 18:00 | <i>Departure or Boat trip to Bled Island and/or Bled Castle and/or Apiculture Museum Radovljica (depend on weather)</i> |
| End of workshop meeting | |
| | |

Registration on site

Registration fee: 50 €

Table of Contents

| | |
|---|----|
| Lima A.S., A.C. Figueiredo and M. Vilas-Boas. Volatile phytochemicals from Portuguese aromatic flora as a potential alternative for <i>Varroa destructor</i> control | 6 |
| Adjlane N., N. Haddad and N. Chenane. Field experiment to determine the efficacy of some acaricides used to control <i>Varroa destructor</i> on <i>Apis mellifera intermissa</i> in Algeria..... | 8 |
| Gregorc A. and Planinc I. Varroa mites control in honey bee colonies using organic and chemical substances | 10 |
| Coffey M.F. and Professor J. Breen. Efficacy and tolerability of authorized Varroa treatments in Ireland | 12 |
| Pietropaoli M., A. Giacomelli, M. Pizzariello, G. Brocherel and G. Formato. Homeopathic approach to the treatment of <i>Varroa destructor</i> : results of Phosphorus 30CH and Nosode field trials..... | 14 |
| Lodesani M., S. Franceschetti, and R. Dall’Olio. “Early brood removal” as alternative drug free strategy for Varroa control..... | 16 |
| Dainat B. and V. Dietemann. Dispenser robustness under different temperature and humidity conditions for Formic acid treatment against <i>Varroa destructor</i> | 18 |
| Blacquièrè T., B. Cornelissen, C. van Dooremalen, C. HokAhin and J. vander Steen. Cookbook based Varroa control for fewer colony winter losses..... | 20 |
| Cabbri R., M.S. Russano and A. Nanetti. Removing heavy summer Varroa infestations with queen caging and oxalic acid | 1 |
| Uzunov A. and R. Büchler. Potential of brood removal method for sustainable Varroa control..... | 24 |
| Pietropaoli M., M. Pizzariello and G. Formato. Comparison of two different methods to estimate the amount of <i>Varroa destructor</i> in honey bees | 26 |
| Kryger P., H. Skovsgård, F. Vejsnæs and O. Kilpinen. Improving the current strategy for Varroa control in Denmark | 27 |
| Tlak Gajger I. and Z. Tomljanović. Varroa disease control and eradication procedures in Croatia | 29 |
| Aldea P. and Rodríguez R. Factors affecting sanitary control of <i>Varroa destructor</i> in Chile | 31 |
| Dietemann V. Varroa control experiments and experiences | 33 |

COLOSS Workshop “**Varroa control strategies**”, 22. – 23. 5. 2014

| | |
|---|----|
| Ruiz J.A., Z. Hernández and A. Bentabol. Strategy to control Varroa in Tenerife Island | 36 |
| Giacomelli A., M. Pietropaoli and G. Formato. Investigation on honey bee colony losses in Italy: the role of Varroaosis..... | 38 |
| Costa C., M. Bienenkowska, R. Büchler, L. Charistos, M. Drazic, F. Hatjina, N. Kezic, M. Mladenovic, B. Panasiuk, S. Rasic, A. Uzunov and J. Wilde. Is the trait “Varroa Sensitive Hygiene” present in European colonies? | 40 |
| Büchler R. Selection on Varroa sensitive hygiene (VSH) in European honey bees | 43 |

Volatile phytochemicals from Portuguese aromatic flora as a potential alternative for Varroa destructor control

A.S. Lima^{1,2}, A. C. Figueiredo¹, M. Vilas-Boas^{2*}

¹ Universidade de Lisboa, Faculdade de Ciências de Lisboa, Departamento de Biologia Vegetal, IBB, Centro de Biotecnologia Vegetal, Campo Grande, 1749-016 Lisboa, Portugal

² CIMO, Centro de Investigação de Montanha, Escola Superior Agrária, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Apartado 1172, 5301-855 Bragança, Portugal

Author for correspondence: mvboas@ipb.pt
Tel. : (+351) 273 303 237

High honeybee mortalities have been reported in the last years in many countries and Portugal is no exception in this matter, although the number of total hives is still fairly constant. In 2013, there were 16.700 registered beekeepers in Portugal, counting more than 566.000 hives, where the Varroa mite is still the greatest threat for Portuguese apiculture (PAN 2011-2013). The Portuguese National Authority for Animal Health authorizes the use of acaricides such as Apistan, Bayvarol, Apivar, Apitraz, Apiguard, Apilife var, Thymovar, and recently MAQS. However, the regular or misapplication of those synthetic and natural compounds has accelerated mite population resistance or tolerance development in the last years. Therefore, alternative and clean acaricides for Varroa control are imperative. With a typically Mediterranean climate, the Portuguese flora is very rich counting with almost 4.000 *taxa*, 385 of them are endemic species (Sequeira *et al.*, 2010). The aim of the present work is the assessment of aromatic plants products, namely volatile phytochemicals such as essential oils (EOs), as well as decoction waters and hydrolates, as a source of acaricide compounds for Varroa control. EOs isolated from Apiaceae, Asteraceae, Lamiaceae, Myrtaceae and Poaceae species from the Portuguese flora were assessed for acaricidal activity. The EOs were isolated by hydrodistillation and analyzed by GC e GC-MS. The EOs effect on Varroa and on the honey bees was evaluated by the complete exposure method, using both Varroa-parasitized newly emergent bees and adult bees. Different EOs concentrations were tested, with five replicates per experiment (n = 25 bees/tested concentration). Blank, negative and positive controls were included. *In vivo* bioassays were done under controlled temperature and RH, and mites mortality and the toxicity effects on bees assessed after 6, 12, 24 and 48h. Some EOs showed repellent and/or lethal effect on mites. Running experiments to evaluate the EOs concentrations with less toxic effects on the bees and the minimum lethal dose for Varroa are being done. The effect of other plant extracts and sub-products of the hydrodistillation process (decoction waters and hydrolates) on Varroa mites and on honey bees are also under evaluation.

