Final report

for the CORE Organic II funded project

"Integration of plant resistance, cropping practices, and biocontrol agents for enhancing disease management, yield efficiency, and biodiversity in organic European vineyards – VineMan.org"

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Project acronym:	VineMan.org			
Title:	Integration of plant resistance, cropping practices, and biocontrol agents for enhancing disease management, yield efficiency, and biodiversity in organic European vineyards			
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Project summary suitable for web publication

The Project aimed at improving disease control, which is one of the main and most difficult tasks in organic viticulture, integrating plant resistance against pathogens, cropping practices, use of weather-driven disease models, and use of biocontrol agents (BCAs). All these aspects were firstly considered separately in each WP by the competent partners, then efforts were made to develop innovative vineyard management strategies that consider all these aspects.

All plants have an innate immunity against pathogenic fungi and oomycetes that is triggered by pathogen associated molecular patterns (PAMPs). Repeated application of these molecules or their structural analogues can activate and enhance the innate defence response against a following infection by pathogens. During the project, 85 substances were tested, 26 were able to induce a pH-shift or demonstrated to have some cytotoxic activities, 11 were further analysed in pathogen-leaf-disc assays and demonstrated to reduce downy mildew sporulation. One substance achieved promising results also in field experiments.

Different methods for manipulating vegetative growth, canopy density, and fruit exposure were evaluated as a means for making the microclimate less favourable to pathogens and more ideal for the ripeness of the grapes. The first leaves of each shoot were removed at pre-flowering (ELR) and at pre-veraison, and compared to control vines in Italian, Spanish and Austrian vineyards. ELR was effective in reducing bunch sensitivity to grey mould by reducing bunch compactness and increasing berry skin thickness. Both treatments did not affect the titratable acidity of the musts, while ELR increased the tartaric acid suggesting the possibility to obtain more balanced wines by preserving acidity. Above-bunch-zone leaf removal applied at pre- and post-veraison was also tested on potted vines and compared to untreated vines. The results are very promising in that defoliation was effective in temporarily delaying technological maturity without affecting bunch colour and the content of phenoles. Other treatments, such as anti-transiprant application and “cutting off parts of bunches” were also tested.

Existing models for predicting plant disease outbreaks/epidemics were evaluated for their ability to support decision-making about crop protection, based on the presence of favorable environmental conditions and/or biological information concerning the disease and/or the host plant. Mechanistic, weather-driven models for downy and powdery mildews were implemented in a web-based platform provided by Horta s.r.l., able to produce decision-aids for crop protection in organic viticulture.

Fitness and efficacy of biocontrol agents, representing formulations of bacteria and fungi already registered in Europe, were evaluated in relation to grape pest and disease control under organic practices. Strain-specific detection systems were developed and tested in controlled and natural conditions. Experiments in controlled and natural conditions were performed to test the compatibility of different BCAs and inorganic fungicides for mixed or sequential application and it was demonstrated that sulphur can enhance the efficacy of the product Serenade™.

Two innovative management strategies were designed, evaluated through an ex-ante assessment, and tested in different experimental vineyards set up in Italy, Austria, Germany and Slovenia. A first “risk-adverse strategy” is based on the combination of: i) fall treatments with the hyperparasite Ampelomyces spp. for the reduction of the overwintering chasmothecia of Erysiphe necator, ii) the web-portal with models for the prediction of downy and powdery mildews to schedule copper and sulphur treatments at label dose during the season; iii) use of BCAs for the control of grey mold. A second “risk-seeking strategy” is based only on: i) low-dose copper and sulphur applications according to the models and ii) early leaf removal for the control of grey mould. In general, the use of epidemiological models allowed a significant reduction of fungicides, with a consequent reduction of the costs for disease management and the ecotoxicity risk, as well as an increased social acceptability of the vineyard management.

Finally, to test whether the innovative management strategies can increase overall numbers of species inhabiting the grapevines, appropriate culture-depended and culture-independent methods were implemented and applied on samples form the experimental vineyard in Slovenia. Unfortunately, the applied methods could not show that the different management strategies had a significant effect on the fungal species profiles, but very interesting fungal species were detected and the retrieved culture collection represents an important resource for future studies.

Pre-project summary

The Project involves 9 research groups in 5 EU Countries. The VineMan.org Project 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 the 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 is organised in 8 Work Packages (WPs), each of them led by a competent partner, and they are closely related one to each other. Management of the project activities, knowledge, IPR and exploitation of the results have a specific WP (WP1). Methods for inducing the innate immunity of plants against fungi and oomycetes pathogenic to Vitis vinifera will be evaluated in WP2. The effect of some viticulture management options on the development of the target diseases will be investigated in WP3, with particular focus on canopy structure and cluster/berry morphology modifications. WP 4 is devoted to the study of the relationships between the target pathogens and the environmental conditions with emphasis to 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, will be evaluated in four BCAs representing bacteria and fungi including yeasts already registered in the EU as microbial biopesticides. WP6 will be focused on the development of new strategies based on design-assessment-adjustment cycle. Two WPs will be aimed to field trials (WP7) and to the evaluation and monitoring of the microbial communities present on grape leaves and berries (WP8).

1. Main results, conclusions and fulfilment of objectives

1.1 Summary of main results and conclusions

First activity of the project was the organization of the “Kick-off meeting” in Italy (Piacenza, 24.01.2012) where all partners met and the plan for the project activities was discussed and agreed. Shortly after, the Consortium Agreement (D1.1.1) was prepared and signed by all participants. Representative persons of organic farmer associations, European retailers, and consumer’s associations were contacted by the coordinator (under suggestion of the partners) for their availability to constitute an external “Advisory Board” for the project (D1.1.2). Three experts accepted the role: a representative person of Italian organic farmer association, a representative person of Italian local wine association, and an adviser at the Austrian Chamber of Agriculture and representative person of Austrian organic farmer association. The project materials were provided to the “Advisory Board” to obtain comment and suggestions; experts were invited to the “Intermediate meeting” in Slovenia and to the final congress in Vienna.
Different dissemination actions were performed within the four indicated “pillars”. In particular, the project website was developed (www.vineman-org.eu) (D1.2.4) and two leaflets were prepared and translated in the partners languages, explaining respectively the objectives and content of the project (D1.2.5), and the main project results and recommendations to stakeholders (D1.2.6). The objectives and the results of the projects were presented in different occasions: i) to students (teaching), ii) to the scientific community, with different presentation in conferences (D1.2.1) and through peer-reviewed articles (D1.2.2), iii) to stakeholders, during several workshops organized in different countries (D1.2.8) and through articles published in professional magazines (1.2.7), and vi) to the “big audience”, through articles in local newspapers. Finally a very successful final congress was organized in Vienna (AGES) and it was attended by around 150 stakeholders (D1.2.3).

The Project aimed at improving disease control, which is one of the main and most difficult tasks in organic viticulture, integrating plant resistance against pathogens, cropping practices, use of weather-driven disease models, and use of biocontrol agents (BCAs). All these aspects were firstly considered separately in each WP by the competent partners, then efforts were made to develop innovative vineyard management strategies that integrate all these aspects.

All plants have an innate immunity against pathogenic fungi and oomycetes that is triggered by pathogen associated molecular patterns (PAMPs). Repeated application of these molecules or their structural analogues can activate and enhance the innate defence response against a following infection by pathogens. During the project over 80 natural substances including lipopolysaccharides from bacteria, extracts from plants and fungi, secondary plant metabolites like flavonoids, phenylpropanoids, polysaccharides, peptides and other molecules were screened in pH-shift bioassays using grapevine cell suspension culture (D2.1.1). From the 85 tested substances 26 were able to induce a pH-shift or demonstrated to have some cytotoxic activities and were therefore further analysed in pathogen-leaf-disc assays (D.2.1.2). Three substances inducing a pH-shift also showed the ability to significantly reduce sporulation of downy mildew, and two extracts, five secondary metabolites and one other substance were identified, which caused a clear reduction of downy mildew sporulation. These promising candidates were further analysed on greenhouse plants and in small plot experiments in the field. Plants were sprayed with the test substances by using a special application device and 24 hours later inoculated with *P. viticola* spores. The results of the greenhouse experiments reflected more or less the results from the leaf-disc assays. All tested substances showed a reduction of sporulation of the pathogen. For the small plot experiments randomized rows of grapevine plants with variable positions in the field were sprayed 7 to 10 times during the season 2013 and 2014. In addition to natural infections an artificial infection with a spore solution of the downy mildew was conducted. Unfortunately the results of the field experiments of the potential resistance inducing substance FR-088 could not confirm the results from the greenhouse and laboratory studies. On the other hand, plants sprayed with the substance FR-

010 showed a clear reduction of disease symptoms compared to the untreated control. (D2.1.3). To investigate a direct toxicity of FR-010 to the downy mildew an incubation assay of zoospores from *P. viticola* with the test substance has been conducted. Even in low concentration (0.001 %) FR-010 inhibited downy mildew sporulation completely. From the literature it is known that the resistance inducing component chitin has also antimicrobial property which makes it in principle difficult to distinguish between the resistance inducing activity of a PAMP and a possible direct toxic effect to the pathogen. To further investigate the resistance inducing capacity of FR-010 semi-quantitative RT-PCR analysis of marker genes of plant defence were conducted and preliminary results have indicated no increase in gene expression in leaf discs after FR-010 treatment. These results suggest that the distinct effect on *P. viticola* sporulation is likely due to a direct toxic reaction to the pathogen rather than a resistance inducing property of FR-010. Additional experiments under natural conditions have to clarify to which extent the reduction of disease symptoms is in general a result of the direct toxicity of the substance to the pathogen or a consequence of induced resistance in the plant.

Different methods for manipulating vegetative growth, canopy density, and fruit exposure were evaluated as a means for making the microclimate less favourable to pathogens and more ideal for the ripeness of the grapes. Primary leaves and second shoots developing from nodes 1 to 6 were removed at pre-flowering (ELR) and at pre-veraison and compared to control vines, in Italian, Spanish and Austrian vineyards. ELR was very effective at increasing berry skin thickness (in Italian and Spanish vineyards), decreasing bunch compactness and, consequently, reducing sensitivity to grey mould (in Italian, Spanish and Austrian vineyards). The beneficial effect was more evident in the Austrian vineyard where the weather was more conducive to this disease. Both treatments did not affect the titratable acidity in musts while in Italian investigations ELR increased the tartaric acid suggesting the possibility to obtain more balanced wines by preserving acidity. The analysis of the RGB imaging system taken at harvest time revealed an average increase in canopy porosity with respect to control vines, in grapevines subjected to defoliation at veraison and at pre-bloom, respectively. The percentage rate of exposed fruits was significantly larger in ELR vines with respect to control and plants defoliated at veraison. Above-bunch-zone leaf removal applied at pre- and post-veraison was also tested on potted vines and compared to untreated vines. The seasonal carbon/yield ratio did not differ between the different treatments and neither berry fresh mass nor relative skin growth, flesh and seeds were affected by the treatments. Above-bunch-zone defoliations were effective in temporarily delaying technological maturity without affecting bunch colour and the content of phenols. Other treatments were tested such as “cutting off parts of bunches” at berries pea-sized and anti-transpirant or gibberellin applications at fruit set and veraison or at flowering, respectively. All these treatments significantly decreased cluster compactness and consequently incidence of botrytis and lowered titratable acidity.

