SUSTAINABLE STORAGE OF GRAINS
BY IMPLEMENTING A NOVEL PROTECTANT
AND A VERSATILE APPLICATION TECHNOLOGY

D1.2 Report on the Resistance Assessment and Research for the Newly Developed Larvicide and Its Combinations with Adulticides

Report on Formulation Development	
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1. Introduction

Stored-product pests cause significant economic damage across Europe, with various species exhibiting distinct geographic distributions, reproductive rates, and economic impacts. Managing these pests is becoming increasingly challenging due to climate change and the diminishing availability of approved insecticides, making insecticide resistance a major concern at both European and global levels. Effective resistance management requires reliable data; however, comprehensive information on stored-product pest resistance is largely lacking across Europe, with only a few exceptions (e.g., Greece). Existing resources, such as the IRAC database, remain incomplete and outdated for stored-product pests in the EU, resulting in significant variations in data availability among member states.

Stored-product pests display considerable variation in both their natural intrinsic sensitivity (i.e., baseline tolerance prior to any insecticide exposure) and acquired resistance (selected through historical or current exposure). Most documented cases of resistance to date involve classic neurotoxic insecticides. To address these challenges, the **novIGRain** project focuses on developing a novel juvenile hormone analogue (juvenoid)-based formulation paired with a specialized Ultra-Low Volume (ULV) application system. Supported by dedicated hardware and software technology developed within the project, this system enables both standalone applications and dual applications in combination with adulticides.

A key objective of the novIGRain research was to evaluate baseline sensitivity and resistance patterns in target pest populations available across Europe. This involved assessing reduced susceptibility to existing active ingredients—such as pyrethroids, organophosphates, and phosphine fumigants—as well as to the newly developed ULV juvenoid product. To compensate for the lack of publicly available current and historical European data, a comprehensive literature review was conducted alongside practical baseline laboratory testing. Understanding the complex interplay between natural sensitivity, resistance status to various active ingredients, technical storage parameters (e.g., storage type and duration), and environmental conditions (e.g., temperature during fumigation) is essential for optimizing treatment efficacy. To make these multifaceted factors accessible and actionable for farmers and end-users, they were translated into a practical, web-based decision-support tool (detailed in Deliverable 1.3). This advisory application integrates data on pest occurrence, infestation risk, storage conditions, product characteristics, and regional resistance levels.

Deliverable 1.2 presents a general summary of resistance assessment data and research findings obtained for the newly developed larvicide and its combinations with adulticides. These comprehensive results serve as a fundamental data baseline and essential technical input for the implementation of the NovIGRain decision-making tool. By guiding end-users in selecting appropriate control methods—including spray-applied protectants and fumigants based on their mode of action and resistance risk—the project establishes evidence-based anti-resistance strategies. Ultimately, this integrated approach contributes to safer, more effective, and sustainable stored-product protection across EU agri-facilities.

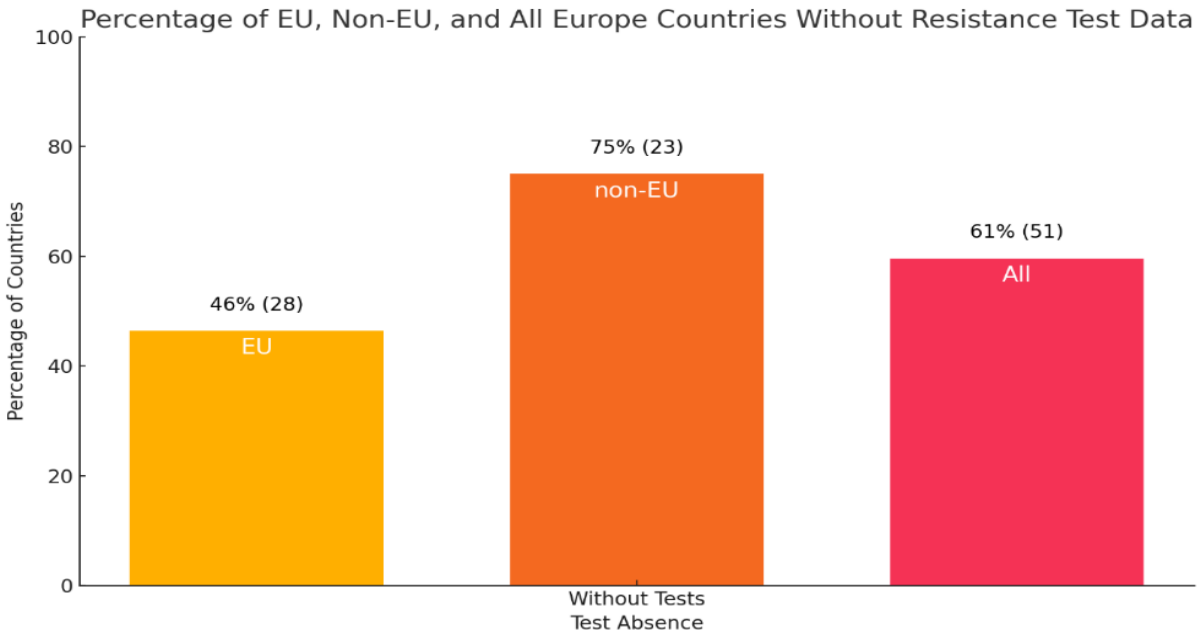
2. Assessment of Resistance in Europe Based on Original novIGRain Results and Historical and Current Literature Data Survey

Understanding resistance status is a fundamental prerequisite for selecting appropriate pest control products for target species, as well as for designing effective rotation and combination strategies. In this context, the project provides a significant contribution to resistance assessment, divided into two key components: First, to address the critical shortage of publicly accessible historical and contemporary European data, a thorough literature review was conducted to compile and analyze existing records. Second, this theoretical baseline is complemented by original empirical research. This section presents novel laboratory testing designed to establish baseline sensitivity and evaluate reduced susceptibility to widely used conventional active substances, specifically pyrethroids, organophosphates, and phosphine fumigants.

