

# CONFERENCE: INNOVATIVE DISEASE CONTROL STRATEGIES FOR EUROPEAN ORGANIC AND INTEGRATED VINEYARDS, VIENNA, JANUARY, 9<sup>TH</sup>, 2015

## VINEMAN.ORG FOUNDED BY CORE ORGANIC II

### SUMMARY OF CONTRIBUTIONS

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In the first part of the conference the project Vineman.org was presented. Subsequently important contributions from the scientific community in the field of organic viticulture (researchers, representatives from European public authorities and professional associations) were presented and evaluated in a panel discussion.

### VINEMAN.ORG: BACKGROUND AND OBJECTIVES

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The VineMan.org project involves eight research groups in five EU Countries and aims at designing, developing, and testing innovative cropping systems for organic vineyards in Europe. In detail, the project focuses on enhancing organic grape production and its stability through a more efficient control of grape diseases. The project aims at improving disease control, which is one of the main and most difficult tasks in organic viticulture, integrating plant resistance against fungal pathogens, cropping practices, and use of BCAs depending on environmental conditions. VineMan.org was organised in eight Work Packages (WPs), each of them led by a competent partner and related to each other. Management of the project activities, knowledge, IPR and exploitation of the results had a specific WP (WP1). Methods for inducing the innate immunity of plants against fungi and oomycetes pathogenic to *Vitis vinifera* were evaluated in WP2. The effect of some viticultural management options on the development of the target diseases was investigated in WP3, with particular focus on canopy structure and cluster/berry morphology modifications. WP4 was devoted to the study of the relationships between the target pathogens and the environmental conditions with emphasis on the development of weather-driven, mechanistic, dynamic models for predicting plant disease epidemics. In WP5, the fitness, impact, and efficacy against the main grape diseases were evaluated in four BCAs representing bacteria and fungi including yeasts already registered in the EU as microbial biopesticides. WP6 was focused on the development of new strategies based on design-assessment-adjustment cycle. Finally, two WPs were aimed on field trials (WP7) and on the evaluation and monitoring of the microbial communities present on grape leaves and berries (WP8).

### ACKNOWLEDGEMENT

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## ENHANCEMENT OF PLANT RESISTANCE

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One of the goals of work package 2 of the VineMan.org project was to identify natural nontoxic compounds, which induce resistance in plants. The idea was to use these substances as resistance enhancer against downy and powdery mildew of grapevine in organic vineyards to reduce sulphur and copper applications. For the identification of potential resistance activator compounds a pH-shift bioassay was used to test over 80 substances. In particular, the pH-values of five to six days old *Vitis vinifera* cv. 'Pinot Noir' cell cultures were measured for 60 to 90 minutes after application of the compound. Approx. 20 % of all tested substances showed a potential resistance inducing activity, indicated by a temporary pH-value increase, whereas 20 % induced a toxic effect and the rest of the substances showed no effect. All positively tested candidates were then further analysed in leaf disk assays. Therefore grapevine leaf disks were sprayed with a specific concentration of the test substances and inoculated with spores from *Plasmopara viticola* 24 hours afterwards. Only three substances showed the ability to reduce sporulation of downy mildew significantly, all other compounds were insufficient, including the known resistance inducing component chitosan. The three promising candidates were subsequently analysed for their potential to induce resistance in leaf tissue. In order to examine the activation of plant defence, semi-quantitative RT-PCR analysis was carried out. Unfortunately none of the candidates did induce gene activation of defence marker genes after leaf application. In order to investigate a potential direct toxicity of the candidates to the downy mildew a direct incubation assay of zoospores with the test substance was conducted. Interestingly all substances seemed to have a direct effect on the pathogen, possibly explaining the good results of the leaf disk assay.

## MODIFICATION OF CANOPY AND CLUSTER STRUCTURE

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The treatment early removal of 6 leaves and of 2° shoots at pre bloom on cv. 'Riesling' and cv. 'Zweigelt' vines (ELR) is a strategy to reduce infestation of Botrytis and to reduce yield and cluster weight as compared with non-defoliated, late defoliated (removal of 6 leaves and of 2° shoots at veraison (LLR)) and moderately defoliated (water shoots, 2° shoots and 1-2 leaves per shoot in the fruiting zone) vines.