Sequeira *et al.* (2010). *Checklist da Flora de Portugal (Continental, Açores e Madeira)*. Associação Lusitana de Fitossociologia (ALFA).

PAN 2011-2013 – Programa Apícola Nacional, Triénio 2011-2013. Gabinete de Planeamento e Políticas, Ministério da Agricultura, do Desenvolvimento Rural e das Pescas.

A. S. Lima is grateful to the FCT for PhD grant SFRH/BD/76091/2011. This study was partially funded by FCT, under PTDC/CVT-EPI/2473/2012, Pest-OE/AGR/UI0690/2011 and Pest-OE/EQB/LA0023/2011.

For your remarks

Field experiment to determine the efficacy of some acaricides used to control *Varroa destructor* on *Apis mellifera intermissa* in Algeria

N. Adjlane^{1*}, N. Haddad² and N. Chenane³

^{1*} Department of Biology, Faculty of Science, M'hamed Bougara University of Boumerdes;
e-mail: adjlanenouredine@hotmail.com

² National Centre for Agricultural Research and Extension, Bee Research Department,
P.O. Box 639, Baq'a 19381, Jordan

³ Institut technique des élevages, Baba Ali, Alger, Algérie

Author for correspondence: adjlanenouredine@hotmail.com

Varroa mite has become a major concern of beekeepers since the discovery of the first cases of infestation in the eastern areas of Algeria in 1982 in hives of honey bee *Apis mellifera intermissa*, and since that time many acaricides were used against it. Due to external factors such as climate and/or the application methods, the effectiveness of these products was fluctuating. The objective of this experiment was to study the efficacy of manufactured plastic strips impregnated with amitraz 500 mg (Apivar®, VETO-PHARM), with flumethrin 0.06% (Bayvarol®, Bayer HealthCare) and tau-fluvalinate 0.8 g (Apistan®, VitaEurope Limited), aside with home made strips of tau-fluvalinate and amitraz; as a common practice, these strips are introduced into the colonies and left several months. The experiment was conducted in a private apiary of a professional beekeeper in the Mitidja area in the region of Blida during the period between August to November 2013 on 70 *Apis mellifera intermissa* colonies kept in standard Langstroth hives. Bayvarol recorded the highest (92.14%) efficacy rate, followed by Apivar (86.35%) and then Apistan (74.65%). These rates are considered very low compared to the actual therapeutic value of three products recommended by manufacturers (99%). The home made traditional treatments had a very low efficacy where it was 32.37% for amitraz and 41.56% for Mavrik. Our study showed a reduction in the efficiency of commercial products (Apistan, Bayvarol and Apivar) and a very low efficiency for amitraz and Mavrik. Such results prove the high demand of searching for more effective treatments against *Varroa*.

For your remarks

Varroa mites control in honey bee colonies using organic and chemical substances

A. Gregorc¹ and I. Planinc²

¹ Agricultural Institute of Slovenia, Hacquetova 17, SI-1000 Ljubljana, Slovenia;

² Veterinary Faculty of the University of Ljubljana, National Veterinary Institute, Ljubljana, Slovenia

Author for correspondence: ales.gregorc@kis.si

Variety of organics and chemicals treatments was assessed in order to establish the efficacy against *Varroa* mites. Oxalic acid solution (OA), consists of 2.9 % oxalic acid and 31.9 % sugar in water and thymol based products Timovar (Andermatt BioVet AG) and Apiguard (Vita Europe Ltd., UK) were applied in a controlling the mite. Flumethrin, fluvalinate or amitraz were also comparatively applied in colonies. The flumethrin or fluvalinate applications triggered an average mite mortality at the two apiaries: 19.11% (± 14.62) and a 39.28% (± 10.47) reduction in the number of mites in slightly infested colonies and 94.30% (± 4.26) and 96.24% (± 3.14) in highly infested colonies.

The efficacy of an August treatment of Apiguard, a thymol-based gel, against the mite *Varroa destructor* was tested. OA treatments were conducted with 50 ml OA and sucrose-in-water solution (w/w), using oxalic acid dehydrate.

The relative mite mortality after four OA applications, two Timovar or two Apiguard applications, in colonies with capped brood was 41.80% (± 14.31), 14.35 % (± 10.71) and 18.93% (± 13.56) respectively. In the separate experiment two Apiguard applications and single amitraz treatment resulted in reducing the mite populations by 19.71% (± 12.61) and 23.89% (± 14.25) respectively. In the Mediterranean located apiary, Timovar and Apiguard treatments triggered 59.02% (± 17.28) and 46.50% (± 13.33) of the total mite reduction. Apiguard treatments resulted in a 3.76-times increase in *Varroa* mortality over the four week treatment period compared to untreated colonies. The rate of *Varroa* mortality was evenly spread among the four weeks of the Apiguard treatment. Apiguard killed 46% of the mites in the colonies, as we inferred from mite mortality following treatment with OA solution a month following the Apiguard treatment. By contrast, only 4% of the mites died during the same period among untreated colonies. Our results suggest that Apiguard is of limited efficacy during the brood season, but supposed to be enough effective in colonies with low level of infestation. The results indicate that OA trickling, two thymol formulations or Amitraz fumigation are of limited use during periods with brood. The possible use of OA and thymol based products against the *Varroa* mite in honeybee colonies and complementary apicultural methods performed in the apiaries is discussed.