2.1. Historical and Current Literature Data Survey

The NovIGRain results confirm that Europe is not an exception to the global resistance crisis observed in stored-product pests. The main problem regarding farm-level perception of stored product pests resistance importance and the fight against it on farms is not the absence of resistance within Europe, but rather the historical lack of systematic resistance monitoring and documentation. Before the NovIGRain project, resistance data for the main insecticide groups used in stored-product protection, including phosphine, pyrethroids, and organophosphates, were missing for a large proportion of European countries and target pest species.

The NovIGRain literature survey conducted within the project showed that resistance data for at least one of these insecticide groups were unavailable for approximately 46% of EU countries, 75% of non-EU European countries, and around 61% of all European countries considered.



The largest knowledge gap was identified for pyrethroid resistance. Approximately 89% of potential resistance data points were missing across Europe, despite the fact that pyrethroids, including deltamethrin, are among the most widely used insecticides for structural treatments and residual protection in grain storage facilities. This lack of information means that many pest management decisions have historically been made without sufficient knowledge of whether applied products remain effective against local pest populations. The NovIGRain project therefore represents an important contribution by providing new resistance data from European populations and improving the basis for resistance management decisions.

2.2. Assessment of Resistance in Europe Based on Original novIGRain Results

Within the NovIGRain project, resistance was evaluated (based on field populations collected from various parts of Europe) for three major insecticide groups: phosphine, pyrethroids (deltamethrin), and organophosphates (pirimiphos-methyl). The results showed substantial differences among pest species and active substances. The most serious situation was observed for phosphine, which remains the dominant fumigant used for stored grain disinfestation worldwide. Recent global reviews and meta-analyses have demonstrated that phosphine resistance is a worldwide phenomenon, particularly among internal grain feeders.

Phoshine - The highest phosphine resistance frequencies in the NovIGRain survey were observed in *Rhyzopertha dominica*, *Tribolium confusum*, and *Sitophilus oryzae*. For *R. dominica*, resistance exceeded 85%, indicating a very high probability of phosphine-resistant populations being present in European grain storage systems. Similarly, *T. confusum* showed a high resistance frequency exceeding 75%, while *S. oryzae* showed resistance exceeding 60%. Resistant populations were detected across several European countries, confirming the broad geographical distribution of phosphine resistance. These results demonstrate that phosphine fumigation can no longer be considered universally reliable without prior verification of pest susceptibility. Global resistance frequencies have been reported at very high levels, including approximately 94% for *Rhyzopertha dominica*, 84% for *Sitophilus oryzae*, and around 28% for *Sitophilus granarius* (Machuca-Mesa et al., 2023).The NovIGRain results show that European resistance levels are closely aligned with these global trends. In tested European populations, phosphine resistance was detected at approximately 89% in *R. dominica*, 62% in *S. oryzae*, and 26% in *S. granarius*.

Deltamethrin - Resistance to deltamethrin was generally lower than resistance to phosphine; however, important differences among pest species were observed. The highest resistance frequency was detected in *Sitophilus oryzae*, where resistance approached 40% of tested populations. Increased resistance was also observed in *Tribolium confusum*, while other species generally showed low resistance frequencies. These results indicate that deltamethrin remains potentially effective against many stored-product pests, but its reliability depends strongly on the target species and local resistance situation. Replacing unsuccessful phosphine treatments with deltamethrin without resistance verification may therefore only transfer the problem from one active substance to another.

Pirimiphos-methyl - Resistance to pirimiphos-methyl was generally lower compared with phosphine and showed a more species-specific pattern. The highest resistance frequencies were observed in *Sitophilus oryzae* and *Tribolium confusum*, where resistant populations represented approximately one quarter of tested populations. In contrast, no resistance was detected in tested populations of *Tribolium castaneum*, suggesting that pirimiphos-methyl remains a potentially effective option for some pest species.

The novIGRain results demonstrate that effective resistance management must account for specific differences among pest species, active substances, and geographical regions. A comprehensive understanding of current resistance status allows for far more targeted product selection, as well as optimized rotation and combination strategies. Species such as *Rhyzopertha dominica* and *Sitophilus oryzae* represent particularly critical challenges due to their high resistance frequencies and potential for multiple resistance. In these cases, reliance on a single insecticide group represents a high-risk strategy, as increasing reliance on a single active substance to replace other failing products could accelerate resistance development in the future. The findings emphasize the necessity of integrated pest management (IPM) approaches based on regular resistance monitoring, rotation of active substances with distinct modes of action, and carefully selected product combinations where appropriate. The variation in resistance patterns across countries and species highlights the vital importance of international cooperation and coordinated monitoring across Europe. Ultimately, combining novIGRain experimental data with historical literature analysis confirms that resistance management can no longer rely solely on assumptions of single-ingredient chemical effectiveness. Continuous monitoring, improved resistance databases, and the implementation of IPM programs are essential to maintain effective protection of stored commodities and prevent future control failures.

This dynamic presents both a significant challenge and a strategic opportunity to utilize a broader spectrum of products and their targeted combinations effectively. While the observed high frequencies of resistance restrict the efficacy of traditional single-agent treatments, they simultaneously highlight the necessity of multi-target management approaches. Integrating products with complementary modes of action—such as pairing specialized larvicides with adulticides or strategically alternating and combining protectants with fumigants—can suppress different life stages, delay resistance development, and enhance overall control efficacy. Based on the resistance frequencies identified in this project, the following section outlines the hypothetical potential for combining, alternating, and complementing these preparations within stored-product protection.

