novIGRain

NOVEL, RENEWABLE PLANT-OIL BASED ULV FORMULATION CONTAINING S-METHOPRENE LARVICIDE FOR POST-HARVEST APPLICATION ON CEREALS AND THE DEVELOPMENT OF A VERSATILE DUAL APPLICATION TECHNOLOGY

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Members of the Consortium:



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INTRODUCTION

In early 2020 the multi member **novIGRain** Consortium has submitted an application to the European Union under the Horizon 2020 scheme with the title „**SUSTAINABLE STORAGE OF GRAINS BY IMPLEMENTING A NOVEL PROTECTANT AND A VERSATILE APPLICATION TECHNOLOGY**".

The EU has evaluated and approved the project proposal and granted a considerable amount for a duration of five years. **30 %** of the overall project value is to be covered by the Consortium members. Following the preparation works and the signing of the Grant Agreement, the Consortium Agreement and the necessary appointments etc., the project has kicked off on **1st October 2020**. What were the reasons behind the proposal?

Cereal production is one of the oldest achievements of mankind in Europe. It has been a matter of life and death, of well-being or tragedies of hungers. By time, the cultivated area, the yield, the varieties, the storage and distribution of cereals have considerably increased and milling technologies developed in parallel. On the other hand, the quantity of chemicals used have increased during the decades to keep up the yield increase and the insect free shelf life of unprocessed cereals.

In post harvest grain protection, organo-phosphates, synthetic pyrethroids, pyrethrins, phosphines, Spinosad and diatomaceous earth are most commonly used. Although their mode of action differs, all of these actives have a strong killing effect, in most cases from early up to the mature stages. Europe is a major producer of grain crops (cereals, oilseed and protein crops). Before Brexit the yearly harvest was around **355 million tons**. One can easily imagine the quantity of fertilizers, plant protection products and post harvest chemicals used before uploading in silos.

The driving force behind the project was that **larvicides** have never been introduced and used in stored grain protection in the EU. The overall idea and the first proposal came from Babolna Bio Ltd., Hungary. Larvicides are widely used in the USA, Canada and Australia. Why hasn't the EU used any of the larvicides previously? This class of insecticide is totally missing from the palette of defence against grain insects.

Overview of the partner regions covered within the project Consortium

- Maxiline / Belgium - coordinating the Project and the consortium
- Babolna Bio / Hungary - the author of the idea, chemical company
- Sojam / France - distributor of grain protectants and machinery manufacturer
- VURV / Czeh Republic - grain protection and crop, resistance research
- UBM / Hungary - grain trading company
- United Experts / Belgium - project experts
- Toxi-Coop / Hungary - phys-chem, toxicological, environmental laboratory
- SCC / Germany - scientific consulting

The targets clearly defined:

- develop a grain protectant insecticide based on an **Insect Growth Regulator larvicide,** which targets precise development stages,
- **S-methoprene** Insect Growth Regulator was chosen as active substance,
- the development of a less hazardous product, with consequently less hazardous residues and therefore expect good MRL values,
- the development of a product with a favourable mammalian toxicological profile, important for the safe use by consumers,
- the use of a solvent from a renewable and natural origin as a carrier, making the product more sustainable.

In parallel additional goals were set:

- The possibility to separately apply multiple grain protection insecticide products in combination (larvicide together with an adulticide for example), which further diversifies the application and reduces the risk of resistance.
- Develop a special, multiple, versatile spraying equipment from which very small amount of larvicide (**2-4 litres/100 tons**) can be evenly applied. The nozzles can be separately adjusted. The equipment is movable and easy to handle.
- Resistance assessment & decision tool development: research and compilation of available information on PPP-resistance in a user-friendly tool or model.
- Perform an impact assessment that will make the comparison between the most common storage management techniques and the product/ application techniques developed by the **novIGRain** project. The impact will be assessed on various levels: economic, social and ecological impacts.
- Stakeholder interaction throughout the project.