ELR on cv. 'Riesling' significantly affected the content of total phenolics in must. Moreover LLR on cv. 'Riesling' reduced must weight in comparison with non-defoliated, early defoliated and moderately defoliated vines.

With respect to RGB imaging system results regarding yield components, cluster compactness and both cluster exposure and canopy porosity, the most suitable canopy management treatment to potentially cope with fungal infections in a more successful way should be the ELR treatment.

The percentage of chicken berries was higher in the ELR (leaf removal was applied in pre-flowering) than in the control. Moreover both bunch and berry weight were lower in the ERL treatments compared to the control and this led to reduced bunch compactness. As expected less compact bunches were less susceptible to Botrytis cinerea and affected disease incidence and severity in the ELR treatment lower than in the control.

The application of Vapor Gard at fruit set and veraison reduced cluster compactness with cv. 'Neuburger' vines in the year 2013. Vapor Gard at fruit set and veraison led to a decrease of titratable acidity with cv. 'Neuburger' only in the year 2013.

In Italy the results of this study show that a mechanical leaf removal at post veraison on cv. 'Sangiovese' vines apical to the bunch zone is a practical strategy to delay sugar accumulation in the berry by about 2 weeks as compared with non-defoliated vines. The technique proved itself as an effective, easy-to-do and economically viable method (it requires only 3 to 4 h/ha to be achieved mechanically) to hinder berry sugar accumulation and to obtain wines of lower alcohol content. Importantly, the technique did not affect the content of total phenolics in grapes and wines or the replenishment of reserves storage in canes and roots. To be effective at significantly delaying sugar accumulation in the berries, it is advised to remove leaves apical to the bunch zone at around 16 to 17 °Brix and ensuring that at least 30 to 35 % of the leaf area is removed.

In Austrian investigations for the treatment "cutting off parts of bunches" at berries pea-sized significantly decreased cluster compactness, grape bunch length and incidence of botrytis.

## ENVIRONMENT AND DISEASE DEVELOPMENT

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Epidemiological models available in literature for the major grapevine diseases were evaluated and the mechanistic models for primary and secondary infection of downy and powdery mildew developed by Rossi et al. (2008) and CAFFI et al. (2011 and 2013), respectively, were considered the most suitable to support decision making about crop protection, in particular when connected to a server repository that stores weather data coming from a network of weather stations, that measure environmental variables in the vineyard. In details, these models provide detailed information on the biological processes of the pathogens in real time and this promptness was considered of high importance in organic viticulture because of the lack of curative plant protection products. Once identified, the models were implemented in an electronic platform able to produce decision aids for organic viticulture. The electronic platform is provided by Horta s.r.l., a spin-off company of the Catholic University of Piacenza (Italy), and is currently available for the project partners through the project website. The use of the epidemiological models' outputs present on the electronic platform was considered as an alternative tool to be tested in experimental vineyards in Italy, Austria and Slovenia for the management of the diseases. In particular, the model outputs should help the vineyard manager to better schedule the plant protection applications against the major pathogens in a way to reduce vain treatments as much as possible.

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## IMPROVEMENT OF FITNESS AND EFFICACY OF BIOLOGICAL CONTROL AGENTS (BCAs)