A literature review (D4.1.1) was performed in order to identify the epidemiological models for the major grapevine disease of potential interest for organic viticulture. These models were evaluated and the two mechanistic models for primary infection of downy and powdery mildew and the model for secondary infection of downy mildew developed by Rossi et al. (2008), Caffi et al. (2011 and 2013), respectively, were considered the most able to support decision making about crop protection. 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 (D4.1.2) and connected to a server repository that stores weather data coming from a network of weather stations installed in the vineyards. The electronic platform is provided by Horta s.r.l., a spin-off company of the Università Cattolica del Sacro Cuore, and is currently available for the project partners through the project website.
The output of the models implemented in the electronic platform was considered as a tool to be tested in WP7 for the management of the diseases. The weather station of each experimental vineyard was connected to the platform and the WP7 partners were trained for the use of the system. In particular, the electronic platform was tested in the experimental vineyards in Austria,

Italy and Slovenia in two grapevine growing seasons (2013 and 2014), under very different epidemic conditions: from almost absence of the diseases in the untreated control to the needs of closing the untreated plots in order to avoid the complete defoliation of the grapevines.
The models confirmed the well-known characteristics of mechanistic models, being able to correctly explain the main events of disease epidemics in different areas without any need of calibration or modification (D4.2.1). The models produced some unjustified alarms, in particular at the beginning of the season, but they extensively confirmed their utility in being used as a support during the decision making process for scheduling fungicides application, not only under IPM conditions (Caffi et al., 2010 and 2012), but also under organic management of vineyards.

Fitness and efficacy of commercially available biocontrol agents were evaluated in relation to grape pest and disease control under organic practices. Strain-specific detection systems were developed and validated for *Bacillus amyloliquefaciens* QST713, the active ingredient of Serenade Max™ (hereafter referred as QST713), *Ampelomyces sp.* AQ10, the active ingredient of AQ10™ (hereafter referred as AQ10) *Aureobasidum pullulans* CF10 and CF40, the active ingredient of Botector™ (hereafter referred as CF10/40). Species-specific PCR assay was developed for *Lecanicillium lecanii*, the active ingredient of Mycotal™ (D5.1.1). To pretest the use of the qPCR protocol for bio pesticide tracking in field, QST713 and AQ10 were applied on grapevine leaves with a potter tower. After two weeks, the amount of QST713 was close to or below the detection level. The persistence of AQ10 was even lower, after two days the fungus could no longer be detected. Greenhouse experiments with potted grape vines under different controlled environments (constant temperature and humidity profile vs. constant temperature but alternating cycles of high and low humidity) were performed to study the fitness of BCAs. Due to the high deviations within the replicates there is no statistical support for oscillating colonization pattern: based on existing data there is no indication on positive or negative effects of varying humidity profiles on persistence of QST713 (D5.1.2). To monitor the ability of the BCA to colonize the leaf surface under natural environment the BCAs were applied on two different cultivars (cv Neuburger and cv Zweigelt) in an Austrian vineyard. In the first year the BCAs were sprayed separately with an application interval of 3 weeks, in the second year an improved control strategy was tested. No reliable qPCR data could be obtained from the bio pesticide tracking in field, because of extremly low DNA concentrations of the samples. The evaluation of the efficacy revealed that the improved strategy using BCAs in combination failed to reduce *E. necator* and *Botrytis cinerea*. It has to be stated that during the investigation the disease pressure was so high that even the efficacy of conventional treatments were insufficient (D5.1.3).
The entomopathogenic fungus *Lecanicillium lecanii* (MycotalTM) was tested against *Scaphoideus titanus*, the vector of the phytoplasma responsible for the grapevine flavescence dorée disease. Results from laboratory tests showed that the *L. lecanii* was virulent to the larvae of *Scaphoideus titanus*. The percentage of the cumulative mortality of *S. titanus* nymphs corrected after Abbot's formula (1925) ranged from 73% (experiment 1), 54% and 35.7% (experiment 2) to 33.3% (experiment 3).
Bacillus amyloliquefaciens (QST713 from Serenade), *Aureobasidium pullulans* (Botector) and *Ampelomyces quisqualis* (AQ10) were tested in an *in vitro* plate test for compatibility using different artificial growth media. All strains grew well on all the media with similar results: *B. amyloliquefaciens* QST713 strongly inhibited the growth of *A. pullulans* and a precipitate formed in the agar in correspondence with the inhibition zone. No precipitate was seen between *B. amyloliquefaciens* QST713 and *A. quisqualis* AQ10. Due to the slow growth of AQ10, no growth inhibition could be recorded and new confrontation assay with decreased distance will be performed. For the strains *A. pullulans* BotA and BotB and *A. quisqualis* AQ10, weak growth inhibition for BotA and BotB was seen at very short distances, but no long distance effects was observed (D5.2.1). To test the compatibility of BCAs and inorganic fungicides for mixed or sequential application a comparison series was conducted. All leaves were sprayed with copper or sulphur respectively and after the leave surfaces were dry, the different BCAs were applied. While copper has a tendency to negatively influence QST713 on vine leaves, sulphur had a

clear positive tendency. Potted grapevines were placed in a semi controlled greenhouse to test improved control strategies against powdery mildew. After the first natural and visible infestation with *Erysiphe necator,* Serenade™ ,AQ 10™, Serenade™ in combination with sulphur respectively with AQ 10™ were applied in the recommended dose by hand. The bio pesticide tracking revealed that AQ 10 could not colonize the leave surfaces inspite high *E. necator* infestation, but a slight reduction of *E. necator* (qPCR and in visual assessment) could be stated. No positive or negative influence of QST 713 on persistence of AQ 10 could be observed. QST731 could colonize the leave surfaces. Positive influence of sulphur on persistence of QST 713 could be confirmed (D5.2.2 and D5.2.3).

Single management components obtained from WP2 to WP5 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 the UCSC 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 the UCSC 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 multi-criteria assessment tool DEXiPM was used to *ex-ante* assess these innovative strategies against the “organic baseline” on economic (labour, yield effect, product quality, product price) environmental and health risk for workers aspects (D6.1.1). Significant differences were found in the social and the economical sustainability while no differences were found in the global environmental sustainability of the considered strategies. 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.
During grape-growing seasons 2013 and 2014 the innovative strategies were tested in different experimental vineyards (D7.1.2 and D7.1.3) and an *ex-post* assessment was performed with the data collected in the fields using the tool DEXiPM complemented by the GIS-based risk assessment tool SYNOPS-WEB, that calculates the potential risk of chemical plant protection products for both terrestrial and aquatic organisms. Under the organic management regimes tested within this project only few significant modifications could be detected: the level of both acute and chronic risk for aquatic organism was reduced in both innovative strategies, and the ecotoxic chronic risk for both Daphnia and algae was reduced within the risk-seeking strategy. Many changes were adopted in the different management strategies, nonetheless the DEXiPM tool did not detect significant differences in the overall sustainability. This could be due to the low sensibility of the model: the model considers classes of attributes, not specific values. The ranges of the classes were developed for Integrated management of vineyards and seems to be not adequate for organic management. For example, the fungicide TFI (Treatment frequency index) assumed different values for the different management strategies, but almost all fell in the same class and therefore no differences could be detected by the model (D6.1.3).

Four experimental vineyards were set up following a common protocol (D7.1.1) in different countries to test the innovative strategies developed in WP6 during grapevine growing seasons 2013 and 2014. Monitoring of the plant phenology and of the principal grapevine diseases was performed regularly. Weather conditions of the different locations and the two seasons triggered very different epidemic: from almost absence of the diseases in the untreated control to the needs of closing the untreated plots in order to avoid the complete defoliation of the grapevines. In general, in all experimental vineyards of all countries the management performed following both risk-adverse and risk-seeking strategies allowed the same (or even better) disease control than the farmer strategy but reducing the number of treatments and application dose (D7.1.2

and D7.1.3). In Italy grapes were harvested separately and yield and must characteristics were analyzed: cluster weight was significantly reduced in S2, while no significant differences were detected in berry weight. Sugar and phenols content in grapes were not influenced by the different management strategies. Only anthocyanin content in grapes seemed to be positively influenced by the leaf removal (increased compared to S1 and Farm). Microvinifications were performed with the separately harvested grapes and the wines characteristics analysed: alcohol and polyphenols content, as well as total titratable acidity were not influenced by the different management strategies, while anthocyanins content resulted to be higher in the innovative strategies (at least in 2014) (D7.2.1). Finally, all relevant data were gathered from each experimental location in order to perform a cost-benefit analysis. The main elements in the analysis were gross yield, material costs and labour/equipment costs. A comparison between the disease management costs and the returns for covering other costs was also performed. The cost of the risk-averse strategy was consistently higher than the baseline, while the risk-seeking strategies demonstrated to effectively protect the production although applying less funcicides, allowing the growers to obtain a significant saving in the cost for diseases control and get a return for covering other costs (D7.3.1).

WP8 addressed the diversity of fungi in an organic vineyard. Two methods were applied in order to isolate fungi from grapes and leaves collected from the experimental vineyard in Slovenia and building up a culture collection of fungal strains: culture-dependent approach based on a dilution to extinction method of pulverized and excessively washed plant particles and a culture-independent approach was based on PCR amplification of DNA extracted from grapes and leaves to analyze mixed fungal communities with PCR primers specific for the fungal internal transcribed spacer region (ITS) 1 that is part of the rDNA gene cluster. The tested organic strategies did not show a clear measurable impact on the fungal diversity in or on grapes and leaves. However, analyses of data from the culture independent approach at least indicated that the taxa composition from strategy 1 (100% dosage and BCAs) treated leaves differed from the compositions retrieved from strategy 2 (reduced pesticide doses and canopy management) and the farmer's treatment in May 2014, while the patterns from strategy 2 and the farmer's treatment were similar. Strategy 1 communities from leaves differed also in September from those of the other treatments. In September 2014, the profiles from strategy 2 replicates 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 caused an increase of fungal diversity in the experiment. A similar indication was also observed on grapes harvested in September 2014. Two replicates from samples obtained from strategy 2 treatments differed qualitatively 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. By inoculating pulverized plant material, slow-growing fungi can be isolated that are usually overlooked if bigger plant pieces are used that in addition may host fast growing other species. Although occurring rather sporadically and also not in high isolation numbers, at least 14 fungal species were retrieved that are most likely new to science. Two 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 (such as *Lecanicillium lecanii*), these taxa may present ecological service providers and their entomopathogenous effect will be tested in future studies. Among others, 12 species of the order Helotiales were isolated equally sporadically that may also present undescribed species. Because they are at least distantly related to Hymenoscyphus plant pathogens, a follow-up project should address the ecological role of these fungi in vineyards. *Aureobasidium pullulans* was the mostly encountered taxon in all experimental plots. Because it was applied as a biological control formulation only on grapevines of the strategy 1 plot, it is possible that *A. pullulans* may present an important ecological service providing species naturally occurring in organic orchards, a hypothesis also supported by the results of other studies. Numerous species of Penicillium and Aspergillus were isolated consistently, however, not mycotoxigenic highly problematic species such as *A. carbonarius, A. ochraceus* or *P. verrucosum* and *P. crustosum*.

1.2 Fulfillment of objectives

The activities foreseen were all successfully performed, thanks to the professional work of each partner and the fruitful collaboration within partners.
The dissemination activities performed, in particular the workshops with stakeholders and the final meeting, gave a good feedback on the activities performed during the project.

The different activities performed within WP2 to WP7 were summarized in the following recommendation to end-users: i) potential use of resistance enhancing compounds in case of low disease pressure (downy mildew); ii) perform leaf removal at pre flowering stage on varieties with very compact clusters for reduced bunch rot sensitivity and increased grape quality; iii) use epidemiological models to better schedule treatments against downy and powdery mildew; and iv) combination with sulphur may increase efficacy of Serenade.