Starting point: S-methoprene as larvicide active substance

S-methoprene (CAS 65733-16-6) is an insect growth regulator that mimics the natural juvenile hormone (JH) in many insect species controlling the metamorphosis. The effect of **S-methoprene** during the sensitive stages produces morphological deformities, abnormalities that lead to death. **S-methoprene** has no killing effect on insects that have reached the pupal or adult stages. Most susceptible species are grain insects, mosquitoes, ants, fleas, bedbugs, etc. **S-methoprene** has favourable toxicity and environmental features.
If a formulation is capable to supress the development of the grain insects with a result of above 90 %, it means that no adulticide is required. If less, than combination treatment may assure a 100 % insect free status of the grain. This is important as most country require an insect free status and certificate when trading. Rotation of insecticides becomes easier and the use of a larvicide most probably decreases the use of adulticides.
S-methoprene is a larvicide approved under the Biocidal Product Regulation and is a widely used active against public health insects and pests. As a result of the very high standard synthesis, the manufactured active substance is 97 – 98 %. Multiple physical-chemical, toxicological, environmental studies show favourable properties. The nature of grain protection and protectants is that they are used **indoors,** within the silos or other closed buildings. Environmental and human issues are less considered as in principle the spray will not enter land and water sites, while the automatization of the treatment is so progressed that the intervention of people is less frequently required.

Work Packages

The operational work is separated into 6 Work Packages, each of which is assigned to a WP Leader who is to steer the execution of their WP. The WP Leaders coordinate the task Leaders who are responsible for the detailed planning, day-to-day coordination and quality of the execution of each task. The task leaders organise - if necessary - skype conferences, extra meetings or workshops within their tasks.

Formulation development

During the first year the specific objectives within the Work Package 2 were:

- Creating a new, plant oil-based ULV formulation containing **S-methoprene** active substance suitable for post-harvest indoor application on cereal seeds in closed grain stores to achieve long-term preventive control of stored grain attacking insects
- Assessing complex behaviour of the formulation and the active substance in living organisms and in the ecosystem and predicting environmental fate by studying biological effects on target and non-target organisms, performing detailed analysis on physico-chemical properties and metabolic, toxicological and ecotoxicological profile and testing efficacy, in laboratory, under simulated-use and practical (field) conditions
- Creating complex data package for dissemination purposes and for building a decision support system.

The **carrier** of the formulation was a major issue. Prior to the start of the development we have drafted and listed our expectations towards the carrier as follows:

- originate from a renewable source
- is easily accessible
- is expected to pose low risks
- the active substance is expected to be well soluble in the carrier
- good chemical compatibility with the active substance
- good sticky capacity on the grain

Lab-scale batches of the developed formulation variants

Plants oil seemed to be a good choice, so formulation development was made with peanut, almond, olive oil and biodiesel. As plant oils can easily become rancid, antioxidants were also trialled. One had to take into consideration that some of the antioxidant may have unfavourable properties, like endocrine disruption.
The formulation is meant to be used as a **ULV** (ultra-low volume) spray to give excellent coverage while keeping the quantity as low as possible.
The residuality target was established at 10 - 12 months.
A number of studies are now planned to be executed by Toxi-Coop Laboratory and many other foreign laboratories. Preliminary efficacy studies are the next steps.

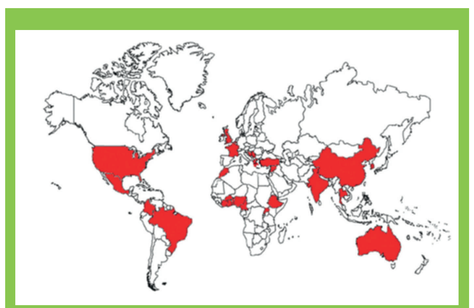
No.	Species
1.	Granary weevil *(Sitophilus granarius)*
2.	Rice weevil *(Sitophilus oryzae)*
3.	Lesser grain borer *(Rhyzopertha dominica)*
4.	Red flour beetle *(Tribolium castaneum)*
5.	Confused flour beetle *(Tribolium confusum)*
6.	Saw-toothed grain beetle *(Oryzaephilus surinamensis)*

Number of tests to prepare a discriminatory dose in the evaluation of the new product.