For your remarks

Efficacy and tolerability of authorized Varroa treatments in Ireland

M. F. Coffey* and Professor J. Breen

University of Limerick, Dept of Life Sciences, Ireland

Author for correspondence: Mary.Frances.Coffey@ul.ie

Varroa destructor was first reported in Ireland in 1998 and subsequently the Irish Medicines Board authorized the use of two Varroacides, the chemical pyrethroid Bayvarol® and Apiguard®, a thymol based product. Initially Irish beekeepers used Bayvarol® almost exclusively and in 2010 following a survey carried out by the Department of Agriculture Food and the Marine the presence of pyrethroid resistant Varroa mites in honeybee colonies was confirmed. Two field trials in 2005/2006 and 2008/2009 also concluded that Apiguard, the alternative authorized treatment was insufficient as a sole treatment in Ireland due to high annual and colony variability, hence in 2010, ApiBioxal®, an oxalic based winter treatment was authorized. In a recent field trial the efficacy and tolerability of this product was assessed at the dosage rate recommended by the manufacturers (6% OA solution dissolved in 1:1 sugar solution or 2.3 g when administered using the vaporiser method) over a two year trial period (winter 2011 and 2012) and at the lower dosage rate recommended by the The Integrated Varroa Control Group (2000) for cool temperate climates (4.5% and 1g using trickling and vaporiser methods respectively). Preliminary analysis of the data indicates when the product was administered at a lower dosage concentration (4.5%) and quantity (1.3g), the mean percent efficacy achieved was not compromised, but the product was better tolerated by the colony. This year another relatively new Varroacide, Mite-Away Quick Strips has been authorized as a Summer/Autumn treatment. In the limited literature available, the reported efficacy and tolerability of this product is inconclusive and if colonies are treated during a honey flow there are some indications that a short withdrawal period maybe necessary before the honey is retailed, hence this year we are carrying out a field trial assess these parameters.

For your remarks

**Homeopathic approach to the treatment of *Varroa destructor*:
results of *Phosphorus 30CH* and *Nosode* field trials**

M. Pietropaoli*, A. Giacomelli, M. Pizzariello, G. Brocherel and G. Formato

Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana
Via Appia Nuova 1411, 00178 Rome
+39 320 60 333 90

Author for correspondence: pietropaolimarco@gmail.com

In summer 2012 IZSLT carried out two field trials to verify the acaricide efficacy and the strength of the bee hives after treatments with *Phosphorus 30CH* (Boiron Ltd) and *Nosode* (galenic drug from Varroa).

Phosphorus 30CH was administered for 35 days to 24 beehives (12 treated beehives and 12 untreated beehives in the same apiary); *Nosode* was administered for 42 days to another apiary of 21 beehives (11 treated and 10 untreated).

To assess the acaricide efficacy of the treatments we followed the EMA Guidelines of 2007 on veterinary medicinal products controlling *Varroa destructor* parasitosis in bees.

The acaricide efficacy obtained both with *Phosphorus 30CH* and *Nosode* resulted unsatisfactory. The strength of the hives of the treated groups were comparable to that of the control groups.

In conclusion, in our field trials, the above mentioned homeopathic treatments couldn't be considered a viable alternative to conventional treatments against *Varroa destructor*.

For your remarks

“Early brood removal” as alternative drug free strategy for Varroa control

M. Lodesani, S. Franceschetti and R. Dall’Olio

Agricultural Research Council, Honey Bee and Silkworm Research Unit (CRA-API)
Via di Saliceto, 80
40128 Bologna – Italy

Author for correspondence: raffaele.dallolio@gmail.com

A two-year experiment is ongoing from early 2013 at the CRA-API lab in Reggio Emilia. We are evaluating the efficiency of a drug free strategy to control Varroa mite infestation that involves removing brood combs in early spring, with the idea of reducing the mite population in a pivotal moment for colony development. Status of the colonies (amount of bees, brood and honey) is assessed at regular intervals using a modified Liebefeld method. Mite infestation levels are monitored on bees and in brood cells. Honey production is also considered as a test parameter.

To evaluate if the mite control technique also affects viral loads for DWV, ABPV and CBPV qRT-PCR is also performed on adult bees.

Preliminary data show a significant effect on mite infestation level in late summer.

For your remarks

Dispenser robustness under different temperature and humidity conditions for formic acid treatment against *Varroa destructor*

B. Dainat^{1,2,*} and V. Dietemann²

¹ Swiss bee health service, Schwarzenburgstr. 161, 3003 Bern, Switzerland

² Swiss bee research Centre, Schwarzenburgstr. 161, 3003 Bern, Switzerland

Author for correspondence: benjamin.dainat@agroscope.admin.ch

Phone: +41 5846 38201

Nowadays, colony survival relies heavily on Varroa treatment and the ability of the beekeeper to perform them successfully. An ideal treatment must be cheap, easy to perform, thereby reducing user error margin, robust for application under any kind of climate type, avoiding residues in hive products and of course highly efficient against the mite. To reach this goal, alternative Varroa control strategies have been developed at the Swiss bee research centre and collaborating laboratories in the last 25 years. The strategy after honey harvesting consists in using formic acid in late summer and oxalic acid in winter. Since the design of the original dispenser for formic acid, new models appeared on the market that were designed to overcome their dependency of the old model to temperature and humidity conditions. In summer 2013, to test their efficacy under various meteorological and beekeeping conditions, the newly funded bee health service and the Swiss bee research centre used 42 colonies, split in 4 apiaries. The beekeepers were given dispensers for random assignment to his colonies: N=13 FAM dispenser (original model developed by the Swiss Bee Research centre), N=13 Liebig dispenser, and N=13 Nassenheider professional. Temperature and humidity were recorded in each hives. Preliminary results show that beekeeper preferred the FAM dispenser for his simplicity of use. The efficiency of the 3 dispensers appears similar even with high humidity and temperature variance. These results and our experience in developing Varroa control methods will be of use for several of the aims of this workshop.

For your remarks

Cookbook based Varroa control for fewer colony winter losses

T. Blacquière*, B. Cornelissen, C. van Dooremalen, C. HokAhin and J. vander Steen

Bees@wur, Plant Research International, Wageningen, the Netherlands

Author for correspondence: tjeerd.blacquiere@wur.nl

Tel. : +31 317 481 330

Since the appearance in 2002 of resistance of the Varroa mite to fluvalinate in the Netherlands we only recommended the use of ‘soft’ acaricides: formic acid, oxalic acid and thymol products. In our three-yearly brochures we explained and recommended many possible ways for Varroa control. In essence we recommended to base application of treatments on Varroa infestation based on mite fall according to IPM principles.