WP8 objectives were achieved although the applied methods could not show that the different management strategies had a significant effect on the fungal species profiles. In fact, very interesting fungal species were detected and the retrieved culture collection represents an important resource for future studies

2. Milestones and deliverables status

Milestones:

No[1]	Milestone name	Planned delivery month[2]	Actual delivery month[2]	Means of verification
M1.1.1	Signature of the Consortium Agreement	2	2	D1.1.1
M1.1.2	Entering of the Advisory Board	2	10	D1.1.2
M1.2.1	Kick-off meeting	1	1	List of participants and Minutes
M1.2.2	Publication of the project website	4	4	D1.2.4
M1.2.3	Intermediate meeting	18	18	List of participants and Minutes
M1.2.4	Final meeting	36	36	List of participants
M1.2.5	Project's Congress	36	36	D1.2.3
M2.1.1	Candidate compounds to be used in WP6 (2nd year)	12	12	Written list within D6.1.1
M2.1.2	Candidate compounds to be used in WP6 (3rd year)	24	24	Written list within D6.1.2
M3.1.1	Candidate canopy management solutions to be used in WP6 (2nd year)	12	12	Written list within D6.1.1
M3.1.2	Candidate canopy management solutions to be used in WP6 (3rd year)	24	24	Written list within D6.1.2
M4.1.1	Electronic platform with disease models available for WP7	12	12	D4.1.2
M5.2.1	Strain-specific qPCR methods available	12	12	D5.1.1
M5.2.2	Candidate BCAs to be used in WP6 (2nd year)	12	12	Written list within D6.1.1
M5.2.3	Candidate BCAs to be used in WP6 (3rd year)	24	24	Written list within D6.1.2
M6.1.1	New strategies to be tested in WP7 (2nd year)	12	12	D6.1.1
M6.1.2	New strategies to be tested in WP7 (3rd year)	24	24	D6.1.2
M7.1.1	Protocols for setting experimental vineyards ready	6	12	D7.1.1.
M8.1.1	Cultivation independent method for fungal analysis available	18	18	D8.1.1
M8.1.2	Cultivation dependent method for fungal analysis available	18	18	D8.1.2
M8.1.3	Methods for the identification of fungi available	18	18	D8.1.2

[1] Please use the numbering convention <WP number>.<number of milestone/deliverable within that WP>. For example, deliverable 4.2 would be the second deliverable from work package 4.
[2] Measured in months from the project start date (month 1).

Deliverables:

No[1]	Deliverable name and language	Nature[3]	Dissemination level[4] and link to the document	Planned delivery month[2]	Actual delivery month[2]
D1.1.1	Consortium Agreement	Other	INT	2	2
D1.1.2	Advisory board (list of members)	Other	PU	2	10
D1.1.3	Project report (mid-term)	Report	RE	18	18
D1.1.4	Project report (final)	Report	RE	36	36
D1.2.1	List of presentations in conferences	Other	PU	36	36
D1.2.2	List of articles in peer-reviewed journals	Other	PU	36	36
D1.2.3	Proceedings of the project congress	Other	PU	36	36
D1.2.4	Project web-site	Web-site	PU	4	4
D1.2.5	Project leaflets (content of the project)	Other	PU	4	4
D1.2.6	Project leaflets (results of the project)	Other	PU	36	36
D1.2.7	List of articles in professional journals	Other	PU	36	36
D1.2.8	List of workshops with stakeholders and end-users	Other	PU	36	36
D2.1.1	Report on efficacy of candidate resistance activator compounds in bioassay	Report	RE	18	18
D2.1.2	Report on efficacy of candidate resistance activator compounds on leaf disks	Report	RE	18	18
D2.1.3	Report on efficacy of candidate resistance activators in small plot experiments	Report	RE	30	36
D3.1.1	Report on canopy management effects on grape diseases	Report	RE	24	24
D3.1.2	Report on infrared system for canopy assessment	Report	RE	24	24
D3.1.3	Report on RGB imaging system for canopy assessment	Report	RE	24	24
D3.2.1	Report on cluster modification effects on grape diseases	Report	RE	24	24
D3.3.1	Report on modification of berry morphology	Report	RE	24	24
D3.3.2	Report on structure of the berry surface	Report	RE	24	24
D4.1.1	Report on models for the target diseases	Report	RE	12	12

[3] Please indicate the nature of the deliverable. For example Report, Paper, Book, Protocol, Prototype, Website, Database, Demonstrator, Meeting, Workshop…

[4] Please indicate the dissemination level using one of the following codes: PU = Public; INT= Internal (Restricted to other project participants); RE = Restricted to a group specified by the consortium; CO = Confidential, only for members of the consortium.

D4.1.2	Electronic platform with disease models	Web-site	RE	12	12
D4.2.1	Report on validated models for the target diseases	Report	RE	36	36
D5.1.1	Report on strain-specific qPCR for BCAs	Report	RE	12	12
D5.1.2	Report on survival and persistence of the BCAs in controlled environment	Report	RE	24	24
D5.1.3	Report on survival and persistence of the BCAs in vineyard	Report	RE	36	36
D5.2.1	Report on compatibility of BCA mixtures	Report	RE	18	18
D5.2.2	Report on efficacy of BCA mixtures in environment controlled experiments	Report	RE	24	24
D5.2.3	Report on efficacy of BCA mixtures in planta	Report	RE	36	36
D6.1.1	Ex-ante assessment of management strategies	Report	RE	12	12
D6.1.2	Ex-post assessment of WP7 and new ex-ante assessment	Report	RE	24	24
D6.1.3	Final ex-post assessment	Report	RE	36	36
D7.1.1	Protocol for setting in-vineyard experiments	Protocol	INT	6	12
D7.1.2	First report on disease control and yield in-vineyard experiments (1st year)	Report	RE	24	24
D7.1.3	Second report on disease control and yield in-vineyard experiments (2nd year)	Report	RE	36	36
D7.2.1	Report on wine quality in-vineyard experiments	Report	RE	36	36
D7.3.1	Report on grower's income in-vineyard experiments	Report	RE	36	36
D8.1.1	Report on methods for cultivation independent analysis of fungi	Report	RE	18	18
D8.1.2	Report on methods for cultivation dependent analysis of fungi	Report	RE	18	18
D8.1.3	Report on methods for the identification of encountered fungi	Report	RE	18	18
D8.2.1	Report on in-vineyard analysis of encountered fungal biodiversity	Report	RE	36	36

Additional comments (in case of major changes or deviation from the original list)

Delay in the delivery of M1.1.2/ D1.1.2: The consortium encountered unforeseen difficulties in contacting representative persons of organic farmer associations, European retailers, and consumer's associations, and in getting their approval to enter the Advisory board. In particular, the consortium was not able to find any available expert working directly in the retailer sector. Therefore, this expertise is not represented in the AB; nonetheless, the other experts are able to give some suggestions also related to that sector. The Advisory board was completed before the intermediate meeting and the members were invited to participate in that meeting; therefore, the consortium does not consider the delay as a relevant issue.

Delay in the delivery of M7.1.1/D7.1.1: The partners of WP7 delayed the design of the protocol for setting in-vineyard experiments, because they considered more appropriate to wait for the results from WP6 before defining the final protocol. This delay was not relevant in that the experimental vineyards had to be set (and have been set) around month 15 and the protocol was delivered in month 12.

Delay in the delivery of D2.1.3: Partner SWI delayed the delivery of D2.1.3 Report on efficacy of candidate resistance activators in small plot experiments because of the general late start of the activities but this was already explained to and agreed with the Secretariat.

3. Work package description and results:

WP 1	Project management and result dissemination
Responsible partner: P 01 – UCSC, V. Rossi	

Original description of work:

WP description: WP1 has two tasks, Task 1.1. is aimed at managing the project, Task 1.2 is aimed at disseminating the project and the results rising from the project.

Task 1.1: Management. The organisational structure of the project was designed in such a way to: i) respond to the project complexity and the degree of integration required, ii) guarantee consistency between the project work plan and the actual implementation, iii) achieve the project goals in a cost effective way according to the agreed time schedule and budget, iv) ensure an effective process of decision-making and internal communication, v) manage knowledge circulation and intellectual property rights (IPR). It includes the coordinator, the steering committee and an external advisory board.

The coordinator of the project: i) is the contact point between the CORE Organic II Secretariat and the partners involved in the consortium for the whole duration of the project; ii) secures that all project milestones and deliverables are met and takes actions if one or more partners fail to fulfil their commitments; iii) compiles and submits reports (deliverables D.1.1.3 and D1.1.4) and other deliverables to the Secretariat on behalf of the consortium. The steering committee (SC) is composed by the responsible of each Partner. The SC: i) takes decisions about the project management; ii) controls the progress of the project activities; iii) guarantees punctuality of reporting; iv) controls achievement of milestones and deliverables; v) suggests to refine work plan based on the achievements to date; vi) discusses dissemination issues; vii) manages risk; viii) discusses other matters which may arise within the project. The SC will keep in touch by regular communication and will formally meet at the project start and once a year, or more frequently if appropriate. SC members will also keep in close contact by e-mail or telephone whenever necessary. The agenda of the meetings will be set by the project coordinator, who will take the minutes and circulate them among the partners within three weeks following the meeting. The SC avails itself of the opinions of an external Advisory Board (AB) (the list of AB members is D1.1.2). The AB will be consulted at least once a year, at the intermediate and final meetings, and will be consulted by phone or web-conference when necessary. In principle, it will be constituted by representative persons of organic farmer associations, European retailers, and consumer's associations. The AB members will not receive remuneration but their travel and allowance costs will be covered by the project. A Consortium Agreement (CA) will be prepared based on section 6.4 of the "Guidelines for applicants" and signed, in order to manage the project activities, finances and IPR (D1.1.1). The CA will i) underpin the partner collaboration and provide the partners with mutual assurance on project management structures and procedures, and their rights and obligations towards one another; ii) assure the funding bodies involved that the project consortium has a satisfactory decision making capacity and is able to work together in a synergistic positive manner; iii) assure the partners and the funding bodies involved that IPR is managed in a way that meets the requirements of the partners involved.

Task 1.2: Dissemination. The dissemination plan includes the various communication routes, organised in four pillars focused on different audiences who have a potential interest in the project results, i.e., i) within-project audience, ii) scientific community, iii) professional users, and iv) big audience.

Pillar 1: Tools for 'within-project audience'. These tools are addressed to the small-groups involved into the project, i.e. partners and members of the Advisory Board, as well as to the funder bodies of CORE Organic II. They include the meetings of Technical committee and Advisory board (kick-off, intermediate and final meetings), and project reports for funders (mid-term and final reports). In addition, since some partners are involved in teaching (UCSC, SWI, UDLR, IFAT-UDG) the project results will be used in teaching, for both under-graduate and

graduate students in such a way to bring the research work closer to the students and to see if the research is understandable.
Pillar 2: Tools for the scientific community. These tools include: min 6 presentation in international and national conferences (D1.2.1) to communicate with other researchers in the early stage of the project and to enter in contact with new possible research partners which are interested in the same research field; min 4 publications in peer-reviewed journals with Impact Factor to show the high quality of the research performed within the project (D1.2.2); a final Congress to present the results and the holistic approach of the research for managing the organic vineyards, and proceedings will be published (D1.2.3)
Pillar 3: Tools for professional users of the results (consultants, farmers, etc.). A project web-site (D1.2.4) will be implemented and regularly updated which shows the project partners, aims, contents, progresses, and results. Considering that farmers are searching more and more information from Internet, the web-site will be enriched with presentations and videos showing results of practical interest for them. Project leaflets will be also prepared in English and translated in Italian, Spanish, German, and Slovenian at the beginning and end of the project to disseminate the project content and results, respectively (D1.2.5 and D1.2.6). Min 5 articles in professional magazines and journals will be published (D1.2.7). The choice of the magazine and journal will be carefully evaluated in each country to reach the target group as best as possible. Min 5 workshops will be organised with stakeholders and end-users, one in each participating country, some of them in form of visits to experimental fields (D1.2.8). Workshops are key ways for involving stakeholders and end-users and for communicating with them in an interactive way which can be helpful also for project planning and realisation.
Pillar 4: Tools for 'big audience'. Min 5 articles will be published in popular magazines or newspapers, at least one in each participant country (D1.2.9), and min 5 presentations will be prepared for broadcasting media (TV, radio, Internet magazines, etc.) in such a way to reach a big audience, including consumers, and make the research area familiar to them (D1.2.10). Special attention will be paid to the style of writing/speaking so that the reading and the understanding will be easy for the unspecialised audience.