Photos taken and used by the permission of Tomas Vendl (Czech Research Institute)
Saw-toothed grain beetle (Oryzaephilus surinamensis),
Lesser grain borer (Rhyzopertha dominica),
Red flour beetle (Tribolium castaneum)

Resistance testing

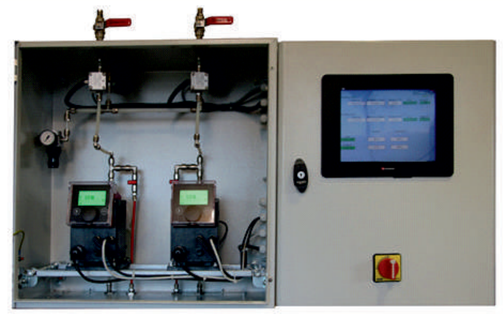
Initial resistance testing was the job and task of the Czech Crop Research Institute. We know that in Europe many Coleoptera species are becoming resistant to many insecticides, especially phosphines, but others, too. Resistance research of the grain industry is weak and the EU lacks comprehensive data. One of the main objectives of the **novIGRain** project is to establish a resistance map of the EU.
With the lead of Vaclav Stejskal a literature survey was followed by the estimation of the discriminatory doses of the sensitive strains. Two insecticides were chosen for the start, pirimifos-methyl and deltamethrin. Field strains were collected from the EU. Unfortunately due to the COVID infestation, less samples have reached the Institute than desired. In total 65 samples had been tested, so supplementary tests will run in 2022.
Discriminatory doses of deltamethrin and pirimiphos-methyl were determined for 6 strains of storage pests in laboratory strains: granary weevil (*Sitophilus granarius*), rice weevil (*S. oryzae*), red flour beetle (*Tribolium castaneum*), confused flour beetle (*T. confusum*), saw-toothed grain beetle (*Oryzaephilus surinamensis*) and lesser grain borer (*Rhyzopertha dominica*).
Resistance to insecticide compounds based on synthetic pyrethroids (deltamethrin - DE), organophosphates pirimiphos-methyl- PM) **was found** in 3 species of stored product Coleoptera pests: *Rhyzopertha dominica* (DE), *Tribolium confusum* (DE, PM), *T. castaneum* (DE). These results will consequently be used as the basic reference values.



Locations of observed resistances in stored product insects.
Countries where major studies of resistance have been carried out since 1995

The development of the spraying equipment

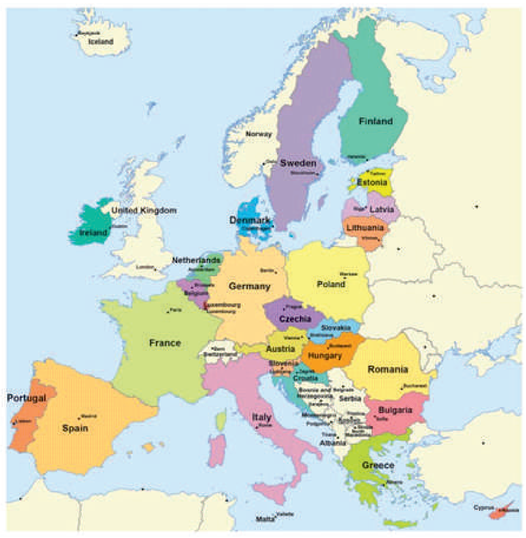
Sojam, the French participant is working to develop a new, mobile and versatile **ULV sprayer system** that is able to spray two different liquid insecticides in parallel without mixing the two. The separate systems will be separately adjustable to larger and smaller quantities as required upon infestation levels and as per labels of insecticides. Nozzles are also adjustable to produce different spray patterns.
The H2020 **novIGRain** prototype is now almost ready. The twin pump and twin meter equipment is adjustable in many ways. One of the main challenges was to precisely adjust the flow rate to a very small amount of ULV formulation.
2 - 4 litres/100 tons clearly shows that technically this is a real task to achieve a uniform coverage of the insecticide on the grain. Another novelty is that sprayer and the nozzles do not spray the conveyor belt but the grain as it falls to the silo pit. This method gives a better coverage.



