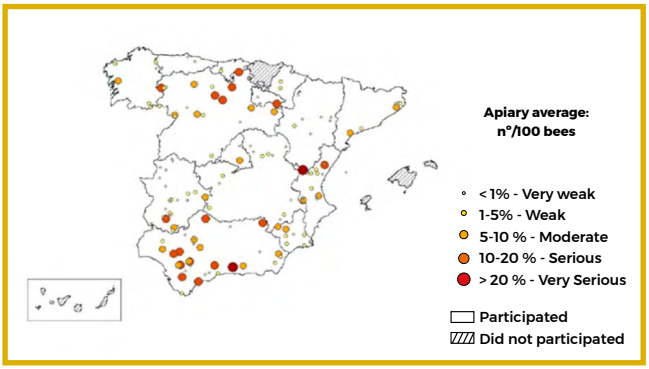
Problem addressed: varroa and treatments

Varroa destructor and bees mortality

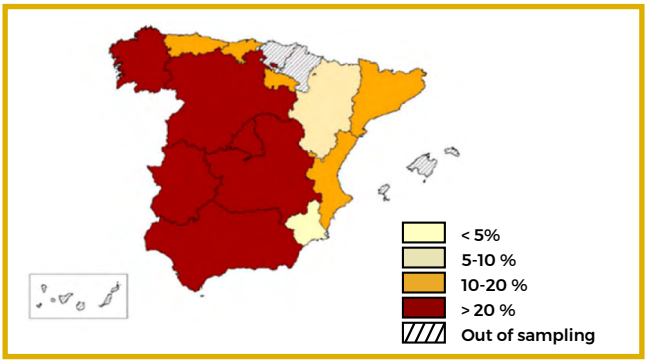
Bees play a fundamental role in food security and nutrition, sustainable agriculture, the environment and ecosystem health, and the conservation and improvement of biological diversity, among other dimensions of the sustainable development. Despite this, the bee population is declining [1].

In Spain, winter bee mortality throughout the 2019-2020 campaign was 19.2% in the apiaries studied by the 2019-2020 Surveillance Program about bee colony losses, the highest since this program began in 2012 [2]. There is not a single cause that justifies this mortality rate. However, different researchers point out *Varroa destructor* mite as the main cause of this situation [3,5].

While comparing both maps below, a clear connection between Varroa infestation in autumn (left map), and mortality of bees in winter (right map) is observed, since the regions with the highest mortality are those with a greater Varroa infestation. In autumn 2019, the mite was detected in 94.5% of the analysed apiaries, of which 27.7% presented levels of parasites from moderate (5-10% of the bees were affected) to serious (>20%). According to the experience of beekeepers, it is currently difficult to find a hive without Varroa, and they assure that "with 20% of varroa, a hive will not live". The detection of Varroa in autumn, although different every year, has remained since 2012 at figures above 70% [2].



V. destructor infestation per apiary in autumn 2019 (Source: [2]).



Winter mortality rate per region (2019-2020) (Source: [2]).

Harm to bees. Besides the harm caused by the mite due to its despoiling action, it weakens the immune system of bees, boosting the generalized appearance of viral, bacterial and fungal infections. The result is a poor sanitary and physiological condition which leads to an important loss of population and production, even with low levels of infestation [6].

Economic losses. The amount of honey in a varroa-infested domestic bee hive in Mediterranean climate has been estimated to decrease by 45% [7]. For a Spanish beekeeper with 500 hives and a usual production of 10kg per hive, this means an economic loss of 16,987€. Considering that the usual profit of that beekeeper is slightly above 13,000€, the economic losses due to varroa are equivalent to 128% the annual profit [6].

Currently, from a health and economic point of view, *V. destructor* mite is the main challenge Spanish beekeeping is facing [6].

Adult females of *V. destructor* (Source: Pacific Pests and Pathogens).

V. destructor in data

- Presence in **94.5%** of apiaries (official data)
- Population losses up to **19.2%** (occasionally up to **70-100%**)
- Production of **45%** less honey, occasionally **100%**
- Sanitary treatments represent **15-26%** of total costs
- Economic losses equivalent to **128%** of the usual annual profit.