Report on results obtained and changes to the original plan/WP aims:

A- results obtained:
During the project several and very important management activities were fulfilled. First of all the partners met at the "Kick-off meeting" in Italy, where the plan for the project activities was discussed and agreed. Shortly after, the Consortium Agreement was prepared and signed by all participants. During all the following 18 months the partners stayed in contact via emails and phone calls and met again all together for the "Intermediate meeting" in Slovenia. The activities went on with no particular issues and the partners met again in Freiburg (month 26) and in Vienna for the "final meeting" in January 2015.

Representative persons of organic farmer associations, European retailers, and consumer's associations were contacted by the coordinator (under suggestion by the partners) for their availability to constitute an external "Advisory Board" for the project. Three expert accepted the role: a representative person of Italian organic farmer association, a representative person of Italian local wine association and an adviser at the Austrian Chamber of Agriculture and representative person of Austrian organic farmer association. The "Advisory Board" was invited to the "Intermediate meeting" in Slovenia and to the "final congress" in Austria.

Different dissemination actions were performed within all the indicated "pillars". In particular, the project website was developed (www.vineman-org.eu) and two different leaflets (the first explaining the content and the second the results of the project) were prepared and translated in the partners languages.
The objectives and the results of the projects were presented in different occasions: i) to students (teaching), ii) to the scientific community, with presentations to national and international congress and through peer-reviewed articles, iii) to stakeholders, during several

workshops organized in different countries, during the final congress organized in Vienna and through articles published in professional magazines, and vi) to the “big audience”, through articles in local newspaper.

B- comments on deviations from the original plan:
Partner IFAT-UDG (Spain) decided to leave the Consortium because he is not able to fulfil the activities foreseen by the project, since he did not receive any funding. All partners agreed with the termination of IFAT-UDG. IFAT-UDG was involved only in WP5 and its planned work was redistributed within the other WP’s partners without any budget change request. The Coordinator sent an official communication to the Core Organic II Secretariat as agreed.

The Consortium encountered some difficulties in getting the approval by representative persons of organic farmer associations, European retailers, and consumer’s associations to enter the Advisory board. In particular, the consortium was not able to find an available expert working directly in the retailer sector, nonetheless the other experts appointed were able to give fruitful suggestions also related to that sector.

The Consortium was not able to perform all dissemination activities foreseen for Pillar 4 “big audience”, nonetheless a higher number of all other kind of dissemination activities were performed, in particular the ones falling into Pillar 3 “for professional users” that was considered the most important audience to be reach and the one that will benefit more from the project results.

WP 2	**Enhancement of plant resistance**
Responsible partner: P 04 – SWI, H-H. Kassemeyer	

Original description of work:
WP description: methods and processes to enhance the plant resistance against the target diseases will be evaluated as induced resistance (Task 2.1).

Task 2.1: Induction of disease resistance. Innate immunity, resistance response, and the molecular patterns which are able to induce defense response against fungi and oomycetes will be studied by means of molecular and biochemical tools. The specific compounds inducing the innate immunity and enhancing the resistance response of grapevine will be analyzed through HPLC, LC-MS, GC-MS and Maldi-Tof. These molecules and structural analogues from microorganisms (including fungi, bacteria, algae, and mosses isolated from the plant surface) will be purified and tested in three steps: i) bioassay with a grapevine cell suspension culture to quantify the potential to activate resistance on a cellular level (e.g., production of phytoalexins and PR-proteins) (D2.2.1); ii) leaf disk assay to test candidate substances after a challenging infection based on microscope analysis and disease severity on leaf disks (D2.2.2); iii) greenhouse and small plot experiments; in this step, the compounds will be formulated with biocompatible detergents ad stickers to enhance/ stabilize their efficacy under field conditions. Candidate compounds, as well as detergents and stickers will be evaluated for the fulfillment of the EC-regulations (D.2.2.3).

Report on results obtained and changes to the original plan/WP aims:

A- results obtained:
Over 80 natural substances including lipopolysaccharides from bacteria, extracts from plants and fungi, secondary plant metabolites like flavonoids, phenylpropanoids, polysaccharides, peptides and other molecules were screened in pH-shift bioassays using grapevine cell suspension culture (D2.2.1). From the 85 tested substances 57 % didn’t show any pH-shift inducing capacity, whereas 21 % demonstrated a potential resistance inducing activity. The last 22 % seemed to have a cytotoxic effect to the cell culture. For some of them semi-quantitative

RT-PCR analysis were conducted to verify the resistance inducing activity.
In the next step 26 substances, including all pH-shift inducing components and some cytotoxic molecules, have been further analysed in pathogen-leaf-disc assays (D.2.2.2). Therefore disease severities of downy or powdery mildew infected leaf-disks were either analysed with an image processing program or by microscopy. In the end only three substances of the pH-shift inducing molecules have shown the ability to reduce sporulation of the downy mildew significantly, whereas all other compounds were insufficient, including the known resistance inducing component chitosan. In addition some of the cytotoxic molecules were also tested in pathogen-leaf-disc assays. As a result of these assays two extracts, five secondary metabolites and one other substance were identified, which caused a clear reduction of the sporulation of the downy mildew.
After testing the potential resistance inducing substances and some of the cytotoxic molecules on leaf discs, promising candidates were further analysed on greenhouse plants and in small plot experiments in the field. Therefore greenhouse plants were first sprayed with the test substance by using a special application device and 24 hours later inoculated with P. viticola spores. The results from the greenhouse plants experiments reflected more or less the results from the leaf-disc assays. All tested substances showed a reduction of sporulation of the pathogen.
For the small plot experiments randomized rows of grapevine plants with variable positions in the field were sprayed 7 to 10 times during the season 2013 and 2014. In addition to natural infections an artificial infection with a spore solution of the downy mildew was conducted. To enhance wettability and water resistance candidates were mixed with a biocompatible detergent. Unfortunately the results of the field experiments of the potential resistance inducing substance FR-088 could not confirm the results from the greenhouse and laboratory studies. The disease severity of Plasmopara viticola infested leaves and grapes were the same for FR-088 treated and for the untreated control. On the other hand grapevine plants treated with the plant protection product Cuprozin® almost didn't show any disease symptoms. So far it is not known which factors are responsible for the breakdown of FR-088 under field conditions. Possible explanations are UV instability of the substance, poor water resistance, degradation by microorganism activity on the leaf surface or insufficient activation of plant resistance. In contrast to the results from FR-088 treated plants, plants sprayed with the substance FR-010 showed a clear reduction of disease symptoms compared to the untreated control. The reduction of disease severity on leaves and grapes of FR-010 applied grapevine plants were almost as good as the approach sprayed with Cuprozin®. To investigate a direct toxicity of FR-010 to the downy mildew an incubation assay of zoospores from P. viticola with the test substance has been conducted. Even in low concentration (0.001 %) FR-010 inhibited downy mildew sporulation completely. From the literature it is known that the resistance inducing component chitin has also antimicrobial property which makes it in principle difficult to distinguish between the resistance inducing activity of a PAMP and a possible direct toxic effect to the pathogen. For future experiments the pathogen-leaf-disc assay will be improved by spraying one side of the leaf with the test substance and the other with zoospores to avoid direct contact of the test substance with the pathogen. To further investigate the resistance inducing capacity of FR-010 semi-quantitative RT-PCR analysis of marker genes of plant defence will be conducted. But preliminary results have already indicated no increase in gene expression in leaf discs after FR-010 treatment. These results suggest that the distinct effect on P. viticola sporulation is likely due to a direct toxic reaction to the pathogen rather than a resistance inducing property of FR-010. Interestingly nearly all substances analysed in this study seemed to have a direct effect on the pathogen in this experimental design, explaining possibly most of the sporulation reduction results from the pathogen-leaf-disc assays. Additional experiments under natural conditions have to clarify to which extent the reduction of disease symptoms is in general a result of the direct toxicity of the substance to the pathogen or a consequence of induced resistance in the plant.

B- comments on deviations from the original plan:
The specific innate immunity inducing compounds were not identified and analysed through

HPLC, LC-MS, GC-MS and Maldi-Tof because of time and financial reasons. Instead, the compounds were supplied by companies and other collaboration partners. Due to substances availability and unreasonable asset cost only two candidates were tested in small plot experiments in the field.

WP 3	**Modification of canopy and cluster structure**
Responsible partner: P 08 – LFZ, F. Rosner	

Original description of work:

WP description: the effect of some viticulture management options on the development of the target diseases will be investigated with new methodologies. Particular attention will be given to canopy structure (Task 3.1), cluster (Task 3.2) and berry morphology (Task 3.3).

Task 3.1: Modification of canopy structure and fruit microclimate. Different methods for manipulating vegetative growth, canopy density, and fruit exposure will be evaluated as a means for making the microclimate less favourable to pathogens and more ideal for the ripeness of the grapes. The effects of canopy management such as defoliation by hand, defoliation by compressed air, side shoots removal, water shoot removal, on three different canopy heights (low, middle and high), and a complete defoliation of the grape zone at veraison on canopy porosity and cluster exposure will be assessed. The effects of cultural practices applied at different severities (low and high) on disease severity (*B. cinerea)*, grape yield, must density, titratable acidity, pH-value, and nitrogen content in the must will be evaluated in Austrian (cv. `Grüner Veltliner` and `Zweigelt`) and Spanish (cv. `*Temparanillo*` and `*Grenache*`*)* vineyards over two grape-growing seasons by LFZ and UDLR, respectively (D.3.1.1). Canopy structure will be evaluated by measuring temperature using an infrared system. A temperature classification system will be developed related to green leaves, clusters, shoots, and canopy gaps (porosity) (D.3.1.2). An RGB imaging system will be also developed to estimate, in a few seconds, different colour pixel classes related to different vine organs (D.3.1.3). These tools could be valuably integrated within weather based disease models (WP4) as well as being used as a tool to judge spraying efficacy. Additionally damages on grapes and berries caused by sunburn will be evaluated.

Task 3.2: Modify cluster morphology. Methods for modifying cluster morphology in a way to increase pre-infectional resistance to bunch rots and to upgrade quality of grapes will be evaluated based on three approaches: i) pre-flowering source limitation through basal leaf removal to reduce both fruit set and berry size on potted vines (by UCSC); ii) targeted application of anti-transpirants at beginning of fruit set and veraison to slow down berry growth rates resulting in smaller final berry size by assessing bunch rot severity after artificial inoculation of *B. cinerea* on potted vines (by UCSC) and under natural inoculum conditions in vineyards (by LFZ); iii) growth regulators like prohexadione – calcium and gibberellin application at the middle or end of flowering to reduce cluster compactness and bunch rot severity in the vineyard (by LFZ). Grape bunch length and compactness will be determined in all the experiments. (D3.2.1). Task 3.2 will also benefit from the tools developed in Task 3.1 for non-destructive, real time estimates of cluster size and compactness, and berry size.