Part of the initial ULV spryer system prototype

Market study and impact assessment

The target of the market study was to draw a background of the European cereal production, warehousing, transporting, treatments etc. to have ideas where and how the novel insecticide and spraying technology will fit in.
The French Izinovation SAS has been subcontracted to prepare a Market Study specifically on the grain industry of France, Germany, Spain, Italy, Romania and Hungary. In 2019/20 the total grain production of the EU was **328.7 million tons**, without the UK. The storage capacity is the largest in France, seconded by Germany. Grain is stored vertically and horizontally alike.
Inland waterways and railways handle almost all long-distance tonnage in the EU (60-70 % for inland waterways, 30-40 % for railways). Trucks play a marginal role, but are important in short distance transport. 70 % of the overall export is intra-EU, while 30 % leaves the EU. Harvesting, transporting, ensilage, treating and moving such an enormous amount of cereals is a trying challenge. No wonder that the **estimated loss of cereals is set approximately at 20 %.**
Compared to 2005 the storage capacities of the EU have considerably increased. For example, France as the largest producer has 91 million, while Hungary has 20 million tons capacity for storing cereals. No data, nor details are available currently though on the treatment of such an amount. Mode and preference of treatment are different country by country. For example, Germany favours fumigation, while in France sprayable insecticides are more popular. Many smallholders do not treat their harvested grain at all.
The grain rotation speed is unknown yet, however the stored cereals in general move within less than a year - with the exception of the strategic reserves. For safety reasons the **novel IGR insecticide has been developed to give a year-long residuality.**



novIGRain Consortium set up a homepage, too:
https://novigrain.eu/

Treatment of the cereals against insects are multivarious and complex, although the number of the available and approved insecticides is rapidly shrinking.

Active substance	*Date of approval*	*End of approval*	*Authorized countries among the 6 listed*
Deltamethrin (IN)	01/11/03	31/10/21	DE, ES, FR, HU, IT, RO
Cypermethrin (AC, IN)	01/03/06	31/10/21	DE, ES, FR, HU, IT, RO
Pirimiphos-methyl (IN)	01/10/07	31/07/22	~~DE,~~ ES, FR, HU, IT, RO
Pyrethrins (IN)	01/09/09	31/08/22	DE, ES, FR, HU, IT, RO
Aluminium phosphide (IN)	01/09/09	31/08/22	DE, ES, FR, HU, IT, RO
Magnesium phosphide (IN)	01/09/09	31/08/22	DE, ES, FR, HU, IT, RO
Sulfuryl fluoride (IN)	01/11/10	31/10/23	DE, ES, FR, ~~HU~~, IT, ~~RO~~
Spinosad (IN)	01/02/07	30/04/22	DE, ES, FR, HU, IT, RO
Kielselgur (Diatomaceous Earth) (IN)	01/01/21	31/01/36	DE, ES, FR, ~~HU~~, IT, ~~RO~~

Currently approved, used and restricted insecicide actives of grain protection

SUMMARY

Grain production as well as protection of the harvested grain are essential. The introduction of an Insect Growth Regulator larvicide as a novel class of active substance would not only broaden the choice, but – when used in combination with different adulticides – would also considerably lessen the insect infected loss, prolonging the shelf life of the stored cereal and would contribute to less adulticide use. Insecticide rotation together with a larvicide will provide a supplementary option in treatments.
The Consortium is working to reach a successful project. After the closure of the project in 2024 the goal is to achieve authorization both for the active substance and for the formulation of Methograin as a plant protection product – PPP.
Larvicides are widely and successfully used throughout the major grain grower parts of the World (USA, Canada, Australia), therefore it is time to introduce this type of protection in the European Union, too. We are confident that the use of larvicides alone or in combination with adulticides would give a much higher protection with less loss of grain.

Ref.:
COVID postponed presentation at the Hungarian Pest Control Association 2021 november - Resistance of storage insects to insecticide sprays and fumigant - STEJSKAL Vaclav; FRYDOVA Barbora; VENDL Tomas; AULICKY Radek (Stejskal, V.; Vendl, T.; Aulicky, R.; Athanassiou, C. 2021. Synthetic and Natural Insecticides: Gas, Liquid, Gel and Solid Formulations for Stored-Product and Food-Industry Pest Control. Insects, 12, 590.)

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