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Inorganic fungicides like copper, sulphur or acid clays should be used to manage fungal problems as a last resort; the use of copper is problematic because of its poisonous effect on the soil's flora and fauna (TRIOLI et al., 2009). The use of microorganisms and the exploitation of beneficial plant/microbe interactions offer promising and environmentally friendly strategies for organic agriculture (BERG, 2009). Biological control can result from many different types of interactions between organisms; the most effective BCAs appear to antagonize pathogens using multiple mechanisms (MONTESINOS et al., 2009). Practical implementation of bio control has been constrained by a number of factors, including efficacy and reliability of BCAs (BONATERRA et al., 2007; PAL et al., 2006). Fitness and efficacy of four biological control agents (BCAs) that are already registered according to the EU regulations, were evaluated in relation to grape disease control under organic practices. Fitness of *Bacillus amyloliquefaciens* QST713 (Serenade<sup>TM</sup>), *Ampelomyces quisqualis* (AQ10<sup>TM</sup>), *Aureobasidium pullulans* CF10 (Botector<sup>TM</sup>) and *Lecanicillium lecanii* (Mycotal<sup>TM</sup>) was evaluated through monitoring by strain or species specific quantitative PCR methods based on DNA markers. In a first stage the validated qPCR protocols were tested with potter tower experiments. Serenade was applied at the recommended dose with a potter tower to detached vine leaves kept on water agar. After two weeks, all samples were close to or below the detection level. The influence of copper and sulphur on the colonization ability of the BCAs was also investigated. While copper has a tendency to negatively influence *B. amyloliquefaciens* QST713 on vine leaves, sulphur has a positive tendency. *Ampelomyces quisqualis* could not colonise the leaves' surface even in presence of *Erysiphe necator*.

Flavescence dorée is a severe grapevine yellows disease caused by Grapevine Flavescence dorée phytoplasma. It is transmitted by its principal vector, the Nearctic leafhopper *Scaphoides titanus*. In organic viticulture the mandatory control of the vector is challenging. Therefore alternative and biocompatible control methods are investigated. Results from laboratory testing showed that *Lecanicillium lecanii* was virulent to the second larva instar of *S. titanus*. The percentage of the cumulative mortality ranged from 60 to 72.2 %.

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## DEVELOPMENT AND TEST OF NEW DISEASE MANAGEMENT STRATEGIES FOR ORGANIC VINEYARDS

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Single management components evaluated within the VineMan.org project were considered for the development of two innovative strategies for a comprehensive organic management of the vineyard diseases. The first strategy is more conservative (risk-adverse strategy) and is based on the combination of i) the use of epidemiological models for downy and powdery mildew to schedule copper and sulphur treatments at label dose; ii) fall treatments with the hyperparasite *Ampelomyces* (AQ10) against powdery mildew overwintering fruiting bodies (if disease severity is high enough at the time of harvest) and, iii) the use of *Aureobasidium pullulans* against grey mould. The second strategy (risk-seeking strategy) is based on the combination of i) the use of epidemiological models for downy and powdery mildew to schedule copper and sulphur treatments at reduced dose, and ii) canopy management (i.e. early leaf removal) for the control of grey mould.

The two innovative strategies were tested in vineyards set up by project partners following a common protocol in grapevine growing seasons 2013 and 2014. Four experimental vineyards were set up, one for each partner/country involved in the project: i) Tuscany (Italy), cv 'Sangiovese', ii) Freiburg (Germany), cv 'Müller-Thurgau', iii) Vipava valley (Slovenia), cv 'Pinela', and vi) Klosterneuburg (Austria), cv 'Chardonnay'. The experimental vineyards were

divided in different plots in order to compare the two innovative strategies with the usual grower strategy and a small untreated control. Monitoring of the plant phenology and of the principal grapevine diseases was performed regularly. The multi-criteria assessment tool DEXiPM (implemented within the EC FP7 founded project ENDURE and then refined for grapevine under integrated production management in the EC FP7 founded project PURE) was used to assess these solutions on economic (labour, yield effect, product quality, product price) environmental and health risk for workers aspects. The two innovative strategies were compared with the “baseline”: the use of epidemiological models allowed a significant reduction of fungicide TFI and fuel consumption that provided a significant increase in the economic sustainability. Both innovative strategies requested a specific training of the farmers and operators: the result was an increase of their knowledge and skills that was reflected in a higher job gratification. The social acceptability of the innovative tools increased significantly because of the reduced use of fungicides, their reduced dose and their substitution with natural bio-control agents.

## MICROBIAL BIODIVERSITY IN ORGANIC VINEYARDS

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Two innovative disease management strategies were tested in organic vineyards in different European countries. The strategies centred on applications of (i) reduced fungicide dosages combined with canopy management consisting of early leaf removal (high risk strategy) and (ii) non-reduced fungicide dosages combined with applications of the biological control formulation Botector against the grey mould agent *Botrytis cinerea* (low risk strategy). Pesticide applications were scheduled according to a weather-driven model for predicting plant disease outbreaks. Workpackage 8 of this core organic II project Vineman.org addressed the diversity of fungi in a vineyard near Ajdovščina, Slovenia. Experiments were performed with the white grape variety ‘Pinela’, an autochthonous breed from the Slovenian Vipava valley.