Task 3.3: Modify berry morphology. Berry morphology will be modified to have higher skin-to-pulp ratio through: i) late-season removal of median and apical leaves (by UCSC); ii) application of an anti-transpirant; iii) cutting off parts of bunches at bunch closing; iv) application of gibberelline; v) use of compressed air at the end of flowering; and vi) application of Regalis® aimed at moderating post veraison flesh enlargement (by LFZ). Experiments will be carried out in the vineyards in Italy and Austria (by UCSC and LFZ, respectively) and their effectiveness will be evaluated by assessing bunch rot severity *(B. cinerea)* under natural inoculum conditions and bunch density before grape-harvest (D3.3.1). The surface structure of both treated and untreated berries at different developmental stages will be characterized by using Low-

Temperature-Scanning-Electron-Microscopy (LTSEM) and Atomic-Force-Microscopy (AFM) by SWI (D3.3.2). All experiments will be replicated over two seasons.

Report on results obtained and changes to the original plan/WP aims:

A- results obtained:

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 moderate defoliated (water shoots, 2° shoots and 1-2 leaves per shoot in the fruiting zone) vines. ELR on cv. `Riesling` significantly affect the contents of total phenolic in must. Moreover LLR on cv. `Riesling` reduced must weight in comparison with non-defoliated, early defoliated and moderate defoliated vines.

The usage of IR-camera to investigate effects of defoliation on canopy biomass distribution and fruit exposure is depended on a wide variety of influences. First results suggest that `early removal of 6 leaves and of 2° shoots at pre bloom` (ELR) in regard to cluster compactness, cluster exposure and canopy porosity could be a possibility to reduce fungal infections. A secured statement would be possible with further investigations. Year-dependent the incidence of sunburn was significantly highest assessing treatment ELR (on cv. `Riesling` in 2012 plot B) while severity of sunburn was significantly highest regarding ELR (on cv. `Riesling` in 2013) and LLR (on cv. `Riesling` and `Zweigelt` in 2013).

With respect to RGB 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 PB6+L defoliation.

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

The application of Vapor Gard at fruit set and veraison reduced cluster compactness on cv. `Neuburger` vines in the year 2013. Furthermore, gibberellin at flowering lowered significantly cluster compactness on cv. `Riesling` vines in both years. The application of gibberellin during flowering reduced significantly the cluster weight. Vapor Gard at fruit set and veraison leads to a decrease of titratable acidity on cv. `Neuburger` only in the year 2013.

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 3–4 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 16–17°Brix and ensuring that at least 30–35% of the leaf area is removed.

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

Application of gibberellin during flowering induced a significant lower cluster compactness, highest berry weight and lowest titratable acidity. The application of anti-transpirant at veraison (T 7) and in combination at fruit set (T 9) led also to significantly lower titratable acidity as well as to lower must weight (T 9).

Cutting off whole clusters effected higher grape bunch length and must weight but yield and cluster number per vine were significantly and confidently lowest. The treatment “cutting off parts of bunches” resulted logically in lower cluster weight.

Prohexadione calcium during flowering reduced both cluster and berry weight as well as must weight and titratable acidity.

The berry pressure resistance of all varieties decrease during ripening. All four grape varieties

show different berry compression strengths. Most of the treatments had no or only a miner effect to the stability of grapevine berries. The grape varieties can differ in their response to a particular berry treatment (e.g. application of gibberellin).

B- comments on deviations from the original plan: No deviation

WP 4	**Environment and disease development**

Responsible partner: P 01 – UCSC, V. Rossi

Original description of work:

WP description: weather-driven, mechanistic, dynamic models for predicting plant disease outbreaks/epidemics will be developed (Task 4.1) and validated (Task 4.2) accounting for the relationships between the target pathogens, the environmental conditions (i.e., temperature, relative humidity, leaf/bunch wetness, and rainfall), and the host plant (i.e., growth stage and canopy development). These models will be used in WP6 and in WP7 for tactical decisions about disease control.

Task 4.1: Model development. Existing models (from literature) will be evaluated for their ability to support decision making about crop protection based on the presence of favourable environmental conditions and/or biological information concerning the disease and/or the host plant. Two models already developed by UCSC for predicting primary infection by both *P. viticola* and *E. necator* are good examples of the kind of models to be used for the VineMan.org project (8,24). New models will be developed, if necessary, using literature data through a 3-step process: i) design the model; ii) acquire data on the weather-pathogen relationships from literature or in ad hoc experiments; iii) develop the mathematical structure of the model (D4.1.1). The models will be implemented in an electronic platform able to produce decision aids for crop protection (D4.1.2). The platform will be accessible by the Partners trough the web site of the Project.

Task 4.2: Model evaluation. The accuracy of the models will be evaluated in the experimental vineyards of WP7. Model output will be statistically compared with the data on disease onset and development collected in the untreated plots. If necessary, the models will be modified and improved (D4.2.1).

Report on results obtained and changes to the original plan/WP aims:

A- results obtained:

A literature review was performed in order to identify the epidemiological models for the major grapevine disease. These models were evaluated and the two mechanistic models for primary infection of downy and powdery mildew and the model for secondary infection of downy mildew developed by Rossi et al. (2008), Caffi et al. (2011 and 2013), respectively, were considered the most able 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 particular, 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., the spin-off company of the Università Cattolica del Sacro Cuore, 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 WP7 for the management of the diseases. In particular, the models outputs should help the vineyard manager to better schedule the plant

protection products applications against the major pathogens in an way to reduce as much as possible the vain treatments.
The weather station of each experimental vineyard was connected to the platform and the WP7 partners were trained for the use of the system. In particular, the electronic platform was tested in the experimental vineyards in Austria, Italy and Slovenia in two grapevine growing seasons (2013 and 2014), under very different epidemic conditions: from almost absence of the diseases in the untreated control to the needs of closing the untreated plots in order to avoid the complete defoliation of the grapevines.
The models confirmed the well-known characteristics of mechanistic models, being able to correctly explain the main events of disease epidemics in different areas without any need of calibration or modification (D4.2.1). The models produced some unjustified alarms, in particular at the beginning of the season, but they extensively confirmed their utility in being used as a support during the decision making process for scheduling fungicides application, not only under IPM conditions (Caffi et al., 2010 and 2012), but also under organic management of vineyards.

B- comments on deviations from the original plan: No deviation

WP 5	**Improve fitness & efficacy of BCAs**
Responsible partner: P 02 - AGES, H. Reisenzein	

Original description of work:
WP description: Fitness and efficacy of BCAs representing formulations of bacteria and fungi already registered in accordance with EU Regulations, will be evaluated in relation to grape disease control under organic practices. The selected BCAs (corresponding to commercial microbial biopesticide products), will consist of the bacterium *Bacillus subtilis* QST713 (Serenade Max, Basf), the filamentouse fungi *Ampelomyces quisqualis* AQ10 (AQ-10, Intrachem) and *Lecanicillium lecanii* (Mycotal, Koppert) and the yeast *Candida oleophila* O (Bionext). These are biocontrol agents of several grape diseases (downy and powdery mildews, grey mould) or have activity against similar pathogens in related hosts. The entomopathogenic fungus *L. lecanii* will be used against the leafhopper *Scaphoideus titanus*. *S. titanus* is the vector of a phytoplasma regulated as a quarantine pathogen within the EC and responsible for the dangerous grapevine flavescence dorée disease. The efficacy of *L. lecanii* against leafhoppers is demonstrated in other crops.

Task 5.1: Fitness of the BCAs. Fitness of selected BCA´s will be evaluated through bio pesticide tracking by combining selective-differential microbiological media with checking by standard PCR methods. Strain-specific qPCRs for quantification of *B. subtilis, A. quisqualis*, and *L. lecanii* will be developed and validated by BOKU (D5.1.1). *C. oleophila* can be tracked by using an established qPCR protocol. A series of comparative survival and environmental fate studies will be performed: i) under controlled environment with potted grape plants (high relative humidity, low relative humidity, alternating cyclic low-high relative humidity) (D5.1.2); ii) in two vineyards in Austria by AGES and in Spain by IFAT-UDG (D5.1.3). The population density of the BCAs will be monitored at appropriate time intervals (days, weeks, months depending on the assay) to determine their fate and residual persistence.

Task 5.2: Biological control of the main target diseases. Strategies will be developed to improve efficacy and reproducibility of the BCAs, and to increase the action spectrum of biological control of grape diseases, using compatible mixtures, sequential applications of BCA´s, or BCA´s in combination with natural products. To develop strain mixtures, a preliminary analysis will be done on compatibility of the BCAs by *in vitro* agar cross-inhibition tests (D5.2.1). Then, optimized mixture of BCA´s will be developed and submitted, together with individual components, to an efficacy assay under controlled environment conditions for control of the target diseases powdery mildew and grey mould (D5.2.2). A subsequent test will be done with

potted plants (two cultivars) under controlled environment by comparing the best mixtures or sequential application with a reference fungicides and untreated controls (D5.2.3) using powdery mildew and/or grey mould as model diseases of grapevine.

Report on results obtained and changes to the original plan/WP aims:

A- results obtained:

Development of strain specific qPCRs for bio pesticide tracking of selected BCAs

Strain-specific detection systems were developed and validated for *Bacillus amyloliquefaciens* QST713, the active ingredient of Serenade Max™ (hereafter referred as QST713), *Ampelomyces sp.* AQ10, the active ingredient of AQ10™ (hereafter referred as AQ10) *Aureobasidum pullulans* CF10 and CF40, the active ingredient of Botector™ (hereafter referred as CF10/40). Species-specific PCR assay was developed for *Lecanicillium lecanii*, the active ingredient of Mycotal™.

Studies on survival and persistence of the BCAs in controlled environment

The active ingredients of BCAs were tested in an in vitro plate test for compatibility. QST713 strongly inhibits growth of CF10/40. No such precipitate was seen between QST713 and AQ10. For the strains CF10/40 and AQ10, week growth inhibition for CF10/40 could only be seen at very short distances, but no long distance effects could be observed.

To pretest the use of the qPCR protocol for bio pesticide tracking in field QST713 and AQ10 were applied on grapevine leaves with a potter tower. After two weeks, the amount of QST713 was close to or below the detection level. The persistence of AQ10 was even lower, after two days the fungus could no longer be detected. To test the compatibility of BCAs and inorganic fungicides for mixed or sequential application a comparison series was conducted. All leaves were sprayed with copper or sulphur respectively and after the leave surfaces were dry, the different BCAs were applied. While copper has a tendency to negatively influence QST713 on vine leaves, sulphur had a clear positive tendency. Greenhouse experiments with potted grape vines under different controlled environments (constant temperature and humidity profile vs. constant temperature but alternating cycles of high and low humidity) were performed to study the fitness of BCAs. The BCAs sprayed by hand. After two weeks, all samples were close to the detection level, after three weeks the colonization increased under both environments, after 4 weeks the population decreased again to the detection limit. Due to the high deviations within the replicates there is no statistical support for oscillating colonization pattern. Based on existing data there is no indication on positive or negative effects of varying humidity profiles on persistence of QST713. Potted grapevines were placed in a semi controlled greenhouse to test improved control strategies against powdery mildew. After the first natural and visible infestation with *Erysiphe necator,* Serenade™ ,AQ 10™, Serenade™ in combination with sulphur respectively with AQ 10™ were applied in the recommended dose by hand. The bio pesticide tracking revealed that AQ 10 could not colonize the leave surfaces inspite high *E. necator* infestation, but a slight reduction of *E. necator* (qPCR and in visual assessment) could be stated. No positive or negative influence of QST 713 on persistence of AQ 10 could be observed. QST731 could colonize the leave surfaces. Positive influence of sulphur on persistence of QST 713 could be confirmed.

Studies on survival and persistence of the BCAs under field conditions

To monitor the ability of the BCA to colonize the leaf surface under natural environment the BCAs were applied on two different cultivars (cv Neuburger and cv Zweigelt) in an Austrian vineyard. In the first year the BCAs were sprayed separately with an application interval of 3 weeks, in the second year an improved control strategy was tested. No reliable qPCR data could be obtained from the bio pesticide tracking in field, because of extremly low DNA concentrations of the samples. The evaluation of the efficacy revealed that the improved strategy using BCAs in combination failed to reduce *E. necator* and *Botrytis cinerea*. It has to be stated that during the investigation the disease pressure was so high that even the efficacy of conventional treatments were insufficient.