Because of the lack of adoption by beekeepers and the increasing severity of the colony losses we decided in 2010 to abandon the IPM strategy based on mite fall, and started with a cookbook three course menu recipe. This recipe was described in the 2010 (and 2013 slightly corrected reprint) edition of our Varroa control brochure. This brochure was sent to all beekeeper association members. The new brochure may have been in use by some / many / all since the 2011 season. We hoped that it would add to lower losses in the springs of 2012-2014.

Since spring 2013 in addition to the Coloss questionnaire, bees@wur carries out an active quick telephone inquiry among a random selection of the members of the associations during the first or second week of April, just to have a fast figure for actual winter losses.

In the years 2003 – 2012 the winter losses ranged from 15 -29%, generally around 20%. Our quick surveys showed ~14% loss in 2013, and ~9 % in 2014. It is tempting to conclude that our cookbook is working, although we cannot prove we can hope this is true. If so we should carry on in order to reach the lowest limit (at zero-infestation) found in the DEBIMO of ~4% (Genersch et al., 2010). A web-based (voluntary) survey did show that >90% of the respondents did trickle oxalic acid in December or January last winter, the ‘Starter’ of our three course menu.

For your remarks

Removing heavy summer Varroa infestations with queen caging and oxalic acid

R. Cabbri, M. S. Russano and A. Nanetti*

Consiglio per la Ricerca e la Sperimentazione in Agricoltura, CRA-API, Research Unit of Apiculture and Sericulture, Via di Saliceto 80, 40128 Bologna, Italy

Author for correspondence: *antonio.nanetti@entecra.it
tel.: +39 051 353103

Uncontrolled Varroa populations, pharmacoresistance and need of organic acaricides are chief problems in many countries. This is particularly true in warm regions, where extended broodright periods let the infestations grow considerably and highly effective control concepts must be applied.

The oxalic acid may be used best once dissolved in sucrose solutions and trickled onto the colony. However the treatments attain high efficacy only in broodless conditions. Indeed, quick pharmacokinetics and incapability to penetrate the brood seals at effective doses make the substance unsuitable to hit reproductive mites.

Full summer infestations must be tackled with efficient treatments. However sufficiently active miticides may be unavailable. This fostered us to search a new organic method to control the summer infestations, when large amounts of brood are normally present.

The queen is caged for 25 days within the colony to let all the brood hatch and the mites turn into phoretic. An oxalic acid treatment is made thereafter.

The trials started in 2007. Initially the effects of oxalic acid administered by trickling and of queen confinement were tested separately.

Subsequent comparative field tests were made in North and South Italy against controls. Api Bioxal, an oxalic acid based acaricide recently registered in Italy, was taken into consideration as well.

The tests repeatedly confirmed high miticidal activity, good tolerability for the individual honey bees and for the colonies, non-significant effects of caging on the queen survival. In the different trials the efficacy averaged in the range 94-99%.

According to our results, this combination between a bio-technique and a treatment with a soft chemical may consistently reduce the severe Varroa infestations often experienced in warm countries.

To our knowledge, this is one of the most effective methods that can be used in an organic control concept against varroosis.

For your remarks

Potential of brood removal method for sustainable Varroa control

A. Uzunov^{2*} and R. Büchler¹

² Faculty of Agricultural Sciences and Food, University Ss. Cyril and Methodius in Skopje, Macedonia (,)

¹ LLH, Bee Institute, Erlenstrasse 9, 35274 Kirchhain, Germany

Author for correspondence: uzunov@zf.ukim.edu.mk

Tel.: 00 389 2 3255 100

Complete brood removal present most effective biotechnical method used for obstruction of mite population development in the colony. The potential of this method significantly depend of climatic conditions, colony's annual developmental trajectories, extend of brood season and food availability. In the pan-European experiment (Costa *et al.*, 2012), executed under different environmental conditions, brood removal was the only method used for Varroa control. In German location MBO 30 colonies from different origin were closely monitored on monthly basis for estimation of Varroa infestation level of adult bees. The colonies' average initial infestation level was 1.2% in October 2009 and progressed to an average of 10% (2.3 - 30.7%) until October 2011. Both years, subsequently to the applied brood removal, the infestation level in the colonies decreased for two (from 2.3 to 1.4%) and four (from 6.9 to 1.9%) folds. Consequently, the mite infestation levels in October in both years reached 13.4 and 10% which is slightly above economic threshold level.

After a single season (autumn 2009 - autumn 2012) 26.6% of the colonies were lost, but at the end of the studying period 33% of the colonies survived. Loss or supersedure of the queens and high mite infestation were dominant causes for observed losses. Our results indicated the potential for incorporation of the brood removal method in the annual integrated Varroa control program in the continental part of Europe.

Moreover, in the southern regions of Europe with prolonged and bimodal brood seasonality this method complements with ordinary colony's decline during the dry mid-summer season. This condition provides higher potential for introduction of the method as a regular api-technical method for Varroa control.

For your remarks

Comparison of two different methods to estimate the amount of *Varroa destructor* in honey bees

M. Pietropaoli, M. Pizzariello and G. Formato*

Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana
Via Appia Nuova 1411, 00178 Rome

Author for correspondence: giovanni.formato@izslt.it
Tel. : +39 320 60 333 90

In summer 2013 we compared two different methods to quantify *Varroa destructor* in beehives: powdered sugar (on-field test) or 70% ethanol solution (laboratory test).

In order to obtain comparable data, adult honey bee samples monthly collected (from May to July 2013) were taken from the same honeycombs of 20 hives located in the same apiary. A total of 60 measurements were realized for each of the two methods.

To statistically compare the goodness of the two methods it has been realized a Bland Altman plot.

The two methods resulted comparable within a *Varroa* infestation level ranging from 0.7% to 1.3%, while the powdered sugar method underestimated the amount of *Varroa* mite when the infestation values were higher than 1.3%.