3. Neurotoxic and Fumigant Resistance Assessment in Terms of Their Combination or Complementary Potential

Based on the baseline datasets evaluated in the previous section, this chapter explores the theoretical potential of neurotoxic active ingredients for use in combined or complementary treatment strategies across six key target species. It is essential to emphasize that the concepts presented herein represent a hypothetical assessment based on observed resistance frequencies in these six species—serving as a strategic brainstorming framework for pest control advisors—rather than direct empirical testing of physical mixtures for practitioners. In practical conditions, field efficacy is governed by considerably more complex dynamics, where factors such as species-specific intrinsic tolerance and environmental conditions play a crucial role, as demonstrated by the contrasting temperature dependencies of pyrethroids (which show higher efficacy at lower temperatures) and organophosphates (which perform better under warmer conditions). Because operational success relies on these multifaceted physical and biological interactions, detailed application scenarios and optimized combination choices are fully elaborated within the novIGRain Decision-Support Tool.

***Tribolium confusum* (confused flour beetle) -** In *Tribolium confusum*, the frequency of resistant populations among the tested populations against phosphine was exceeding 75%, representing a significant potential risk of control failure due to the possible presence of phosphine-resistant populations. In comparison, deltamethrin showed a low frequency of resistant populations (exceeding 10%), while pirimiphos-methyl showed an intermediate frequency (exceeding 25%). From the perspective of possible resistance occurrence, the combination deltamethrin + pirimiphos-methyl can be considered a potentially suitable combination, as both active substances showed lower frequencies of resistant populations compared with phosphine. Combinations containing phosphine can be considered potentially less suitable, because their effectiveness may be reduced by the possible presence of a high proportion of phosphine-resistant populations. The use of phosphine alone or strategies relying mainly on phosphine should be considered potentially risky from the perspective of possible resistance occurrence.

***Tribolium castaneum* (red flour beetle) -** In *Tribolium castaneum*, the frequency of resistant populations among the tested populations against phosphine was approaching 60%, indicating an increased potential risk of control failure due to the possible presence of phosphine-resistant populations. In contrast, deltamethrin showed a very low frequency of resistant populations (below 10%), and no resistant populations were detected for pirimiphos-methyl (0%). From the perspective of possible resistance occurrence, the combination pirimiphos-methyl + deltamethrin can be considered a potentially suitable combination. Combinations containing phosphine can be considered potentially less suitable, because a substantial proportion of the tested populations showed resistance to this active substance. Phosphine-based strategies should be considered potentially risky from the perspective of possible resistance occurrence.

***Oryzaephilus surinamensis* (sawtoothed grain beetle) -** In *Oryzaephilus surinamensis*, the frequency of resistant populations among the tested populations was the lowest among all evaluated species, with phosphine resistance below 15%, and resistance to both deltamethrin and pirimiphos-methyl below 5%. From the perspective of possible resistance occurrence, all evaluated combinations can be considered potentially suitable, with deltamethrin + pirimiphos-methyl representing the combination with the lowest potential resistance risk. Combinations containing phosphine can be considered potentially less suitable only because resistant populations were detected. Based on the observed resistance frequencies, no evaluated combination can currently be considered potentially risky from the perspective of possible resistance occurrence.

***Rhyzopertha dominica* (lesser grain borer) -** In *Rhyzopertha dominica*, the frequency of resistant populations among the tested populations against phosphine was exceeding 85%, representing a very high potential risk of control failure due to the possible presence of phosphine-resistant populations. In contrast, resistance frequencies for deltamethrin and pirimiphos-methyl remained below 10%. From the perspective of possible resistance occurrence, the combination deltamethrin + pirimiphos-methyl can be considered a potentially suitable combination. Combinations containing phosphine should be considered potentially less suitable, because the presence of phosphine-resistant populations is highly probable. The use of phosphine alone should be considered potentially risky from the perspective of possible resistance occurrence.

***Sitophilus granarius* (granary weevil) -** In *Sitophilus granarius*, the frequency of resistant populations among the tested populations was around 25% for phosphine and around 20%

for pirimiphos-methyl, while resistance to deltamethrin remained below 10%. From the perspective of possible resistance occurrence, combinations including deltamethrin, particularly deltamethrin + pirimiphos-methyl, can be considered potentially suitable. The use of phosphine alone or pirimiphos-methyl alone can be considered potentially less suitable due to the presence of resistant populations. Long-term repeated use of the same active substance, especially phosphine, should be considered potentially risky from the perspective of possible resistance occurrence.

***Sitophilus oryzae* (rice weevil) -** In *Sitophilus oryzae*, the most concerning resistance profile was observed. The frequency of resistant populations among the tested populations against phosphine was exceeding 60%, while resistance to deltamethrin was approaching 40% and resistance to pirimiphos-methyl was above 25%. These results indicate a high potential risk of control failure due to the possible presence of resistant populations against all three evaluated active substances. From the perspective of possible resistance occurrence, the combination pirimiphos-methyl + deltamethrin can be considered a potentially more suitable combination compared with phosphine-based strategies. However, it remains only relatively suitable because resistant populations were detected for all tested active substances. Combinations containing phosphine should be considered potentially less suitable I many cases, while phosphine-based strategies and repeated use of a single active substance should be considered potentially risky from the perspective of possible resistance occurrence.