### MATERIALS AND METHODS

Culturable fungi were isolated from bulk samples (either leave or grape, collected on May, 30, and September, 01, 2014) pulverized with a homogenizer and sieved for receiving 75 to 150 µm plant pieces. Procedures during sample preparation included multiple washing steps for removing fungal spores and propagules allowing a focus on fungi present as hyphae or mycelium on plant pieces (COLLADO, 2007). Small pieces of substrata were generated to maximize the chance of retrieving a single mycelial species per plant piece. Suspensions of plant pieces were diluted to ca. 10 pieces per 20 µl used for the inoculation of media containing tubes of 96-well plates. A total of 4800 tubes were inoculated with plant material either from leaves or grape samples. Fungi growing in tubes of these plates were sub-cultured and identified on the basis of morphological characters and diverse DNA barcodes, e.g. internal transcribed spacer regions and the nuclear 5.8S rRNA gene (ITS) widely used as barcode for fungal species identifications. A cul-

ture independent approach for biodiversity assessments was based on DNA extracted from 30 to 50 mg of plant pieces and a nested PCR for the amplification of molecular barcodes consisting of the internal transcribed spacer regions and the nuclear 5.8S rRNA gene. For visualizing the diversity of retrieved fragments, each of which representing a purportedly single species, PCR products were separated with denaturing gradient gel electrophoresis (PCR-DGGE) (ANDERSON et al., 2003).

RESULTS AND DISCUSSION

DGGE profile analyses based on Pearson similarities did not consistently distinguish differences between the fungal communities retrieved from the three experimental plots. However, the taxa composition from strategy 1 treated leaves (100 % pesticide dosage plus BCA) differed from the compositions retrieved from strategy 2 and the farmer’s treatment in May 2014. At that time, the results from several profiles of the latter two (reduced pesticide dosage plus canopy management and farmer’s treatment) nested together indicating similar fungal communities. Strategy 1 communities from leaves differed also in September from those of the other treatments, although one replicate from strategy 2 was similar to the three strategy 1 treatments. In September 2014, the profiles from at least two replicates of strategy 2 (reduced pesticide dosage plus canopy management) showed several additional bands when compared to the September profiles from strategy 1 and the farmer’s treatment. It is possible that the treatments in strategy 2 (reduced pesticide doses and canopy management) caused an increase of fungal diversity in the experiment (Fig. 1A). A similar indication was also observed on grapes harvested in September 2014. Two replicates from samples obtained from strategy 2 treatments differed qualitatively clearly from the other samples in September 2014 suggesting that the reduced pesticide dosages plus canopy management caused a change of fungal species composition on grapes. The