Standardize a non-destructive method to quick on-field assess the infestation level of *Varroa* mite infestation is crucial (e.g. to verify the acaricide efficacy of the acaricide treatments). At the same time it could represent a starting point to define thresholds detailed to the season of the year beyond which it is not guaranteed the survival of colonies.

In the presentation the field trials results will be described and discussed.

For your remarks

Improving the current strategy for Varroa control in Denmark

P. Kryger¹, H. Skovsgård¹, F. Vejsnæs^{2*} and O. Kilpinen²

¹ Aarhus University, Agroecology, 4200 Slagelse Denmark

^{2*} Danish Beekeepers Association, Fulbyvej 15, 4180 Denmark

Author for correspondence: Per.Kryger@agrsci.dk

In Denmark there is a strong tradition for using a combination of drone brood removal, formic acid and oxalic acid to control Varroa mites in the hives. The vast majority of beekeepers use all or some of the methods in order to keep their bees healthy. The above system has been very successful over the years, but for some beekeepers the treatments seems not to work satisfactory. These beekeepers have high loss rates and very many mites in their colonies. In order to assist the beekeepers we have initiated a common project with support of the EU programme 1234/2007 to better understand the function of the organic acids, both on a fundamental level and from an applied point of view. Our current knowledge of the physical processes involved in the evaporation and dissipation of the acids within the hives does not allow us to make estimation of the functional constrains.

We have established a network of beekeepers across Denmark, with minimal variety in the type of hives, but with rather different timing of the Varroa treatment due to variation in the honey flow. This will allow us to make measurements in the field of the effect of Varroa treatment in relation to variable external conditions, in particular temperature and humidity.

In the lab we aim to conduct a series of experiments, with better control of parameters like humidity and temperature, in order to measure the effect on both Varroa mites and honey bees. In particular we have ordered a tool to measure the air concentration of formic acid to asses more accurately the variation within a cluster of honey bees.

For your remarks

Varroa disease control and eradication procedures in Croatia

I. Tlak Gajger^{1*} and Z. Tomljanović²

¹Department for Biology and Pathology of Fish and Bees, University of Zagreb Faculty of Veterinary Medicine, Heinzelova 55, 10 000 Zagreb, Croatia

² Advisory Service, 10431 Sv. Nedelja, Croatia

Author for correspondence: ivana.tlak@vef.hr

Tel. : 00385 1 2390 151

Varroosis is a disease of honeybee colonies (*Apis mellifera* L.) caused by the haemophagous mite *Varroa destructor* and is considered a major threat for apiculture and needs to be controlled because untreated colonies typically die within a few years due to damage to both pupae and adult bees, as well as consequences of secondary virus infections. A high rate of mite infestation in a honeybee colony and poor colony management have an important influence on beekeeping because damage to the host caused by Varroa disease results in decreasing numbers of honeybee colonies, so it is necessary to evaluate chemical and biological methods of treatments.

Currently in Croatia the unique model for control of Varroa disease is emphasized. This model include implementation of equivalent registered veterinary medical product in all honeybee colonies of all areas at “the same time”. Moment of treating colonies is different for most areas according different geographically and climatic regions, and consequently different honeybee pastures, but always after honey flow and harvesting honey. Aim is to do efficiently Varroa treating, but also production of honey without residues of acaricides and without risk for human health.

Last few years the Varroa problems are increasing because of resistance to approved veterinary medical products at some areas, multiple using some unapproved preparations in inappropriate dosages, effect of adverse environmental factors and different xenobiotic. Also, as a consequence of all above mentioned and additional stress caused by inappropriate technological activities there is higher prevalence of secondary honeybee diseases.

Research of Varroa control in Croatia include efficiency of different acaricides (active ingredients) at five testing apiaries situated in different geographically and climatic regions, especially in active beekeeping season. Aim of this research is to establish more appropriate period of treatments for every of regions and to determine which veterinary medical product can give the best result. Also, comparison of used acaricides at one apiary and all other testing apiaries is possible. Because some honeybee colonies coexist sustainably with Varroa mite without any chemical treatments we established one more testing apiary situated in continental part of Croatia. There are untreated honeybee colonies from all parts of country monitored for biological and rearing parameters. Material for honeybee rearing originated from surviving colonies with the best production and rearing characteristic.

For your remarks

Factors affecting sanitary control of *Varroa destructor* in Chile

P. Aldea* and R. Rodríguez

Centro para el Emprendimiento Apícola de la Universidad Mayor - Camino La Pirámide
5750, Huechuraba. Santiago, Chile

Author for correspondence: patricia.aldea@mayor.cl
Tel. : 56-2-23281241 – FAX: 56-2-23281258

Currently, the sanitary control of Varroa mite has become more difficult in Chile. Even more, the average infestation level is twice or more than few years ago, increasing the percentage of mortalities of the hives around the country since 20% in some parts of Chile to almost 78% in the south. However, the causes of such high levels of infestation were unclear because usually professional beekeepers have application records of different treatments. We determine the most common causes of colony losses in apiaries in different areas of Chile into which a high percentage of resistance to synthetic products (in average a 40 to 65% of resistance to flumethrin, amitraz and coumaphos was detected), failures in the implementation of organic treatments, limited monitoring of mite parasite load was found, among others. An important effect of small beekeepers or nonprofessional producer was found as a factor of dispersal of mites o reservoir to near apiaries. It was developed a pilot beekeeping management and Varroa control to the beekeepers participating in this initiative program. Also, a proposal for selection and breeding program of hives was prepared. Now, we are starting with the selection of strong families and the elimination of weaker hives inside the apiaries.