4. Efficacy of an S-Methoprene Larvicide (Methograin) Against Resistant Field and Susceptible Laboratory Strains of Six Stored-Product Species

The objective of this study was to assess the relative efficacy of Methograin IGR 30 ULV (Batch No. FN-1066) on field-resistant and laboratory strains. Methograin IGR 30 ULV is a juvenile hormone analogue formulation containing S-methoprene, applied to wheat at a nominal rate of 4 L per 100 metric tonnes, with its performance evaluated against 1 standard laboratory strain and 29 selected resistant strains of adult stored-product beetle pests. The tested species included the granary weevil (*Sitophilus granarius*), rice weevil (*Sitophilus oryzae*), lesser grain borer (*Rhyzopertha dominica*), red flour beetle (*Tribolium castaneum*), confused flour beetle (*Tribolium confusum*), and sawtoothed grain beetle (*Oryzaephilus surinamensis*). To achieve the most uniform distribution of the treatment product throughout the treated grain, the treatment solution was prepared in the laboratory (in large laboratory bottles) by diluting 40 µL of Methograin IGR 30 ULV in 100 mL of acetone, corresponding to the target application rate for 1 kg of wheat. The control treatment consisted of 100 mL of pure acetone. To support the development of secondary pests, 50 g of crushed wheat was added to each 1 kg batch of whole wheat in a 5 L glass container. The respective treatment solution was applied to the grain, which was then manually mixed and shaken for 1 minute to ensure uniform distribution. The treated grain was subsequently spread in a large Petri dish for 24 hours to allow complete evaporation of the acetone before testing. As outlined in the project plan, thirty populations were evaluated for each of the six species. For each species and strain, five 50 g replicates of treated grain and five acetone-only solvent control replicates were prepared in plastic containers fitted with perforated lids. Twenty adult beetles were introduced into each container. The experimental units were maintained in a temperature-controlled chamber at 25 °C for an exposure period of two months for most

tested species and three months for species belonging to the genus Tribolium. At the end of the exposure period, samples were inspected according to the established protocol. Surviving or emerged adult beetles were counted and classified as either alive or dead.

4.1. Susceptibility of Species and Populations

Ranking of Species Susceptibility - At this single application rate, the six species naturally segregate into three distinct response tiers based on median live adults per replicate. The first tier represents complete suppression, comprising *R. dominica* (0.0) and *O. surinamensis* (0.0). The second tier consists of near-complete suppression, including *T. confusum* (1.0) and *T. castaneum* (1.1). The final tier corresponds to partial suppression, containing *S. oryzae* (22.5) and *S. granarius* (137.7), with the latter identifying as the least sensitive species overall. This hierarchy demonstrates a consistent pattern: the genus *Sitophilus* represents the least sensitive group, with *S. granarius* being roughly six times less suppressed than *S. oryzae* and two orders of magnitude less suppressed than species in which no adults survived. Both *Sitophilus* species develop internally within intact grain kernels, which restricts the direct exposure of immature developmental stages to surface-deposited chemical residues. While this offers a plausible partial explanation, *R. dominica* also undergoes internal kernel development yet experienced total suppression; thus, concealed development alone cannot fully account for the observed susceptibility ranking.

Interspecific and intraspecific variability ranged from nil in *R. dominica* and *O. surinamensis* to negligible levels in *Tribolium*—where relative scatter between replicates appeared high only because total counts were close to zero. In contrast, *Sitophilus* exhibited substantial variation, with the coefficient of variation across populations reaching 56% in *S. oryzae* and 47% in *S. granarius*.

Complete Suppression - In *Rhyzopertha dominica* and *Oryzaephilus surinamensis*, the outcome was unambiguous: not a single live adult was recovered from any of the 60 evaluated populations (0.0 \pm 0.0 live adults across all five replicates of all 30 populations of each species). This total suppression encompassed populations with documented resistance to phosphine alone, to phosphine combined with deltamethrin, and to all three resistance factors recorded in the dataset. Consequently, there is no variance to report or test to run—the treatment demonstrated absolute and uniform efficacy across all tested strains.

Near-Complete Suppression - In the two *Tribolium* species, adult counts remained extremely low, ranging from 0.2 to 1.8 live adults per replicate in *Tribolium confusum* (laboratory strain Tolab: 0.4 \pm 0.5) and 0.2 to 2.0 in *Tribolium castaneum* (laboratory strain Tclab: 0.8 \pm 0.7). Analysis of variance across the 30 populations yielded only marginal significance ($F(29,120) = 1.7$; $p = 0.028$ for *T. confusum* and $p = 0.022$ for *T. castaneum*). Furthermore, no individual field population differed significantly from its respective laboratory reference strain. Variations of one or two beetles out of the twenty initially introduced, following a three-month exposure period, lie well within experimental noise and carry no practical biological significance.

Partial Suppression with Strong Population-to-Population Variation - The two *Sitophilus* weevil species exhibited a distinctly different response, with surviving adult counts being an order of magnitude higher and displaying substantial variation among populations. For *Sitophilus oryzae*, live adult numbers ranged from 6.8 (Soge7, Germany) to 76.6 (Sofr1, France)—an 11-fold span—with the laboratory reference strain Solab averaging 23.4 \pm 6.0

(F(29,120) = 15.0, p < 0.001$). For *Sitophilus granarius*, survival ranged from 43.0 (Sgge4, Germany) to 362.4 (Sgfr1, France). Overall, roughly half of the field populations of both weevil species fell below their laboratory reference baseline, while the other half lay above it.

4.2. Resistance Status Did Not Predict Treatment Response

This constitutes the central finding of the study. In none of the six evaluated species did populations with documented resistance to phosphine or contact insecticides survive the treatment better than the susceptible laboratory strains or field populations lacking such resistance records. In the three species where both groups could be directly compared—*T. confusum*, *S. oryzae*, and *S. granarius*—resistant and non-resistant field populations showed no statistically significant differences in survival (Mann–Whitney test on population means, p = 0.86, p = 0.16, and p = 0.72, respectively). In *O. surinamensis*, both groups were identical as every count was zero, whereas in *R. dominica* and *T. castaneum*, every field population carried recorded resistance yet none differed significantly from the susceptible laboratory strain. In the weevils, the trend ran even in the opposite direction. For both *S. oryzae* and *S. granarius*, the highest survival rates occurred in populations classified as fully susceptible (Sofr1 and Sgfr1, both originating from France), whereas the strongest population suppression was observed in phosphine-resistant strains from Germany (Soge7 and Sgge4).