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Sanitary treatments

At present, only 14 treatments based on 9 active substances are authorised by the Spanish Agency of Drugs and Sanitary Treatments (AEMPS, for its acronym in Spanish) [8]. In Spain, the Royal Decree 608/2006 of May 19th, establishes that it is mandatory to carry out at least one treatment per year against Varroa [9].

Despite that, the annual Results Report of the Surveillance Program on Colony Loss in Bees reveals that these treatments are not being effective [2]. This is due to different causes [6]:

1. Biological factors. Treatments do not penetrate through larvae operculated cell, which is where the reproductive stage of the Varroa takes place. Thus, treatments are only effective against phoretic Varroa [4].
2. Climatic factors. When winters are warm, acaricides lose their effectiveness as there are still young in the hive. On the other hand, when winters are cold, they have distribution problems within the hive.
3. Inefficacy of the treatments. Considering the limited number of treatments available (and the even more limited number of active substances), their effectiveness is reduced due to the development of resistance by the mite [4]. Resistance of Varroa against tau fluvalinate, amitraz and coumaphos has been demonstrated [10-13]. Moreover, 70% of the beekeepers choose amitraz as active substance in their practices. Furthermore, in the last three campaigns, more than 30% of beekeepers applied the treatment incorrectly [2].

Sanitary treatments against *V. destructor* authorised in Spain. (Source: own elaboration from [8]).

Active substance	Commercial name
Amitraz	Apivar Apitraz 500mg/strip for bees Amicel Varroa
Coumaphos	Checkmite
Thymol	Apiguard Thymovar
Flumethrin	Bayvarol 3,6mg strip for hives Polyvar 275mg strip for hives
Oxalic acid	Ecoxal
Tau fluvalinate	Apistan
Formic acid	Maqs formic acid 68,2mg. Strips for hives.
Formic acid/Oxalic acid dihydrated	Varromed 5mg/mL + 44mg/mL; and 75mg + 660 mg. Dispersion for hives.
Oxalic acid dihydrated	Oxybee 39,4mg/mL.

Adult bee with Varroa mite (Source: El Cortijuelo de San Benito).

Hive infested with *V. destructor*.

Additionally, current treatments entail another series of drawbacks:

- **The cost of the sanitary treatments** against Varroa accounts for 15-26% of total costs in a sector, beekeeping, with a low net returns. This expense is increased by the cost of repopulating the hives, treating secondary diseases, travels and labour to perform the treatments, etc. [2].
- **Toxicity to bees, mainly larvae.** The presence of residues in larvae has been detected. Some fungicides and acaricides have been shown to cause toxicity in bee larvae [14,15].
- **Residues in honey and wax.** Chemical residues from the treatments have also been detected in the products of the hive, carried by the bees in their body. Moreover, there is an accumulation of residues in the wax when the same treatment is used in different years [14].

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VAROAFORM innovation

There is an urgent need to find new active compounds that do not present the mentioned disadvantages of current sanitary treatments, while ensuring antivarroa efficacy [4].

STARTING POINT

VARROATOSIS

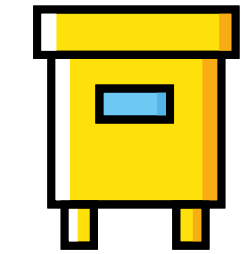
Caused by the mite *V. destructor*, is the main disease affecting bees. It weakens grievously their immune system, causing the weakness of the beehive, deformities, reduction of the population, etc. Its incidence varies according to the management of the beekeeper, the reserves of honey and pollen and the acaricidal effectiveness of the treatments.

CURRENT TREATMENTS

Currently, only 14 sanitary treatments are authorised in Spain, which are not effective in some cases, besides further problems such as: toxicity in bees, residues in beehive products, inadequate dosage, appearance of resistances, etc.