For your remarks

Varroa control experiments and experiences

V. Dietemann

Agroscope – Swiss Bee Research Centre, Schwarzenburgstrasse 161, 3003 Bern,
Switzerland

Author for correspondence: vincent.dietemann@agroscope.admin.ch

The Swiss Bee Research Center has been involved in the development of the so called alternative Varroa control methods developed together with several European bee institutes in the frame of a former COST Concerted Action “Coordination in Europe of integrated control of Varroa mites in honeybee colonies”. We have thus developed a deep knowledge of the necessary experimentation. Based on this experience and the information shared with the community, we have helped establishing standard methods through the BEEBOOK. In the past, we have been able to test and establish the Varroa control strategy across Switzerland and to monitor its efficiency through our own observations as well as more recently through the COLOSS survey. We thus have a good sense of the constraints and usability of Varroa control strategies in the field. We remain available for collaborations, be it towards the establishment and improvement of standards or for common experiments that will be discussed at this meeting.

For your remarks

STRATEGY TO CONTROL VARROA IN TENERIFE ISLAND

J.A. Ruiz¹, Z. Hernández² and A. Bentabol²

¹ Beekeeper Associations in Tenerife (APITEN)

² Casa de la Miel. Cabildo de Tenerife

Author for correspondence: jaruiz65@gmail.com

Honeys from Tenerife just got the Protected Designation of Origin. Environmental conditions in our Island offer great diversity of flora with many endemic species. However, these same conditions that make Tenerife the called “Island of Eternal Spring”, allow colonies can breed around year, but too, unfortunately, Varroa can go on reproducing continuously. So an integral and coordinated strategy to control Varroa has been proposed by veterinarian services of APITEN (Beekeeper Association in Tenerife). The basic sanitary calendar pays special attention to adapt therapeutic treatment to the environmental conditions and timing of blooms, the multiplication methods and the supplied feeding.

In this strategy, it is very important the synergy of sanitary plan to control Varroa and improve beekeeping practices in order to lead to get better productivity and save time and money.

In this sense, we should consider if financial support or funding to beekeepers in EU takes into account the number of hives is according to the law of Farrar and aiming to reach adequate performance, vigor and tolerance to pathologies.

Finally, it would be convenient to achieve a major European coordination of Vets and Beekeeping Technicians to share experiences and participate in an European Experimental Network to control Varroa.

For your remarks

Investigation on honey bee colony losses in Italy: the role of Varroaosis

A. Giacomelli*, M. Pietropaoli and G. Formato

Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana
Via Appia Nuova 1411, 00178 Rome

Author for correspondence: alessandra.giacomelli@izslt.it
Tel.: +39 320 60 333 90

The multidisciplinary project three and half years long (2009-2012) financed by the Italian Ministry of Health to investigate cases of honeybee losses and depopulations, provided studies through inspections in the apiaries with > 10 beehives and interested by mortality/depopulations > 20%. On-field investigations in apiaries were realized with clinical visits and samplings of bee matrixes for laboratory analysis to detect the causes of bee losses. In total, 496 beehives affected by abnormal mortality or depopulation were studied.

Except of clear episodes of pesticides poisonings (determined by the neonicotinoids: imidacloprid and acetamiprid) observed in the 12% (59 beehives) of the events, the major causes of honeybee losses (41,1%, equal to 205 beehives) of the causes were due to Varroaosis associated to virosis.

In many cases (46,6%) we recorded beekeepers mistakes in the management of the Varroa mite treatments. So far, a crucial point to solve honey bee losses still seems due to *Varroa destructor* and to availability of effective acaricide treatments.

In the presentation the field trials results will be described and discussed.

For your remarks

Is the trait “Varroa Sensitive Hygiene” present in European colonies?

C. Costa¹, M. Bienenkowska², R. Büchler³, L. Charistos⁴, M. Drazic⁵, F. Hatjina⁴, N. Kezic⁶, M. Mladenovic⁷, B. Panasiuk², S. Rasic⁷, A. Uzunov⁸ and J. Wilde⁹

¹ Consiglio per la ricerca e la sperimentazione in agricoltura – Unità di ricerca di apicoltura e bachicoltura, via di Salicero 80, 40128 Bologna, Italy

² Research Institute of Horticulture, Apiculture Division, 24-100 Pulawy, Poland

³ Landesbetrieb Landwirtschaft Hessen, Bee Institute, Erlenstrasse 9, 35274 Kirchhain, Germany

⁴ Hellenic Institute of Apiculture (N.AG.RE.F.), N. Moudania, Greece

⁵ Croatian Agricultural Agency, Ilica 101, 10000 Zagreb, Croatia

⁶ Faculty of Agriculture, University of Zagreb, Svetosimunska 25, 10000 Zagreb, Croatia

⁷ University of Belgrade - Faculty of Agriculture, Institute of Pomology & Viticulture, Department of Beekeeping

⁸ Faculty for Agricultural Science and Food, bul. Aleksandar Makedonski b.b., 1000 Skopje, Republic of Macedonia

⁹ Apiculture Division, Warmia and Mazury University, Sloneczna 48, 10-710 Olsztyn, Poland

Author for correspondence: cecilia.costa@entecra.it

Tel. :+39 0522 285532

Soon after the arrival of *Varroa destructor* on European honey bees, honey bee scientists noticed that the reproduction rate of *Varroa* in colonies could differ quite greatly. Two scientists working in the USDA bee laboratory in Baton Rouge, Harbo and Harris, focused on this trait to breed *Varroa*-resistant stock. Initially it was thought that some colonies were able to reduce reproduction of *Varroa* mites inside the cell, and the trait was named “Suppression of Mite Reproduction”, and selection was performed by testing colonies for rate of unfertile mites in portions of capped brood. Studies on colonies thus selected showed that they performed well in hygienic behavior tests and specific experiments suggested that the low proportion of fertile mites was due to preferential removal of reproducing mites by worker bees, so the trait was renamed “*Varroa sensitive hygiene*”. In the United States much attention has been dedicated to this trait by scientists and breeders, and VSH stock is used in commercial operations. Some studies have tried to investigate the genetic control of this trait, however the mechanism by which *Varroa* population growth in these colonies is reduced is not yet well understood, as the specific hygiene behavior has not been confirmed by subsequent experiments, and a certain role of the brood in suppressing mite reproduction seems to be present.