In summary, **the data therefore provide no evidence of cross-resistance between established resistance mechanisms and S-methoprene, which aligns with its entirely distinct mode of action. From a practical standpoint, this outcome supports the integration of an insect growth regulator (IGR) as a rotation or complementary component within an integrated resistance management program.**

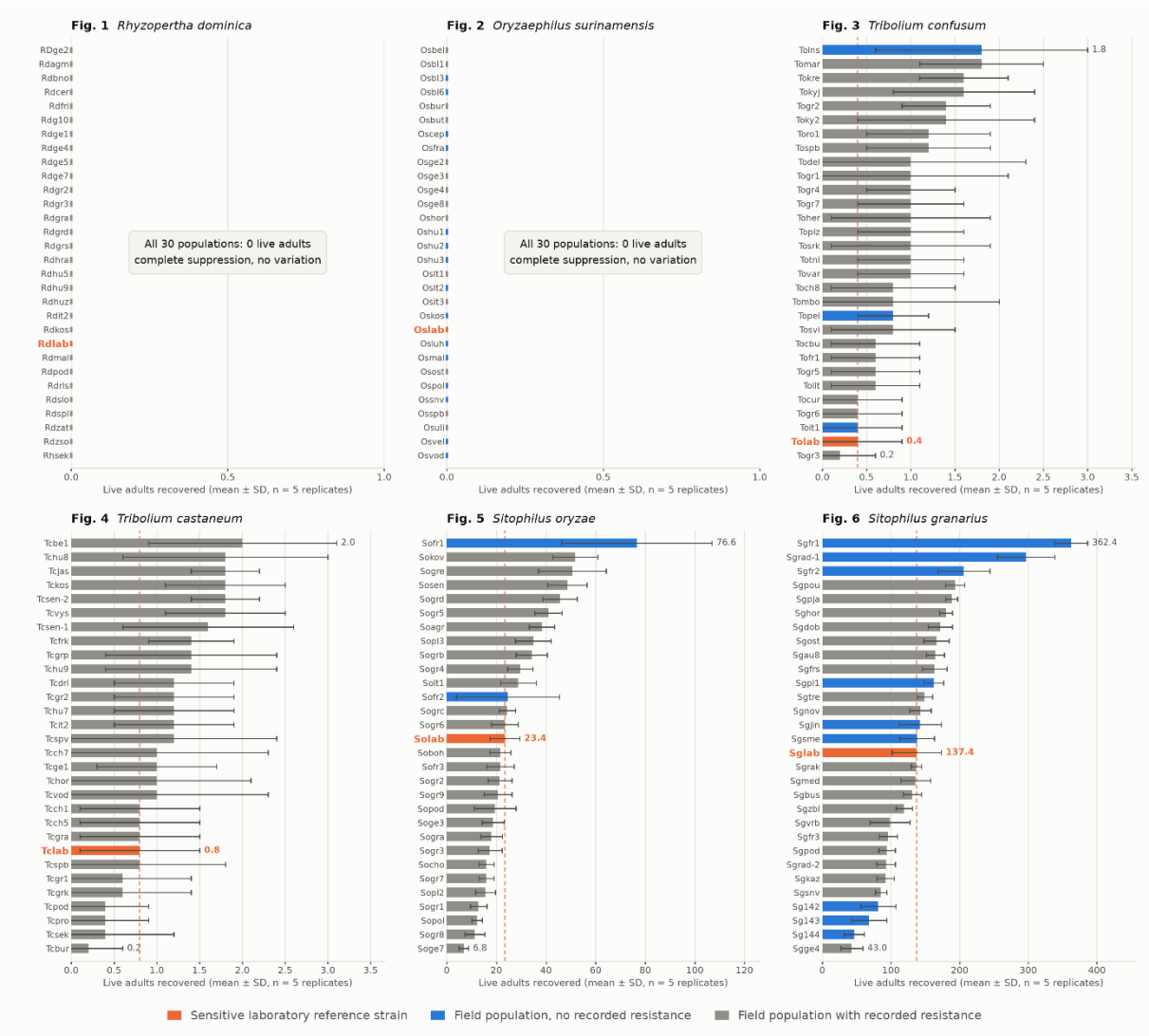
4.3. Conclusions and Limitations

Under standardised laboratory conditions, Methograin IGR 30 ULV at 4 L / 100 t completely suppressed adult development in *R. dominica* and *O. surinamensis* across all 30 populations of each, and suppressed *T. confusum* and *T. castaneum* to within one or two individuals of zero. The two *Sitophilus* weevils were markedly less sensitive — *S. granarius* least of all — and showed genuine, statistically significant variation between populations, an 8- to 11-fold spread of which part is likely to reflect differences in reproductive potential rather than in sensitivity as such. Critically, **that variation was not explained by resistance status**: populations resistant to phosphine, deltamethrin or pirimiphos-methyl responded as well as, or better than, fully susceptible ones, and the weakest suppression in both weevils occurred in populations with no recorded resistance at all. The findings are therefore best stated as a comparison of *relative laboratory efficacy* between species and populations, indicating no cross-resistance between conventional resistance mechanisms and S-methoprene.