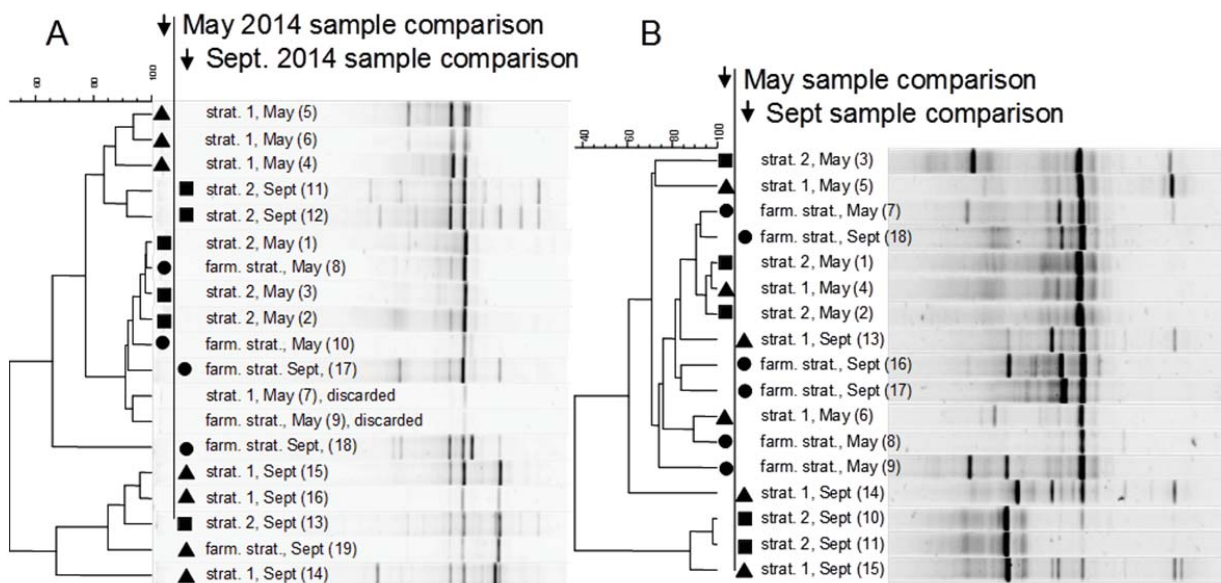


Fig. 1. Dendrograms based on UPGMA clustering using Pearson correlation coefficient of DGGE profiles showing fungal diversity similarities in leaf (A) and grape (B) samples collected in May/Sept. 2014. ▲ = strategy 1, full pesticide dose + BCA; ■ = strategy 2, reduced pesticide dose + canopy management; ● = farmer’s treatment



exception consisted of one replicate from strategy 1 treatments that nested together with the banding profile obtained from strategy 2 samples (Fig. 1B).

The culture dependent inventory of fungi from leaves and grapes yielded approximately 600 strains in 2014: (i) Three non-identifiable members of the Cordycipitaceae were isolated sporadically from leaves or grape particles deriving from all three plots. Because Cordycipitaceae are frequently associated with insects and accommodating biological control agents against insect pests, these taxa may present ecological service providers and their entomopathogenous effect will be tested in future studies. (ii) The order of the Helotiales was represented by *Chalara holubovae*, previously described as an endophyte from pine needles (KOUKOL, 2011), *Cistella acuum* and 12 sporadically retrieved other species. These other species could not be identified on the bases of ITS and LSU rDNA sequences through Blast searches in DNA sequence reference databases and may present undescribed species. Because they are at least distantly related to the *Hymenoscyphus* plant pathogen, a follow up project should address the ecological role of these fungi in vineyards. (iii) *Aureobasidium pullulans* was the mostly encountered taxon in all experimental plots. Two strains of the species present the active components in the biological control formulation Botector that was applied to the strategy 1 plot in 2013 and 2014. It is likely that *Aureobasidium pullulans* presents an important naturally occurring component in vineyards as was already suggested by others (MARTINI et al., 2009; SCHMID et al., 2011). (iv) Although numerous species of *Penicillium* and *Aspergillus* were isolated including *A. jensenii*, *A. sclerotiorum*, *P. glabrum*, *P. bialowiezense*, *P. brevicompactum*, *P. olsonii*, *P. parvulum*, *P. sumatrense*, *P. paraherquei*, *P. citreonigrum*, and *P. polonicum*, there was no indication for the presence of any of the most mycotoxigenic species such as *A. carbonarius*, *A. flavus*, *A. parasiticus*, *A. ochraceus*, *P. verrucosum*, and *P. crustosum*.

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## **OBC GUIDELINES FOR INTEGRATED PRODUCTION OF GRAPES: LESSONS TO LEARN AND EXPERIENCES TO SHARE BETWEEN OR GANIC AND INTEGRATED PRODUCTION**