Bioassay assessment on the ability of *Lecanicillium lecanii* (Mycotal™) to infect *Scaphoideus. titanus* larvae. Results from laboratory tests showed that the entomopathogenic fungus

Lecanicillium lecanii (Mycotal™) was virulent to the larvae of *Scaphoideus titanus*. The percentage of the cumulative mortality of *S. titanus* nymphs corrected after Abbot's formula (1925) ranged from 73% (experiment 1), 54% and 35.7% (experiment 2) to 33.3% (experiment 3).

B- comments on deviations from the original plan:
There were some difficulties encountered during the reporting period. One issue was that one of the selected and still registered BCAs (LexyTM) was not available on the European market. This difficulty could be handled by replacing Candida oleophila O (LexyTM) with Aureobasidum pullulans (BotectorTM, Bio-ferm).

WP 6	**Design new management strategies**
Responsible partner: P 01 – UCSC, V. Rossi	

Original description of work:
WP description: New disease management strategies will be designed for organic vineyards following a design-assessment-adjustment cycle (Task 6.1).

Task 6.1: Design-assessment-adjustment cycle. The first step is to design management solutions based on current knowledge. The second step is an *ex-ante* assessment of the solutions and current practices using appropriate indicators and other available assessment tools (D6.1.1). Subsequently solutions resulting from WP2 to WP5 will be tested in WP7 (second year of the project). The final step is an *ex-post* assessment of these solutions. Following the *ex-post* assessment, a new design-assessment-adjustment cycle will be initiated incorporating the information from the previous cycle to as well as new knowledge and tools developed within and outside the project (D6.1.2). At the end of the project, a final *ex-post* assessment will be provided (D6.1.3).

Report on results obtained and changes to the original plan/WP aims:

A- results obtained:
Single management components obtained from the other project WPs were considered for the development of two innovative strategies for a comprehensive organic management of the vineyard. The first strategy is more conservative, i.e. risk-adverse strategy, while the second one is a more risk-seeking strategy.

- The **risk-adverse strategy** is based on the combination of i) the use of the UCSC epidemiological models for downy and powdery mildew to schedule copper and sulphur treatments at label dose; ii) fall treatments with the hyperparasite *Ampelomyces* spp. (AQ10) against powdery mildew overwintering fruiting bodies (if disease severity is high enough at the time of harvest) and, iii) the use of a specific biocontrol agent (*Aureobasidium pullulans*) for the control of grey mould.
- The **risk-seeking strategy** is based on the combination of i) the use of the UCSC 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 multi-criteria assessment tool DEXiPM (implemented within the DEXi decision support system, within the EC FP7 founded project ENDURE and then refined for grapevine under integrated production management in project PURE) was used to *ex-ante* assess these innovative strategies against the "organic baseline" on economic (labour, yield effect, product quality, product price) environmental and health risk for workers aspects (D6.1.1). Significant differences were found in the social and the economical sustainability while no differences were found in the global environmental sustainability of the considered strategies. In particular, the use of epidemiological models allowed a significant reduction of fungicide TFI and fuel

consumption that provide a significant increase in the economic sustainability but did not affect the global environmental 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.
During grapegrowing seasons 2013 and 2014 the innovative strategies were tested in different experimental vineyards (D7.1.2 and D7.1.3) and an *ex-post* assessment was performed with the data collected in the fields using the tool DEXiPM complemented by the GIS-based risk assessment tool SYNOPS-WEB, that calculates the potential risk of chemical plant protection products for both terrestrial and aquatic organisms.
Under the organic management regimes tested within this project only few significant modifications could be detected: the level of both acute and chronic risk for aquatic organism was reduced in both innovative strategies, and the ecotoxic chronic risk for both Daphnia and algae was reduced within the risk-seeking strategy. Many changes were adopted in the different management strategies, nonetheless the DEXiPM tool did not detect significant differences in the overall sustainability. This could be due to the low sensibility of the model: the model considers classes of attributes, not specific values. The ranges of the classes were developed for Integrated management of vineyards and seems to be not adequate for organic management. For example, the fungicide TFI (Treatment frequency index) assumed different values for the different management strategies, but almost all fell in the same class and therefore no differences could be detected by the model.

B- comments on deviations from the original plan: No deviation

WP 7	**Test new strategies**
Responsible partner: P 02 - CRA-VIC, P. Storchi	

Original description of work:
WP description: Innovative management strategies developed in WP6 will be tested in the 2nd and 3rd year of the project, by in-vineyard experiments located in high-quality vine areas of Germany (Rhine Valley), Italy (Chianti), Austria (Lower Austria), and Slovenia (Primorska). Strategies will be applied on large plots, homogeneous for variety, age, training system, and terroir. At least two innovative strategies will be tested and compared with the usual practice of the grower, following an On-farm experimental design. Additional strategies will be implemented by each Partner, if necessary, to better tackle differences between the various environments and test additional strategies. A small plot with untreated plants will be also considered for assessing the environmental conduciveness for the target diseases. Effectiveness of the different disease management strategies will be evaluated during the grape-growing season (Task 7.1), as well as the quality and safety of the wine (Task 7.2). The innovative strategies will be also evaluated for their ability to increase grower's income (Task 7.3).

Task 7.1: Effect on disease severity and grape yield: incidence and severity of the target diseases will be evaluated on a representative sample of leaves and bunches during the grape-growing season at minimum three growth stages of vines. Grape yield and quality will be evaluated at harvesting (D7.1.2 and D7.1.3). These experiments will be done by CRA-VIC, LFZ, SWI, and KIS by following common simple protocols (D7.1.1) and the data will be analysed conjunctly by CRA-VIC.

Task 7.2: Effect on grape and wine quality and safety: the must will be analyzed during the first harvest season (soluble sugar content, titratable acidity, pH, etc.) as well as the resulting wine made from 100 kg of grapes for each plot by using the typical local oenological techniques. This Task will be performed only in the vineyards managed by CRA-VIC (D7.2.1).

Task 7.3: Effect on grower's income: data on the monetary costs of usual vs. innovative strategies will be collected in the different vineyards of Task 7.1 and elaborated by CRA-VIC. The relative impact of the different strategies adopted on the final price will be also evaluated (D7.3.1).

Report on results obtained and changes to the original plan/WP aims:

A- results obtained:

Each partner involved in the WP set up an experimental vineyard and tested the innovative strategies developed in WP6 during grapevine growing seasons 2013 and 2014. In total, four experimental vineyard were set up:

- CRA-VIC: Tuscany (Italy), plot of around 5 ha surface, grape cultivar Sangiovese.
- SWI: Freiburg (Germany), plot of around 0,4 ha, grape cultivar Müller-Thurgau.
- KIS: Vipava valley (Slovenia), plot of around 0,5 ha, grape cultivar Pinela
- LFZ: Klosterneuburg (Austria), plot of around 0,35 ha, grape cultivar Chardonnay.

The experimental vineyards were divided in different plots (as indicated in the protocol, D7.1.1) 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.

Weather conditions of the different locations and the two seasons were triggered very different epidemic: from almost absence of the diseases in the untreated control to the needs of closing the untreated plots in order to avoid the complete defoliation of the grapevines.

For example in 2013 in the Italian vineyard the first infections of *Plasmopara viticola*, the grapevine downy mildew causal agent, occurred in mid-June in the untreated plot. During the same period minor damage were also recorded in plots treated with the two innovative strategies, as well as in the plot managed as usual by the grower. No powdery mildew infections were recorded. In the German vineyard the first appearance of *Plasmopara viticola* was already on the 11th May and of *Erysiphe necator* on the 19th June. In the Slovenian vineyard no disease symptoms were recorded until mid-July and only very few powdery mildew symptoms were found in all plots of the Austrian vineyard.

In general, in all experimental vineyards of all countries the management performed following both risk-adverse and risk-seeking strategies allowed the same (or even better) disease control than the farmer strategy but reducing the number of treatments and application dose (D7.1.2 and D7.1.3).

Moreover, in Italy grapes were harvested separately and yield and must characteristics were analyzed: cluster weight was significantly reduced in S2, while no significant differences were detected in berry weight. Sugar and phenols content in grapes were not influenced by the different management strategies. Only anthocyanin content in grapes seemed to be positively influenced by the leaf removal (increased compared to S1 and Farm). Microvinifications were performed with the separately harvested grapes and the wines characteristics analysed: alcohol and polyphenols content, as well as total titratable acidity were not influenced by the different management strategies, while anthocyanins content resulted to be higher in the innovative strategies (at least in 2014) (D7.2.1).

Finally, all relevant data were gathered from each experimental location in order to perform a cost-benefit analysis. The main elements in the analysis were gross yield, material costs and labour/equipment costs. A comparison between the disease management costs and the returns for covering other costs was also performed. The cost of the risk-averse strategy was consistently higher than the baseline, while the risk-seeking strategies demonstrated to effectively protect the production although applying less funcicides, allowing the growers to obtain a significant saving in the cost for diseases control and get a return for covering other costs (D7.3.1).

B- comments on deviations from the original plan:
In Italy, the meteorological conditions were particularly adverse, with low spring temperatures which delayed the vegetative growth. In addition there were several hailstorms, which partially damaged the basal leaves in the different plots. In all countries, season 2014 was recorded as one of the most rainy season in the last decades and on average more treatments were performed.
In Germany, a randomized experimental field was used instead of large "on-farm" plots and an artificial infection with *Erysiphe necator* (25000 spores/ml) was made to achieve a homogenous infection in the field. This deviation don't affect the validity of the experiment.
In Germany, different epidemiological models for the major grapevine diseases are used to schedule the fungicide treatments because these models are currently used to warn growers in that area. This deviation don't affect the meaning of the comparison between different vine management strategies because the focus is on using models for scheduling fungicides, irrespective of the model used.

WP 8	**Microbial biodiversity in organic vineyards**
Responsible partner: P 07 - KIS, H.-J. Schroers	

Original description of work:
WP description: The impact of the disease management strategies implemented in the field trials of WP7 on the diversity of fungi will be explored. We aim at testing whether the novel cropping methods can increase overall numbers of species inhabiting the grapevines and evaluate selected fungi encountered for their potential suppressiveness against pathogens. Specifically, the grape canopy and berries are inventoried. For this purpose, appropriate culture-depended and culture-independent methods will be implemented (Task 8.1) and applied (Task 8.2).

Task 8.1. Develop methods: methods adopted are the cultivation-independent Denaturing Gradient GelElectrophoresis (DGGE) system for community structure analysis (15, 16) (D8.1.1) and a cultivationdepended dilution-to-extinction technique (28). Gold standard methods are applied for the identification ofthe encountered fungi (D8.1.3).

Task 8.2. Testing the impact of disease management strategies on fungal communities: the methodsdeveloped in Task 8.1 will be tested in the Slovenian vineyard of WP7. Canopy and bunch samples will becollected during the two experimental seasons through appropriate bulk sampling strategies (D8.2.1).