VARROAFORM SOLUTION

1. Increased effectiveness (acaricidal activity)
2. Reduction of the frequency of administration
3. Continuous release throughout the Varroa life cycle (30 days)
4. Not toxic for bees
5. Low cost
6. No residues generation in honey or other beehive products (wax, propolis)
7. Suitable for ecological apiculture
8. Easy handling, application and dosage



INNOVATIVE SOLUTION

VARROAFORM aim is to develop a controlled-release solution as an alternative to conventional treatments in apiculture for the control and prevention of varroatosis in domestic bee based on natural substances and incorporated into biodegradable systems/supports.

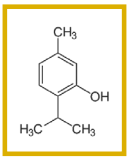
HOW?

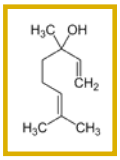
ACTIVE SUBSTANCES

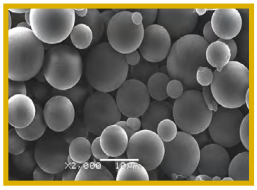
In addition to those authorised and compatible with organic production (thymol), solutions based on substances of natural origin (carvacrol, menthol, linalol or eucalyptol) are developed.

SUPPORT

The active substances are incorporated into suitable vehicles for their application (protection against oxidation, photodegradation, hydrolysis, volatilization) and that allow these substances to be released at effective doses during the duration of the cycle (28-30 days): gels, microcapsules, cyclodextrin complexes.







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ApiGranca

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What is an Operational Group?

Operational Groups are one of the key tools for the execution of the National Rural Development Programme 2014-2020 for promoting innovation in the agri-food and forestry sectors within the European scope. They gather agents of different profiles with common interests, such as farmers, ranchers, companies, researchers or training and dissemination actors, who are associated to implement an innovation project in order to provide a joint and multi-sectorial response to a problem or need.

VARROAFORM is a supraautonomous Operational Group with a multidisciplinary team which covers the regions of Galicia, Castilla y León, Castilla-La Mancha and Canarias.

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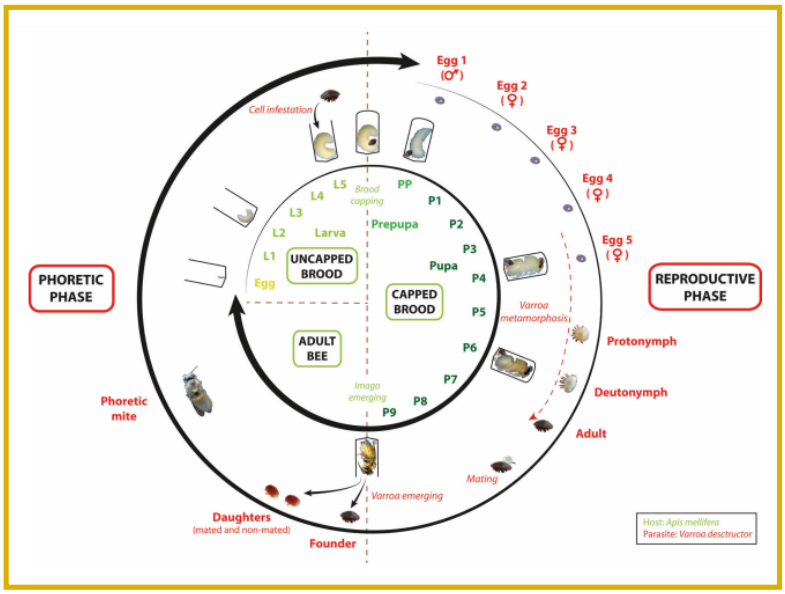
How?

Design of alternative solutions for the control and prevention of varroatosis

Active substance

To overcome the disadvantages of current treatments, VARROAFORM uses the main components found in essential oils as acaricides, the anti-varroa activity of which has been demonstrated in numerous studies [4, 16-19]. Furthermore, these compounds have the advantage that the generation of residues is very low, and the development of resistance by Varroa is unlikely [20].

However, many of these studies have evaluated the acaricidal activity of these compounds in *in vitro* tests, not in real conditions. Another aspect to consider is the effect that the toxic dose for Varroa may have on adult bees and larvae [21]. Therefore, an evaluation in real conditions and in a standardized way is necessary.