While these findings provide critical insights, they originate from a controlled laboratory environment and should not be interpreted as a direct prediction of the efficacy achievable under practical storage conditions. Rather, they establish the relative sensitivity of species and populations under a standardized experimental setup. A primary consideration when interpreting these results is the carrier vehicle utilized. In this trial, the active ingredient was applied using an acetone carrier, which evaporates completely to leave an exceptionally uniform deposit on the grain surface. Under operational conditions, commercial formulations are applied with organic oil carriers. This alters active ingredient distribution across the kernel surface, modifies bioavailability to insects, and influences residue persistence over time. Consequently, absolute efficacy in commercial storage facilities may vary relative to the

figures reported here. Large scale confirmation under operational conditions with the oil-based carrier, including untreated controls and a dose–response series, is the logical next step.

Graph 1. Emergence of laboratory and field-resistant strains across six target species of stored-product beetle pests on laboratory Methograin-treated grain



***Note**: The susceptible laboratory reference strain — the benchmark for its species — is orange, Populations run from the least to the most suppressed, and error bars show the standard deviation between the five replicates. Each species has its own scale; the scales differ by more than two orders of magnitude between figures.*

5. Assessment of Efficacy of Grain Treated with Methograin Alone and in Combination with K-Obiol ULV Against Pests

The tested species included the granary weevil (*Sitophilus granarius*), rice weevil (*Sitophilus oryzae*), lesser grain borer (*Rhyzopertha dominica*), red flour beetle (*Tribolium castaneum*), confused flour beetle (*Tribolium confusum*), and sawtoothed grain beetle (*Oryzaephilus surinamensis*). To achieve the most uniform distribution of the product throughout the

substrate, the treatment solution was prepared in large laboratory glass bottles by diluting 40 µL of Methograin IGR 30 ULV in 100 mL of acetone, corresponding to the target application rate per 1 kg of wheat. The control treatment consisted of 100 mL of pure acetone. To support the development of secondary pests, 50 g of crushed wheat was added to each 1 kg batch of whole wheat in a 5 L glass container. The respective treatment solution was applied to the grain, which was then manually mixed and shaken for 1 minute to ensure uniform distribution. The treated grain was subsequently spread in a large Petri dish for 24 hours to allow complete evaporation of the acetone prior to testing.

As outlined in the project plan, thirty populations were evaluated for each of the six species. For each species and strain, five 50 g replicates of treated grain and five acetone-only solvent control replicates were prepared in plastic containers fitted with perforated lids. Twenty adult beetles were introduced into each container. The experimental units were maintained in a climate-controlled chamber at 25 °C for an exposure period of two months for most tested species, and three months for species belonging to the genus Tribolium. At the end of the exposure period, samples were inspected according to the established protocol, and all surviving or emerged adult beetles were counted and recorded as live adults.

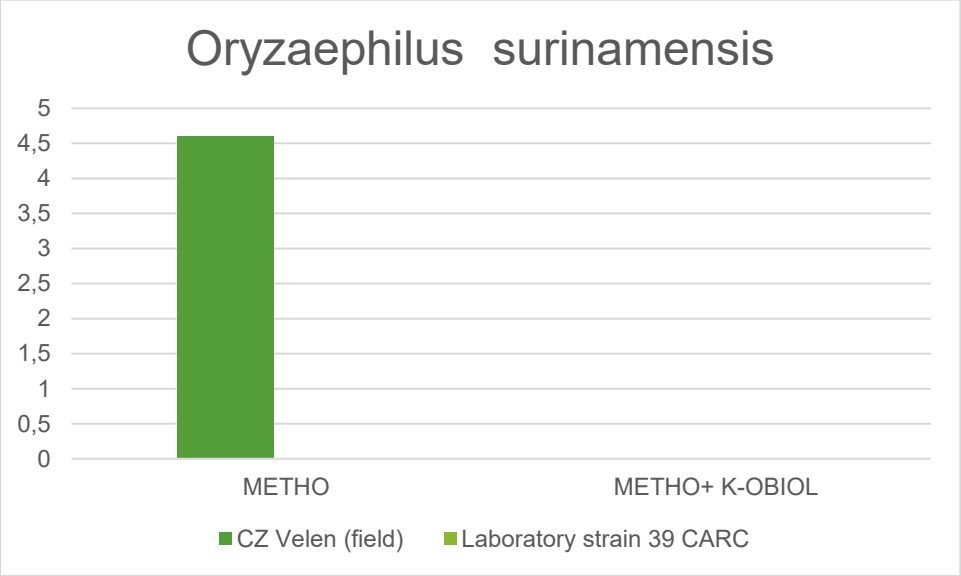
Combination with K-Obiol ULV Against Various Pest Species and Strains: Silo Treatments and Laboratory Assays

For the final phase of the project, a preliminary orientation test was included. The test was initiated using 20 adults per strain across three planned strains to mimic initial infestation. The observation period was extended to a minimum of two months, with regular monitoring of population size and structure in both treated and artificially infested grain samples. This setup allowed for the observation of population dynamics by enabling the initially introduced adults to establish a population, if viable, within the treated grain, thereby simulating natural field infestation behavior. The objective of this experimental design was to demonstrate the preventive efficacy of the novel combined treatment, even against resistant field insect populations. Semi-field trials were conducted in two operational Bábolna grain silos in Hungary to evaluate the grain treatments. The first silo was treated using SOJAM technology with Methograin (S-methoprene) at a rate of 4 L / 100 tonnes, while the second underwent a dual SOJAM application combining standard dosages of both Methograin at 4 L / 100 tonnes and K-Obiol ULV (deltamethrin) at 8.4 L / 100 tonnes, in accordance with the product label in France. Both the treated wheat and untreated control grain were provided by Bábolna Bio Ltd. Representative composite samples from both silos were subsequently transported to the CARC in Prague, where they were artificially infested with various pest strains according to the study protocol. To enhance reproducibility beyond the initial three planned strains, additional strains were evaluated. Most notably, extra strains were included for *Sitophilus granarius*, a species known from literature and laboratory assays to exhibit variable tolerance to juvenile hormone analogues, making the combination with complementary products such as K-Obiol ULV particularly crucial for its control. In total, the evaluation encompassed multiple strains, including two strains of *Oryzaephilus surinamensis* and five strains of *Rhyzopertha dominica*. Tests were conducted by introducing 20 adults per strain into vials containing 50 g of treated or control substrate across five replicates, with live and dead individuals recorded following a two-month exposure period.