Report on results obtained and changes to the original plan/WP aims:

A- results obtained:
First year activities focused on the implementation of methods. For "pulverizing" plant material (stem, leaves, grapes) and for obtaining environmental DNA from plant material, different methods were tested. Serial sieving with Retch sieves allowed collection of plant pieces with a size range of 75-125 µm or 100-200 µm. Aliquots of washed particles were used for DNA extraction. After serial dilutions and for the culture-dependent experiments, aliquots of particle suspensions were also used as inoculum pipetted in 96-well plates with wells containing yeast extract / malt medium supplemented with the antibiotic chloramphenicol. By using 20 plates, a number of near 2000 vials were inoculated and incubated for testing. Culture independent approach: DNA extracted from environmental samples was used for PCR amplifications of the ITS1 fragment of the rDNA gene cluster. Obtained mixtures of PCR fragments were analyzed on DGGE gels. Results indicated that the methodology worked well: (i) similar DGGE pattern occurred when DNA from different extraction methods were used for plant material from the same location. (ii) different DGGE profiles (i.e., different species compositions) were

encountered when samples from different locations were compared. (iii) similar DGGE profiles were obtained from stems and leaves, but the DGGE profiles obtained from grapes differed. In order to test the quality of the obtained environmental DNA extracts, ITS fragments were also cloned and the obtained clones were sequenced. ITS sequences were then used for identifications that yielded species known to inhabit grapevines or above surface material of other plants, including: *Ampelomyces* sp., the grapevine powdery mildew *Erysiphe necator*, *Epicoccum nigrum*, *Alternaria* spp., *Phoma macrostoma* (all asomycetes), and the basidiomycete yeast *Sporidiobolus pararoseus*.
Second and 3rd year activities took place in an organic vineyard selected for the Slovenian field experiments of WP7/8. Bulk samples of leaves and grapes were collected from the experimental plots just before the biological control agent Botector was applied and shortly before grape harvest. Bulk samples were subdivided into 3 biological replicates, each consisting of 12-15 g and processed as described above and following the methodology described in Collado et al. (FEMS Microbiology Ecology 60: 521–533, 2007) and Anderson et al (Environm. Microbiol. 5: 1121-1132). In culture dependent approaches a single replicate was processed, in culture independent, all three replicates). Although the same procedures were used as in the first year, only poor DGGE profiles were encountered that could not be used for interpretations in the 2nd year. Also the culture dependent approach yielded few fungi only. *Aureobasidium pullulans* was isolated from leaf samples of the strategy 1 plot before and after the grapevines were treated with Botector. Potentially undescribed species of the Cordycipitaceae were encountered that may present novel candidates for the biological control of insect pests. Other taxa encountered included *Diaporthe viticola*, *Doratomyces stemonitis*, *Beauveria bassiana*, *Stachybotrys* spp., *Phanerochaeta sordida*, and *Meyerozyma quilliermondii*. In the third year, near 600 fungal strains were isolated in the culture dependent approach. Because *Aureobasidium pullulans* was the dominating species in all three experimental plots, it may present an important ecological service provider as it may occur also naturally in organic vineyards. The same undescribed species of the Cordycipitaceae as in the 2nd year of the project were isolated at least sporadically. Twelve undescribed species of the Helotiales were encountered. Preliminary results suggest that they can suppress *Botrytis cinerea* in dual culture system, however, they also could present currently unrecognized plant pathogens. Their ecological role in grapevines will be the topic of follow-up studies. Many species of *Penicillium* and *Aspergillus* were isolated and identified to species level; the highly relevant ochratoxigenic species *Aspergillus carbonarius* or *A. ochraceus* were not encountered. Analyses of the 2014 data from the culture independent approach did not clearly show that the different organic strategies had a clear measurable impact on the fungal diversity. The taxon composition from strategy 1 treated leaves (100% pesticide dosage plus BCA), however, differed from the similar compositions retrieved from strategy 2 and the farmer's treatment. In September 2014, the profiles from strategy 2 plants (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 strategy 2 treatment caused an increase of fungal diversity in the experiment.

B- comments on deviations from the original plan: A critical element in the culture dependent approach is the selection of the particle concentration that followed recommendations from literature. The little number of fungi isolated in the second year indicates that either only few fungi were present or the quality of the inoculum was suboptimal. In the third year of the project, different dilutions were tested in parallel; however, 2014 was also a more humid year and consequently supporting fungal growth in the field in general. As a result, too many fungi were retrieved. Although it is not possible to link the occurrence of the currently undescribed species of the Cordycipitaceae and Helotiales with the activities in the different strategies, they present valuable examples of fungal diversity from organic vineyards that we will explore in follow-up experiments.

4. Publications and dissemination activities

4.1 List extracted from Organic Eprints

(Publications affiliated to European Union > CORE Organic II > “project acronym”, grouped by EPrint type, with date of extraction)

The list can have these headers:

http://orgprints.org/view/type/

publications that are not allowed as open access should be deposited as “Visible to: Depositor and staff only.”. The funding bodies and project evaluators will be granted access to these during the evaluation. Guidance on the use of Organic Eprints can be found here: http://orgprints.org/help/ .

Last Modified	EPrint Type	Title	Item Status
28 Mar 2015 18:57	Newspaper or magazine article	Entblätterung vor der Blüte: eine Möglichkeit zur Reduktion von Botrytis, Traubendichte und Ertrag	Under Review
28 Mar 2015 18:54	Newspaper or magazine article	EU-Projekt VineMan.org – Forschung für den ökologischen Weinbau	Under Review
28 Mar 2015 18:52	Newspaper or magazine article	VineMan.org - Europäisches Projekt zur Verbesserung des Pflanzenschutzes im ökologischen Weinbau	Under Review
28 Mar 2015 18:48	Journal paper	Vite: posticipare la maturazione con la defogliazione tardiva	Under Review
28 Mar 2015 18:44	Newspaper or magazine article	Projekt “VineMan.org” – biologische Bekämpfung von Rebkrankheiten	Under Review
28 Mar 2015 18:40	Proceedings	Innovative disease control strategies for European organic and integrated vineyards`. Proceedings of the VineMan.org project congress	Under Review
28 Mar 2015 18:36	Journal paper	Investigations into the effect of pre-bloom defoliation on quality and yield of organically grown `Riesling` grapes as well as `Zweigelt` grapes grown according to integrated plant protection guidelines	Under Review
28 Mar 2015 18:29	Journal paper	Interactions of summer pruning techniques and vine performance in the white cultivar Ortrugo (Vitis vinifera L.)	Under Review
09 Aug 2013 09:54	Journal paper	Influence of mechanical postveraison leaf removal apical to the cluster zone on delay of fruit ripening in Sangiovese (Vitis vinifera L.) grapevines	User Workarea
09 Aug 2013 09:50	Conference paper, poster, etc.	VineMan.org: innovative strategies for enhancing disease management in organic vineyards	Live Archive
09 Aug 2013 09:49	Newspaper or magazine article	La difesa del vigneto biologico: l'unione fa la forza!	Live Archive
09 Aug 2013 09:47	Newspaper or magazine article	VineMan.org, nuovo slancio per la viticoltura biologica	Live Archive
09 Aug 2013 09:45	Newspaper or magazine article	VineMan.org: Europe supports the research on organic viticulture	Live Archive

4.2 Additional dissemination activities

Pillar 1: ‘within-project audience’

Project meetings:

Kick-off meeting: 24th January 2012 – Piacenza, Italy

Intermediate meeting: 21st - 22nd February 2013, Ljubljana, Slovenia

Second intermediate meeting: 26th and 27th February 2014, Freiburg, Germany

Final meeting: 8th January 2015, Vienna, Austria

Teaching:

In the following teaching activities the respective partner explained the project's aims and activities.

Dr. Fuchs (SWI): "Molekulare Mechanismen der Interaktion zwischen Pathogenen und Pflanzen" to biology master students of the University of Freiburg (Germany)

Dr. Rosner (LFZ) 14th May 2013: "Organisch biologischer Weinbau" (course for grapevine growers on organic viticulture at LFZ)

Seminar for technical students (winegrowers), 12.12.2014 in Freiburg at the SWI: „VineMan.org – Europäisches Projekt zur Verbesserung des Pflanzenschutzes im ökologischen Weinbau"
Seminar for biology master students, 25.11.2014 in Freiburg at the university: "Molekulare Mechanismen der Interaktion zwischen Pathogenen und Pflanzen – Teil Nichtwirtsresistenz"

Pillar 2: scientific community

Presentation in international and national conferences (6):

Reisenzein H. Ersatzstrategien von Kupfer im Weinbau. Tagung „Kupfer im Pflanzenschutz", , 26.09.2012, Wien, Austria

Dr. Legler (UCSC) 10th December 2012: poster presentation at "Meeting with the Italian Environment Minister", Piacenza, Italy

Dr. Legler (UCSC) 26th August 2013: accepted oral presentation at "10th International Congress of Plant Pathology – Disease management in Organic Farming session", Beijing, China

Strauß G., Reisenzein H., Steffek R. 2013. Current research activities on *Scaphoideus titanus* and Grapevine flavescence dorée phytoplasma in Austria. Conference of the IOBC/WPRS Working group „Integrated Protection and Production in Viticulture", 16.10.2013, Ascona, Switzerland

Strauss G. 2013 Neuer Ansatz zur biologischen Kontrolle der Amerikanischen Rebzikade durch einen insektenpathogenen Pilz. "54. Österreichische Pflanzenschutztage 2013", 28.11.2013, Ossiach, Austria

Reisenzein H., Strauß G. VineMan.org project. „Forschungsgespräche", 29.11.2013 LFZ Klosterneuburg, Austria

Dr. Rosner (LFZ) 23rd January 2014: Presentation of the results and experiences of WP3 and WP7 at the `Meeting of the managers of the grapevine protection areas in Austria`.

Rossi V., Legler S.E., Gatti M. e Caffi T. 2014. Vineman.org: nuove strategie per la difesa dei vigneti biologici europei. Giornate Fitopatologiche 18-21 march 2014, Chianciano Terme (POSTER)

Fuchs R. and Kassemeyer H.H. "Activation of disease resistance in grapevine plants by PAMP/DAMP-application" oral presentation at the 7th GDPM in Vitoria-Gasteiz (Spain) - June 30th to July 4th, 2014

Fuchs R. and Kassemeyer H.H. „VineMan.org – Europäisches Projekt zur Verbesserung des Pflanzenschutzes im ökologischen Weinbau“ poster presentation at the 59. Deutsche Pflanzenschutztagung in Freiburg (Germany) – 23. - 26. September 2014

Pelengić R., Schroers H.-J. 2015. Vineman.org: Integration of plant resistance, cropping practices, and biocontrol agents for enhancing disease management, yield efficiency, and biodiversity in organic European vineyards. Poster presentation, submitted to and accepted by the organizers of the PHSA Conference, Integrated Approaches for Sustainable Crop Production in Slovenia, Session 5, Prognosis & Decision Supporting Systems, May 11./12.05.2015. Ljubljana. Slovenia.

Publications in peer-reviewed journals (4):

Palliotti A., Panara F., Silvestroni O., Lanari V., Sabbatini P., Howell G.S., Gatti M. and Poni S., 2013. Influence of mechanical postveraison leaf removal apical to the cluster zone on delay of fruit ripening in Sangiovese (*Vitis vinifera* L.) grapevines. Australian Journal of Grape and Wine Research DOI: 10.1111/ajgw.12033

Poni S., Gatti M., Bernizzoni F., Civardi S., Bobeica N., Magnanini E., and Palliotti A., 2013. Late leaf removal aimed at delaying ripening in cv. Sangiovese: physiological assessment and vine performance. Australian Journal of Grape and Wine Research DOI: 10.1111/ ajgw.12040

Gatti M., Garavani A., Cantatore A., Parisi M.G., Bobeica N., Merli M.C., Vercesi A., and Poni S. 2015. Interactions of summer pruning techniques and vine performance in the white cultivar Ortrugo (*Vitis vinifera* L.). Australian Journal of Grape and Wine Research 21 (1): 80–89.