In Europe breeding programs in several countries have included hygienic behavior and / or *Varroa* infestation levels or growth indexes, but testing for VSH / SMR has not yet been applied large scale. Some scientists and breeders have started assessing the presence of this trait and many questions have arisen as to the best way to proceed. Within the Research network for Sustainable Bee Breeding we now plan to develop a protocol to evaluate this trait with a view to including it as a basic trait in breeding programs. During the workshop we will share our experiences so far and discuss the possible methods to evaluate the characters linked to reduction of *Varroa* population growth within a colony.

For your remarks

Selection on Varroa sensitive hygiene (VSH) in European honey bees

R. Büchler

Landesbetrieb Landwirtschaft Hessen, Bee Institute, Erlenstrasse 9, 35274 Kirchhain, Germany

Author for correspondence: ralph.buechler@llh.hessen.de
Tel. : ++49 6422 940613

Harbo & Harris (2005) achieved a strong expression of Varroa sensitive hygiene (VSH) by selecting colonies for a high level of nonreproducing Varroa mites (SMR). Those bees proved to be highly resistant against Varroosis under US field conditions.

During 1991 – 1993, we firstly investigated the removal of Varroa infested brood cells in 144 Carnica colonies of different breeding lines and found 0 to about 50 % of the single infested cells removed within 7 to 10 days. Significant effects of the genetic origin showed the inheritance of the trait and qualified it as a relevant character for selection on mite resistance.

Different methodical approaches were tested in order to establish a simple and standardized method for the estimation of brood hygiene behavior in large scale performance testing. With regard to a reliable repeatability of test results and a significant correlation to the removal of Varroa infested brood a pin test assay was finally established. It became part of the breeding program of the AGT breeder association (www.toleranzzucht.de) with several thousand colonies tested each year. The results are involved in the central estimation of breeding values for Varroa resistance (www.beebreed.eu).

In 2013, we started to test for SMR in our preselected Carnica population. From 26 colonies, 26,9 % showed increased levels of SMR with up to 42 % of nonreproducing mites in worker brood. We found a weak but positive correlation between SMR and pin test results ($r=0,25$, $p=0,224$) and a negative correlation with the mite infestation of the colonies ($r=-0,47$, $p=0,016$).

Suggestions will be discussed for further selection on VSH/SMR in European honey bees .

For your remarks

List of participants:

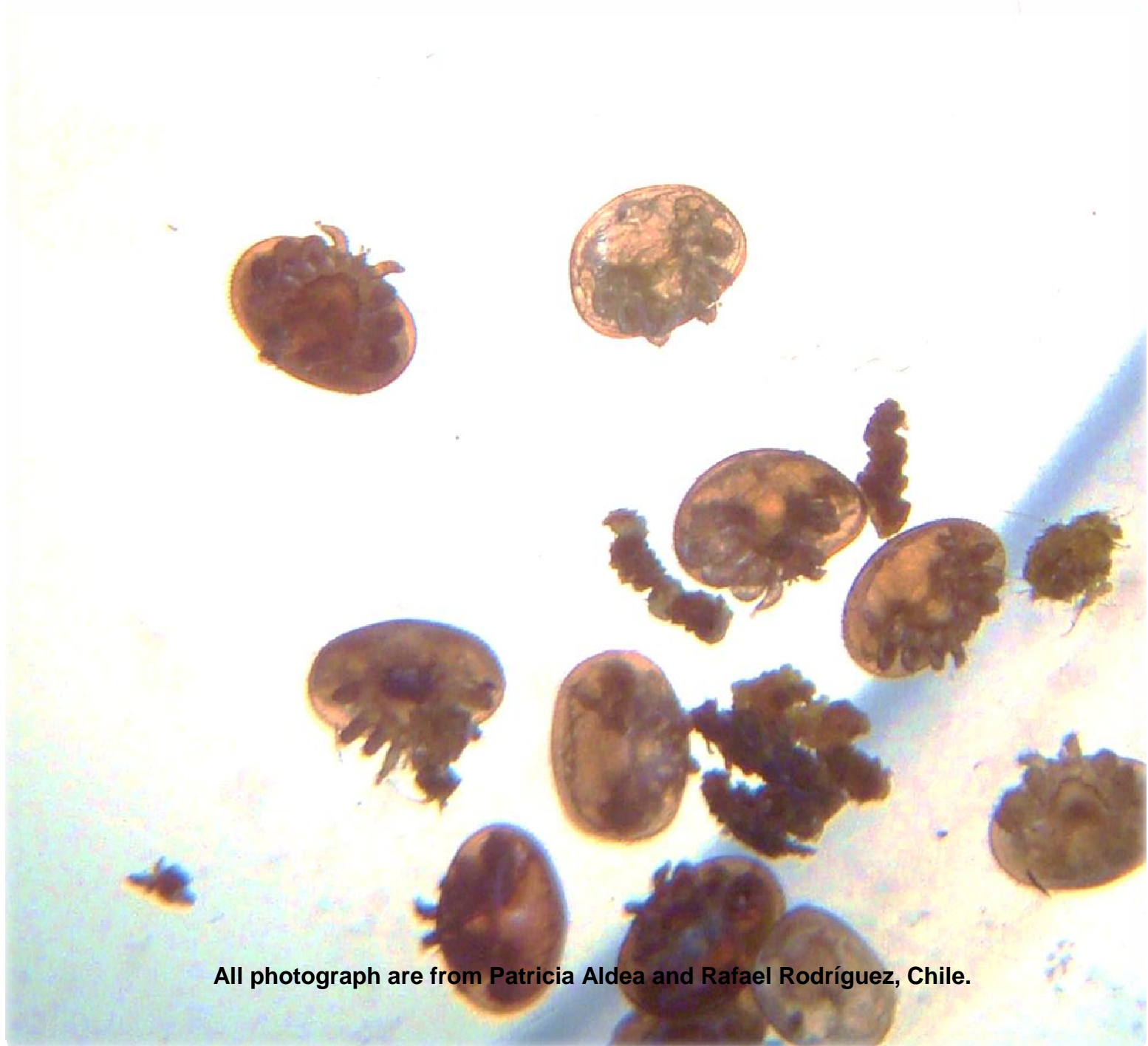
| Name | | Email | Address |
|-----------|-------------|--------------------------------------|--|
| Per | Kryger | Per.Kryger@agrsci.dk | Aarhus University, Agroecology, 4200 Slagelse Denmark |
| Ivana | Tlak Gajger | itlak@vef.hr | Department for Biology and Pathology of Fish and Bees, University of Zagreb Faculty of Veterinary Medicine, Zagreb, Croatia |
| Zlatko | Tomljanović | zlatko.tomljanovic@savjetodavna.hr | Savjetodavna služba, Stilinovićeva 17, 10431 Sveta Nedjelja Hrvatska |
| Raffaele | Dall'Olio | raffaele.dallolio@gmail.com | Consiglio per la ricerca e la sperimentazione in agricoltura – Unità di ricerca di apicoltura e bachicoltura CRA-API, via di Saliceto 80, 40128 Bologna, Italy |
| Ana Sofia | Lima | sofiaborges1@gmail.com | Universidade de Lisboa, Faculdade de Ciências de Lisboa, Departamento de Biologia Vegetal, IBB, Centro de Biotecnologia Vegetal, Campo Grande, 1749-016 Lisboa, Portugal |
| Benjamin | Dainat | benjamin.dainat@agroscope.admin.ch | Zentrum für Bienenforschung; Eidgenössisches Departement für Wirtschaft, Bildung und Forschung WBF Agroscope |
| Cecilia | Costa | cecilia.costa@entecra.it | Consiglio per la ricerca e la sperimentazione in agricoltura – Unità di ricerca di apicoltura e bachicoltura CRA-API, via di Saliceto 80, 40128 Bologna, Italy |
| Ralph | Büchler | ralph.buechler@llh.hessen.de | Landesbetrieb Landwirtschaft Hessen, Bee Institute, Erlenstrasse 9, 35274 Kirchhain, Germany |
| Stefan | Berg | stefan.berg@lwg.bayern.de | Bayerische Landesanstalt für Weinbau und Gartenbau, Fachzentrum Bienen, An der Steige 15 D-97209 Veitshöchheim |
| Antonio | Nanetti | antonio.nanetti@entecra.it | Consiglio per la ricerca e la sperimentazione in agricoltura – Unità di ricerca di apicoltura e bachicoltura CRA-API, via di Saliceto 80, 40128 Bologna, Italy |
| Patricia | Aldea | patricia.aldea@mayor.cl | Centro para el Emprendimiento Apícola de la Universidad Mayor - Camino La Pirámide 5750, Huechuraba. Santiago, Chile |
| Flemming | Vejsnæs | fv@biavl.dk | Danish Beekeepers Association; Beekeeping Adviser; Biologist, M.Sc., Fuldbyevej 15, DK-4180 Sorø |
| Vincent | Dietemann | vincent.dietemann@agroscope.admin.ch | Agroscope, Federal Department of Economic Affairs, Education and Research EAER; |