5.1. Assessment of the Efficacy of Grain Treated with Methograin Alone and in Combination with K-Obiol ULV Against Laboratory and Field Strains of *Oryzaephilus surinamensis*

The preliminary and very limited results showed clear differences in susceptibility between the two *Oryzaephilus surinamensis* populations. The field population (CZ Velen) showed only very limited survival after treatment with Methograin (=METHO) (4.6 alive adults on average). However, the combination of METHO + K-OBIOL ULV resulted in complete mortality (0.0 alive adults), demonstrating improved efficacy of the combined treatment. The laboratory strain (39 CARC) showed no surviving adults after either treatment (0.0 alive adults), indicating high susceptibility to both METHO alone and the METHO + K-OBIOL ULV combination. Overall, the results indicate that the addition of K-Obiol ULV enhanced the efficacy of Methograin against the more tolerant field population.

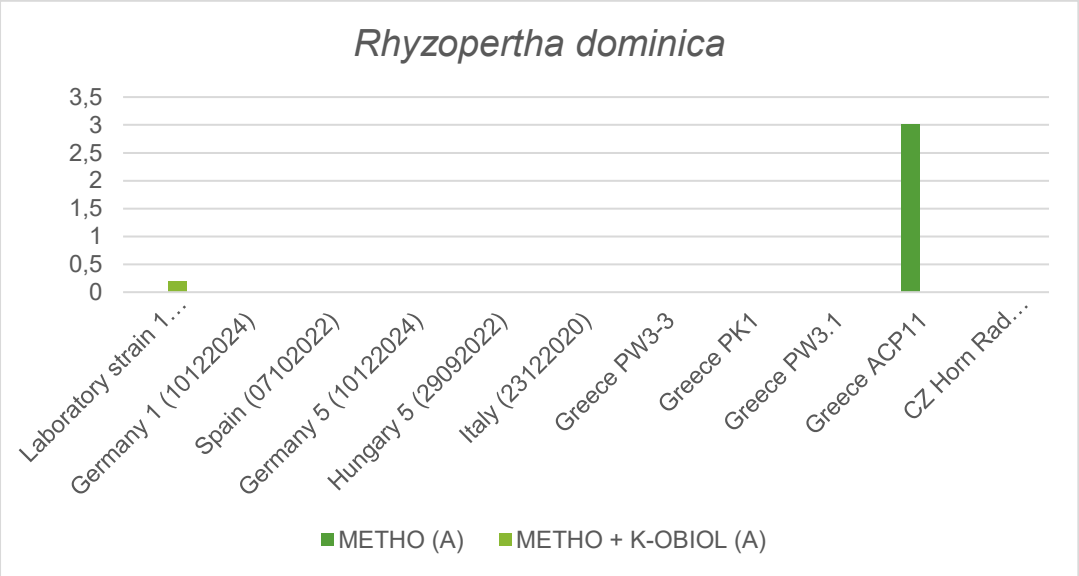
Graph 2. Comparison of *Oryzaephilus surinamensis* emergence in laboratory (blue) and field (orange) populations following exposure to Methograin alone or Methograin combined with K-Obiol ULV



5.2. Assessment of the Efficacy of Grain Treated with Methograin Alone and in Combination with K-Obiol ULV Against Laboratory and Multiple Field Strains of *Rhyzopertha dominica*

The preliminary and limited results showed that most *Rhyzopertha dominica* populations showed complete mortality after both treatments, with an average of 0.0 alive adults (A). The Greece ACP11 population showed survival after METHO treatment (3.0 alive adults), indicating reduced susceptibility to METHO. However, the combination METHO + K-OBIOL caused complete mortality in this population (0.0 alive adults), demonstrating improved efficacy of the combined treatment. The Laboratory strain 1 CARC was highly susceptible, with only a negligible survival level after METHO + K-OBIOL (0.2 alive adults).

Graph 3. Comparison of *Rhyzopertha dominica* emergence in laboratory (blue) and multiple field (orange) populations following exposure to Methograin alone or Methograin combined with K-Obiol ULV.



5.3. Efficacy Assessment of Grain Treatments Using Methograin Alone and Combined with K-Obiol ULV Against Laboratory and Multiple Field Strains of *Sitophilus granarius*