Schematic representation of the life cycle of the mite *V. destructor*, which is synchronized with the life cycle of its host, the honey bee (Source: [20]).

An adequate system/support

The active substance is as important as the support that contains it and serves as the basis for its application. There have been tests of the application of essential oils in powder form, diluted in syrup or dissolved in ethanol, although it is concluded that more research is needed in this regard [16]. The usual method of applying formulations based on these active principles, mainly thymol, is by incorporating them into a solid or semi-solid vehicle, which limits the antivarroa action to the phoretic phase, greatly reducing the effectiveness of the treatment [4].

It is therefore essential to design a formulation that adapts to the life cycle, and that is effective both in the phoretic and reproductive phases, where it is physically protected within the operculum.

VARROAFORM intends, through the appropriate support design, to develop an adjuvant treatment supplement that is effective in both phases of the Varroa life cycle. A support/system ensuring a continuous and homogeneous release of the active substance in the hive throughout 30 days (life cycle of the Varroa) is required. It must keep the characteristics of the active ingredients intact and offer protection against external factors such as oxidation, photodegradation, hydrolysis, climate, etc. In addition, it must be a natural and biodegradable support so that, like the active ingredient, do not generate residues in the products of the hive or generate toxicity in bees.

This **innovative design** will increase the efficiency against Varroa mite and reduce the number of applications, resulting in **an important save for beekeepers.**

Reproductive stage of *V. destructor* inside the operculum (Source: Bayer AG).

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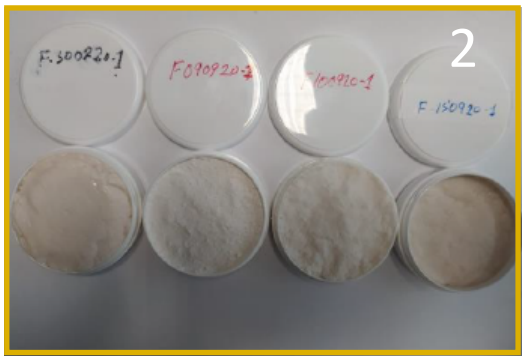
How?

Development of the new solutions

1. Gel type supplement. Thymol is used as active substance and cyclodextrine as support, to enhance the solubility and the amount of active substance in the formulation.

2. Pasta type supplement. Solid supports of high consistency as they contain high proportions of finely dispersed solids in the excipients. Mixtures of essential oils are used as active substance and microcrystalline cellulose and sodium carboxymethyl cellulose are used as support.

3. Oleogel type supplement. Small sized particles in which essential oil is incorporated within a nucleus covered by a protective layer formed by a biodegradable and natural origin polymer.



Evaluation of the treatments

These supplements are evaluated in the experimental apiaries to conduct a screening and select one (or various) supplements which satisfies the conditions regarding acaricidal activity, lack of toxicity for bees and larvae, absence of residues in hive products, ease of application and full-scale production:

- Acaricidal effectiveness, by counting Varroa both in the beehive and on the bees themselves.
- Resistance test to supplements, by counting the number of living mites which remain after the application of the supplement.
- Toxicity on bees, by analysing seminal quality and the influence on the queens rearing.
- Quantification of residues of the supplement in honey, wax and propolis.
- Ease of application on each type of hive used: Layens, Dadant or Langstroth.

Once preliminary results of these tests are obtained, the supplements will be assessed under real conditions in other apiaries of the Operational Group, to further study their behaviour under different climatic conditions.