Mehofer M., Hanak K., Schmuckenschlager B., Vitovec N., Schober V., Wendelin S., Prinz M. 2015. Investigations into the effect of pre-bloom defoliation on quality and yield of organically grown `Riesling` grapes as well as `Zweigelt` grapes grown according to integrated plant protection guidelines. Mitteilungen Klosterneuburg 65, (in press, ISSN 0007-5922)

Reisenzein H. and Gorfer M. 2015. New approaches for improved control strategies in organic viticulture using strain specific qPCRs for bio pesticide tracking. Letters in Applied Microbiology (in preparation)

Final Congress (proceedings):

Description of the research activities in the frame of the project and information about final Congress:
(http://www.ages.at/themen/landwirtschaft/agrarische-forschung/forschung-weinbau/forschung-oekologischer-weinbau/)

Rosner F. and Mehofer M. Innovative disease control strategies for European organic and integrated vineyards`. Proceedings of the VineMan.org project congress (Vienna, 9th January 2015). Mitteilungen Klosterneuburg (in press, ISSN 0007-5922)

Pillar 3: professional users (consultants, farmers, etc.).

Project website: **www.vineman-org.eu**

Project leaflets (2): Two leaflets were developed and translated into different languages; a first leaflet explaining the objectives of the project and a final leaflet reporting the main results obtained and recommendations to stakeholders.

Professional magazines and journals (5):

Rossi V., and Legler S. E. 2013. How to improve disease control in European organic viticulture? Newsletter CORE Organic April 2013: 5-7.

Legler S. E., Caffi T., Gatti M., e Rossi V. 2013. La difesa del vigneto biologico: l'unione fa la forza! BioAgricoltura, 140-141:39-41.

Fuchs R. and Kassemeyer H.H. "Projekt "VineMan.org" – biologische Bekämpfung von Rebkrankheiten" AGRO journal issue 6, January 2014.

Rossi V., Legler S. E., Caffi T., Gatti M. 2014. Vineman.org: nuove strategie per la difesa dei vigneti biologici europei. Atti Giornate Fitopatologiche, estratto Poster.

Galbignani M., Poni S., e Palliotti A. 2014. Vite: posticipare la maturazione con la defogliazione tardiva. Informatore Agrario 18/2014

Fuchs R. VineMan.org - Europäisches Projekt zur Verbesserung des Pflanzenschutzes im ökologischen Weinbau. Landinfo Ausgabe 3:2014 Informationen für die Landwirtschaftsverwaltung.

Fuchs R. and Kassemeyer H.H. EU-Projekt VineMan.org – Forschung für den ökologischen Weinbau. Der Badische Winzer, November 2014

Mehofer M. and Rosner F. 2015. Entblätterung vor der Blüte: eine Möglichkeit zur Reduktion von *Botrytis*, Traubendichte und Ertrag (Leaf removal at pre bloom: an opportunity to reduce *Botrytis*, cluster cumpactness and yield). Der Winzer, in press

Workshops for stakeholder (5):

Dr. Rosner (LFZ) 29th March 2012: Forschungsgespräche (annual meeting of leading people in the wine sector for discussions of new and running projects at LFZ)

Dr. Maria Paz Diago (UDLR) 14th September 2012: Beneficios de la partecipacion de la Universidad de La Rioja en un proyecto de Investigacion en Beneficia de las PYMEs. Proyectos MODEM_IVM y VineMan.org – Jornada ADER: Oportunidades de participación en el VII Programa Marco

Dr. Kassemeyer (SWI) 13th November 2012: "Möglichkeiten und Grenzen der biologischen Bekämpfung von Rebenperonospora und Echtem Mehltau" – extension service day at Staatliches Weinbauinstitut, Freiburg (Germany)

Strauss G., Altenburger J.: Erste Ergebnisse zur biologischen Kontrolle der Amerikanischen Rebzikade durch *Lecanicillium lecanii*; „Rebschutzgebietsleitertagung 2013", 17.01.2013, Wien, Austria

Dr. Schroers (KIS) 12th April 2013: SUSVIT 2: a Grundtvig Learning Partnership for organic viticulture and wine-making – On farm training in Slovenia

Schroers H.J., Pelengić R., Legler S.E., Rossi V. Integration of plant resistance, cropping practices, and biocontrol agents for enhancing disease management, yield efficiency, and biodoversity in organic European vineyards : Core organic II project : The meeting was part of the European Commission Education and Training lifelong learning programme : predavanje na SUSVIT 2, A grundtvig learning partnership for organic viticulture and wine-making, Slovenia, 12. apr. 2013. 2013. [COBISS.SI-ID 4184680]
kategorija: SU (S)

točke: 0.25, št. avtorjev: 4

Dr. Kassemeyer (SWI) 12th June 2013: Ansätze für eine biologische Bekämpfung von Peronospora und Echtem Mehltau im Weinbau – Biolandbau unter bioÖkonomischen Aspekten, Landwirtschaftlicher Hochschultag 2013

Dr. Rosner (LFZ) 29th November 2013: Presentation of the results of WP3 at a seminar for grapegrowers at the Federal College and Institute for Viticulture and Pomology Klosterneuburg

Dr. Rosner (LFZ) 25th August 2014: Presentation of the experiences and results of WP7 to grapegrowers, who attended the `Certification course for organic viticulture` organized by the `Rural Training Institute of Lower Austria`

Pelengić, R. Izboljšava varstva vinske trte, količine in kakovosti pridelka ter biodiverzitete v evropskih ekoloških vinogradih : predavanje za Društvo vinogradnikov in vinarjev Polič, Štore, 24. apr. 2014. 2014. [COBISS.SI-ID 4464232]
kategorija: SU (S)
točke: 1, št. avtorjev: 1

Fuchs R. Workshop for organic winegrowers, 13.03.2014 in Freiburg at the SWI: „ Biologische Bekämpfung von Rebkrankheiten"

Fuchs R. Workshop for advisers, 12.11.2014 in Weinsberg at the LVWO:" VineMan.org – Europäisches Projekt zur Verbesserung des Pflanzenschutzes im ökologischen Weinbau"

Pillar 4: 'big audience'

Articles in popular magazines or newspapers (5):

Short article on the project contents on the UCSC Newsletter "Cattolica news", available online at: http://www.cattolicanews.it/studi-e-ricerche-vigneti-biologici-progetto-europeo?utm_content=257%2520-%2520Newsletter%2520161%2520%25282012-02%252009%2529&utm_medium=email&utm_source=MagNews&utm_campaign=My%2520Campaign&utm_term=10202%2520-%2520Agraria.%2520Vigneti%2520biologici,%2520progetto%2520internazionale

Presentations for broadcasting media (5):

Short article on the project contents and Kick Off meeting in Piacenza:
http://www.infowine.com/default.asp?scheda=10905
http://agronotizie.imagelinenetwork.com/attualita/2012/02/09/vinemanorg-nuovo-slancio-per-la-viticoltura-biologica-14803.cfm

Dr. Gudrun (AGES). Gute Nachrichten aus der Forschung: Amerikanische Rebzikade biologisch bekämpfen? Der Winzer, 24.07.2013 online
http://www.der-winzer.at/?id=2500%2C5242718%2C%2C%2CY2Q9MzU0JnhfX1NFVF9TVEFSVFtoaXRib3hdPTM0MCZ4X19TRVRfRU5UUllbaGl0Ym94XT0zNTUmaW50PTE%3D

Public Open day at the SWI, 8.09.2013 in Ihringen (Germany): "Induzierte Pflanzenabwehr"

Public Open day at the SWI, 17.05.2014 in Freiburg: "Induzierte Pflanzenabwehr"

AGES Newsletter 02/15 - 24.02.2015

http://www.ages.at/service/service-presse/newsletter/newsletter-uebersicht-2015/newsletter-0215-24022015/

4.3 Further possible actions for dissemination

- *List publications/deliverables arising from your project that Funding Bodies should consider disseminating (e.g. to reach a broader audience):* Final leaflet, all publications in professional magazines and journals and the final congress proceedings and presentations available on the dedicated webpage developed by AGES

- *Indicate publications/deliverables that could usefully be translated (if this has not been done, and indicate target language):* Both "Legler S. E., Caffi T., Gatti M., e Rossi V. 2013. La difesa del vigneto biologico: l'unione fa la forza! BioAgricoltura, 140-141:39-41" and "Galbignani M., Poni S., e Palliotti A. 2014. Vite: posticipare la maturazione con la defogliazione tardiva. Informatore Agrario 18/2014" could be translated on the different partners language and on French.

4.4 Specific questions regarding dissemination and publications

- *Is the project website up-to-date?* Yes, the website is updated with the results obtained in the different WPs, with the main disseminations activities performed and some pictures are also available to document the field activities. A restricted area is also present on the website that allows the partners to access the electronic platform developed in WP4.

- *List the categories of end-users/main users of the research results and how they have been addressed/will be addressed by dissemination activities.* Grapevine growers: workshops organized in the different countries and in particular the final congress. Scientific community: international congress and peer-reviewed articles. Big audience: articles in local newspaper and some presentations on broadcast media.

- *Impact of the project in relation to main beneficiaries of the project results (Note: for the different categories of end-users/main users of the research results, explain how well the project has been able to reach these target groups, and any known impact).* Very promising results have been obtained and disseminated mainly to grape-growers and scientific community. Both confirmed the importance of an integrated approach for disease control in organic viticulture and the usefulness of the recommendations resulted from the project.

5. Added value of the transnational cooperation in relation to the subject

(max 1 page)

The Project focuses on the protection of the environment and on sustainable and organic growth. Transnational cooperation represents an added value at the European scale and guaranties a higher impact, compared to current activities at regional levels which are too scattered and ineffective.
The perspective of a common management of the research activities at the European scale is mandatory for achieving long-lasting improvements in organic production and protection of the environment. In all, the added values for the participating countries are:

i) the better deployment of technologies and innovations with a relatively small investment of resources (for instance, repeated experiments in the different countries makes it possible to collect more robust results in a shorter time);
ii) the avoidance of duplicative research (each partners has a clear role and there is no duplication of the research activities);
iii) the access to additional advanced equipment and technical skills (each partner has specific expertise in research areas which are not equally explored by the other partners; for instance, SWI has specific expertise in plant-pathogen interactions, KIS has expertise and equipment for molecular analysis at population level; UCSC has specific expertise in disease modelling);
iv) the development of positive multilateral and unilateral relationships between and among countries;
v) the benefits to individual researchers thank to international exposure (i.e., reputation building);
vi) the increase of the chances for “transformative” research results.

Thanks to Vineman.org project activities, BOKU (Tulln, Austria) and the laboratory of Levente Kiss (MTA, Budapest, Hungary) successfully applied for a cooperative project. Project manager Sara Legler performed part of her studies on the biology of *Erysiphe necator* at the laboratory of Levente Kiss. The BOKU/MTA collaborative project will deal with phylogenetics of the mycoparasite *Ampelomyces quisqualis*.

Project partners Hans-Josef Schroers (KIS, Ljubljana, Slovenia) and Markus Gorfer (BOKU, Tulln, Austria) applied for a joint Slovenian-Austrian research project on further characterization of fungal isolates obtained in the Vineman.org project. Application is now in the first stage.

Project partners also prepared together a proposal for the Call Best Practices EXPO 2015 (https://www.feedingknowledge.net/best-practices). The proposal included also a video with an interview to a grapegrower that explains the benefit in using the results of the project (https://www.youtube.com/watch?v=OPmom6AQN2M). Unfortunately the proposal was not accepted.

The development of sustainable food production systems and environmentally friendly plant protection strategies is a main aim of all the involved partners. Participation in the project vineman.org contributed to the buildup of knowledge relevant for these fields in collaboration with international specialists. The collaborative nature of the project with scientists from other European countries is of high relevant for networking activities.
Moreover, advanced disease forecasting methods are key factors in ecological production systems. The access to the recently developed models by collaborators of the project and their implementation on the Decision Support System web portal presented an important additional value.