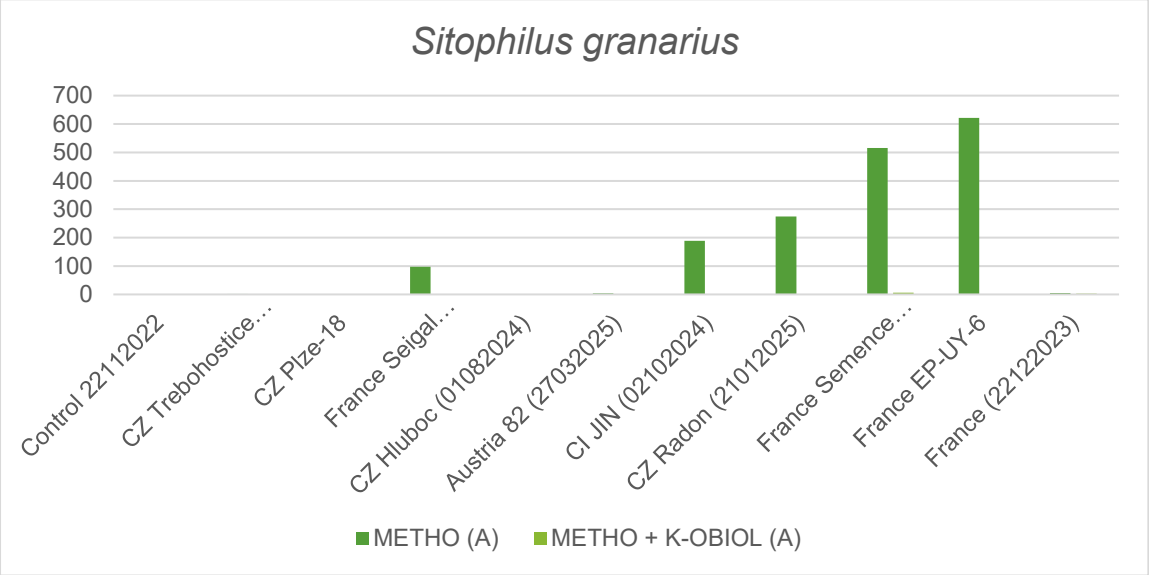
Most populations showed high susceptibility to Methograin combined with K-Obiol ULV, with 0.0 live adults recorded in several strains. The combined treatment of Methograin and K-Obiol ULV substantially reduced survival across all tested populations. Nevertheless, despite the overall high efficacy of the dual formulation treatment, minor survival persisted in a few instances, specifically in the France Semence (6.0 live adults), France 22122023 (3.0 live adults), and France Seigal (0.6 live adults) populations. Although based on limited experimental data, these findings indicate a significant beneficial effect when combining S-methoprene and K-Obiol ULV, particularly against *Sitophilus granarius*.

However, the susceptibility of *Sitophilus granarius* populations to Methograin treatment alone varied considerably among strains. Most populations displayed reduced or very low sensitivity to Methograin alone, particularly France EP-UY-6 (621.4 live adults), France Semence (516.0 live adults), CZ Radon (274.0 live adults), and CI JIN (188.6 live adults), indicating a high level of natural tolerance to this single treatment. These results align with previous findings by Mian and Mulla (1982), who noted that methoprene alone exhibits reduced effectiveness against *Sitophilus* species at standard application rates.

For strains where S-methoprene alone was not satisfactory, the combined standard-dosage treatment achieved nearly complete population suppression in most cases. Overall, the addition of K-Obiol ULV improved treatment efficacy and provided significantly better control of naturally less juvenile hormone-susceptible *S. granarius* populations. The preliminary data thus indicated that most *Sitophilus granarius* populations included in this limited experiment were fully controlled by the combined standard-dosage treatment. However, in a few specific cases, minor survival was observed even when both preparations were combined. Although the limited scope of the experiment cannot fully explain the precise underlying causes, it

highlights that while the combination provides strong overall control, complete suppression may still be hindered by factors such as uneven active ingredient distribution or heightened pyrethroid tolerance within specific resistant strains.

Graph 4. Comparison of *Sitophilus granarius* emergence in laboratory (blue) and multiple field (orange) populations following exposure to Methograin alone or Methograin combined with K-Obiol.



Summary

Deliverable 1.2 of the NovIGRain project delivers the first comprehensive European dataset evaluating resistance patterns in key stored-product pests against major grain-protectant insecticides, establishing a vital foundation for effective chemical rotation or combination strategies. The findings reveal that insecticide resistance is already widespread across tested European countries rather than being an isolated, local problem, posing a substantial challenge to single-agent chemical control strategies. Southern European regions demonstrated the highest frequency of resistant populations, though resistance was confirmed across various geographic regions. Notably, no single registered chemical protectant or fumigant remains universally effective without some degree of existing resistance in Europe. The risk, however, varies significantly by species: *Rhyzopertha dominica* exhibits severe resistance to phosphine, while *Sitophilus oryzae* displays elevated resistance across multiple active substances, indicating the development of multi-resistance. In contrast, species such as *Tribolium castaneum* and *Oryzaephilus surinamensis* remain more or less susceptible to conventional contact insecticides.

A critical finding from this research is that baseline resistance to traditional chemistry does not predict a population's response to the juvenile hormone analogue *S*-methoprene, confirming a total absence of cross-resistance between conventional mechanisms and this insect growth regulator. Strains resistant to phosphine, deltamethrin, or pirimiphos-methyl responded to *S*-methoprene as well as, or even better than, fully susceptible laboratory strains. Under standardized laboratory conditions, Methograin IGR 30 ULV applied at standard rates achieved complete adult development suppression in *R. dominica* and *O.*

surinamensis, alongside near-total suppression in *T. castaneum* and *T. confusum*. Weevils of the genus *Sitophilus* showed inherently lower sensitivity and significant population variability, though this variation was attributed primarily to differences in reproductive potential rather than genuine physiological resistance to the active substance itself.

To address the limitations observed with single-agent treatments, particularly in species with variable tolerance like *Sitophilus granarius*, the final phase evaluated semi-field silo applications using SOJAM technology. The dual application combining Methograin (*S*-methoprene) at 4 L / 100 t and K-Obiol ULV (deltamethrin) at 8.4 L / 100 t demonstrated substantially enhanced preventive efficacy across artificially infested grain samples under extended two-month monitoring. By incorporating complementary active substances, the combined formulation successfully controlled challenging field strains—including multiple strains of *R. dominica*, *O. surinamensis*, and *S. granarius*—suppressing population establishment even in strains exhibiting reduced sensitivity or resistance to individual compounds. Ultimately, managing resistance across European grain storage requires selecting targeted treatments based on the dominant pest species present, rotating active ingredients with distinct modes of action, and integrating these combined chemical approaches with rigorous preventive hygiene measures.

6. References

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