COLOSS Workshop “**Varroa control strategies**”, 22. – 23. 5. 2014

| | | |
|--|--|--|
| | | Schwarzenburgstrasse 161, CH-3003 Bern |
|--|--|--|

List of participants:

| Name | | Email | Address |
|--------------|--------------|---------------------------------|--|
| Małgorzata | Bieńkowska | malgorzata.bienkowska@inhort.pl | Research Institute of Horticulture, Apiculture Division, 24-100 Pulawy, Poland |
| Mary Frances | Coffey | Mary.Frances.Coffey@ul.ie | University of Limerick, Dept of Life Sciences, Ireland |
| Aleksandar | Uzunov | uzunov@zf.ukim.edu.mk | Faculty of Agricultural Sciences and Food, University Ss. Cyril and Methodius in Skopje, Macedonia |
| Aleš | Gregorc | ales.gregorc@kis.si | Agricultural institute of Slovenia |
| Marco | Pietropaoli | pietropaolimarco@gmail.com | Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana Via Appia Nuova 1411, 00178 Rome |
| Giovanni | Formato | giovanni.formato@izslt.it | Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana Via Appia Nuova 1411, 00178 Rome |
| Ole | Kilpinen | ole@biavl.dk | Danish Beekeepers Association |
| Vlasta | Jenčič | Vlasta.Jencic@vf.uni-lj.si | Veterinary Faculty, Uni. Ljubljana, Slovenia |
| Riccardo | Cabbri | riccardo.cabbri@entecra.it | Consiglio per la ricerca e la sperimentazione in agricoltura – Unità di ricerca di apicoltura e bachicoltura CRA-API, via di Saliceto 80, 40128 Bologna, Italy |
| William | Blomstedt | wblomst@gmail.com | MSc at the University of Edinburgh |
| Maja | Šmodiš Škerl | maja.smodis.skerl@kis.si | Agricultural institute of Slovenia |
| Mitja | Nakrst | Mitja.Nakrst@kis.si | Agricultural institute of Slovenia |
| Nikola | Kezic | nkezic@agr.hr | Faculty of Agriculture, University of Zagreb, Svetosimunska 25, 10000 Zagreb, Croatia |
| Martina | Sakač | martinasakac89@gmail.com | Department for Biology and Pathology of Fish and Bees, University of Zagreb Faculty of Veterinary Medicine, Zagreb, Croatia |
| Ivo | Planinc | Ivo.Planinc@vf.uni-lj.si | Veterinary Faculty/National Vet. Institute, Uni. Ljubljana, Slovenia |
| Mira | Jenko Rogelj | Mira.JenkoRogelj@vf.uni-lj.si | Veterinary Faculty/National Vet. Institute, Uni. Ljubljana, Slovenia |



All photograph are from Patricia Aldea and Rafael Rodríguez, Chile.