Apiaries of the Operational Group VARROAFORM in Ourense (A), Guadalajara (B), León (C)

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References

1. Food and Agriculture Organization of the United Nations (FAO). Comité de Agricultura. 25° período de sesiones. Celebración del Día Mundial de las Abejas. 2016, COAG/2016/14.
2. Ministerio de Agricultura, Pesca y Ganadería. Informe de resultados del programa de vigilancia 2019-2020 sobre las pérdidas de colonias en abejas. 2020
3. Dietemann, Vincent & Pflugfelder et. al. V. *destructor*: Research avenues towards sustainable control. Journal of Apicultural Research. 2011, 51, 125-132.
4. Noël A. et al. V. *destructor*: how does it harm Apis mellifera honey bees and what can be done about it?. Emerg Top Life Sci. ; 2020, 4(1):45-57.
5. Ziegelmann, B. et al. Lithium chloride effectively kills the honey bee parasite V. *destructor* by a systemic mode of action. Sci Rep 2018, 8, 683.
6. Subdirección General de Productos Ganaderos, Dirección General de Producciones y Mercados Agrarios. Programa Nacional de Medidas de Ayuda a la Apicultura. España 2020-2022. Madrid, 2019.
7. Manuel Murilhas, A. V. *destructor* infestation impact on Apis mellifera carnica capped worker brood production, bee population and honey storage in a Mediterranean climate. Apidologie 2002, 33 (3) 271-281
8. Agencia Española de Medicamentos y Productos Sanitarios (AEMPS) Listado de Medicamentos Veterinarios autorizados por la AEMPS para abejas.
9. Real Decreto 608/2006, de 19 de mayo, por el que se establece y regula un Programa Nacional de lucha y control de las enfermedades de las abejas de la miel. Boletín Oficial del Estado, 131, de 2 de junio de 2006. 20839-20842.
10. Patti E. et al. Control of Varroa jacobsoni Oud. resistant to fluvalinate and amitraz using coumaphos. Apidologie, Springer Verlag, 2000, 31 (3), pp.437-441.
11. Maggi, M.D. et al. Resistance phenomena to amitraz from populations of the ectoparasitic mite V. *destructor* of Argentina. Parasitology Research, 2010, 107(5), 1189-1192.
12. Norberto M. The resistance of Varroa jacobsoni Oud. to acaricides. Apidologie 1999, 30 (2-3) 229-234.
13. Pettis, J. A scientific note on V. *destructor* resistance to coumaphos in the United States. 2004, 35. 10.1051/apido:2003060.
14. Bajuk B. et al. Coumaphos residues in honey, bee brood, and beeswax after Varroa treatment. Apidologie. 2017, 48. 1-11. 10.1007/s13592-017-0501-y.
15. Zhu W. et al. Four Common Pesticides, Their Mixtures and a Formulation Solvent in the Hive Environment Have High Oral Toxicity to Honey Bee Larvae. PLoS ONE 2014, 9(1): e77547.
16. Emsen B. et al. The effect of three methods of application on the efficacy of thymol and oxalic acid for the fall control of the honey bee parasitic mite V. *destructor* in a Northern climate. American Bee Journal. 2007, 147. 535-539.
17. Razavi SM. et al. The field efficacy of Lepidium latifolium and Zataria multiflora methanolic extracts against V. *destructor*. Parasitol Res. 2015, 114(11):4233-8.
18. Solis Riusech, N. Varroa mite control in honey bee colonies: The use of a fatty acid blend (C8910) for Varroa mite control and exploring management practices used by beekeepers. 2017.
19. Tiwari, R. et al. Efficacy of eco-friendly formulations against honeybee mite, V. *destructor* in Apis mellifera colonies in Uttarakhand - A novel approach. Indian Journal of Agricultural Sciences. 2015, 85. 883-887.
20. Mondet F. et al. Parasites. Chapter: Bee diseases (phatogenesis, epidemiology, diagnosis, therapy and prophylaxis). En: Wolfgang Ritter, editor. Bee Health and Veterinarians. 2014 (pp. 131-141)
21. Brasesco, Constanza & Gende et al. Assessing in Vitro Acaricidal Effect and Joint Action of a Binary Mixture Between Essential Oil Compounds (Thymol, Phellandrene, Eucalyptol, Cinnamaldehyde, Myrcene, Carvacrol) Over Ectoparasitic Mite V. *destructor* (Acari: Varroidae). Journal of Apicultural Science. 2017, 61.

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