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The International Organisation for Biological and Integrated Control-WPRS is one of six Regional Sections of the International Organisation for Biological Control (IOBC). IOBC was established in 1955 to promote environmentally safe methods of pest and disease control in plant protection. Members of WPRS are individual scientists, governmental, scientific or commercial organisations from 24 countries of Europe, the Mediterranean region and the Middle East. IOBC-WPRS encourages collaboration in promoting feasible and environmentally safe methods of pest and pathogen control. Integrated Production is a concept of sustainable agriculture developed in 1976 which has gained international recognition and application. The concept is based on the use of natural resources and regulating mechanisms to replace potentially polluting inputs. The agronomic preventive measures and biological/physical/chemical methods are carefully selected and balanced taking into account the protection of health of both farmers and consumers and of the environment. The principles and objectives of IP evolving during the 1980s have been compiled, analyzed and formulated by an IOBC panel of experts in 1992, and first been published in 1993 (IOBC-WPRS Bull. Vol. 16 (1), 1993). The document and vision was updated in the 2<sup>nd</sup> edition of 1999 and in the 3<sup>rd</sup> edition 2004). The 2004 IOBC Standard for Integrated Production covers ecological, ethical and social aspects of agricultural production as well as aspects of food quality and safety. It is presently one of the highest international food production standards and unique in the way comprehensive sustainability ambitions are coupled with effective and practicable approaches on the farm. The current set of IP guidelines and related tools has proven helpful and inspirational for farmers' organisations looking for a feasible way to work with integrated production in the premium food segment.

All information is available at [http://www.iobc-wprs.org/ip\\_ipm/index.html](http://www.iobc-wprs.org/ip_ipm/index.html).

Integrated production and Organic production can support each other in research/experimentation and also in advisory service because of several common problems/solutions.

Some possible technical synergies between Integrated production and Organic production are the following:

- Ecological infrastructures
  - Composition of infrastructures
  - Population dynamics/spreading of beneficial organisms
- Soil/weed management
  - Effect on soil/plant/pest
- Fertilisation plan/organic matter managementPlant protectionAgronomic preventive methods
  - DSS/forecasting models
  - Biopesticides/similar techniques/selectivity
- Irrigation methods
  - Water balance
  - Relationship with plant/pest/etc.

## STRATEGIES TO DEVELOP EFFECTIVE, INNOVATIVE AND PRACTICAL APPROACHES TO PROTECT MAJOR EUROPEAN FRUIT CROPS FROM PESTS AND PATHOGENS (DROPSA)

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DROPSA is a 4-year project involving 26 partners from Europe, North America, Asia and New Zealand. It will focus on the spotted winged Drosophila fruit fly, *Drosophila suzukii*, and the bacterial pathogens *Pseudomonas syringae* pv. *actinidiae*, *Xanthomonas fragariae* and *X. arboricola* pv. *pruni*. These pathogens and *D. suzukii* are a major concern and challenge to the fruit industry because their eradication or containment are no longer possible.

*Drosophila suzukii* is a highly polyphagous invasive pest native to Asia that causes significant damage to a wide variety of berry and stone fruit crops. Females have a prominent serrated ovipositor, enabling it to pierce the skin and deposit eggs inside ripening fruit. The larvae feed and develop within the fruit, causing it to become soft and rot rapidly, resulting in reduced crop yields and significant financial losses. The production and quality of fruit in the EU is also threatened by bacterial and fungal diseases. The pathogens *P. syringae*, *X. fragariae* and *X. arboricola* are behind the most recent and devastating outbreaks of disease to affect European fruit production, and options for their control are limited.

The aims of the project are:

- To determine the pathways of introduction and spread of *D. suzukii* and pathogens across the European fruit sector and develop preventative strategies and recommendations against the introduction of other dangerous fruit pests and diseases.
- To identify the key mechanisms within the biology and ecology of *D. suzukii* and the life cycles and epidemiology of *P. syringae*, *X. fragariae* and *X. arboricola*, to provide a platform for the development of preventative and sustainable control methods.
- To develop practical, effective and innovative solutions for their control for inclusion in integrated pest management (IPM) strategies.
- To develop forecasting and decision support systems and risk management as a component of IPM.
- To provide economic analyses of proposed strategies for crop protection to support decision making in the implementation of practical solutions and IPM strategies to protect the EU fruit sector.

Using molecular markers, progress has been made towards determining the areas of origin and spread of pathogens and *D. suzukii* in Europe. Biological control agents, semiochemicals and chemicals, including commercialized compounds and novel materials, are currently being evaluated in the laboratory and under field conditions, for their management.

This project has received funding from the European Union's Seventh Framework programme for research, technological development and demonstration under grant agreement number 613678 (DROPSA).