EUPHRESCO Final Report

PROPSCAPH
Evaluation of the risk of spread of *Scaphoideus titanus*, the vector of grapevine flavescence dorée, with commercial grapevine propagation material
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2. Executive Summary

Project Summary

Title
Evaluation of the risk of spread of *Scaphoideus titanus* (ST), the vector of Grapevine Flavescence dorée (FD), with commercial grapevine propagation material

Main objectives and methods

1. Provision of project management and coordination. Communication and cooperation were provided during one joint meeting and many phone calls and e-mails.
2. Study of the colonization history of ST in Europe based on microsatellite and mitochondrial markers. Adult samples were collected at 21 sites in France, Slovenia, Italy and Switzerland. DNA was extracted and characterized genetically by means of the appropriate analytical software.
3. Evaluation of insecticide treatment efficacy against immigration of ST adults in nurseries. Adults were monitored regularly before and after treatment using yellow sticky traps on a grid within and outside of nurseries. Insecticide treatments were applied in nurseries according to national practices.
4. Study of ST egg distribution on propagation material. Material was collected during winter in nurseries in France, Slovenia, Italy and Switzerland and untreated or abandoned vineyards in Italy and stored in cold chambers. Egg hatching was observed by inspecting in the laboratory fresh grapevine shoots added to wood material in rearing cages kept at 24°C and by counting in the field larvae in sticky traps.
5. Evaluation of ST egg mortality due to heat treatment. Egg hatching was observed as above on hot water treated (HWT) and untreated material from nurseries and abandoned vineyards. In March, half of the material was treated according to standard procedures (50°C - 45 minutes) in a commercial HWT unit.

Results and Conclusions
As results were based on one year's experiences, some require confirmation or deserve further investigations.

1. No major problems occurred during the project and all partners were motivated to collaborate. Project coordination should be funded by a common pot.
2. The overall genetic diversities estimated with the nine polymorphic microsatellite loci were similar. Genetic differentiation between the 21 populations studied was low but significantly different from zero. Low levels of genetic variability among populations and significant patterns of isolation by distance suggest that the ST has substantial dispersal abilities, naturally or on propagation material. The analysis on mitochondrial data showed the existence of one haplotype common to all European populations tested here and three additional haplotypes, two of

them were present only in the South of the Swiss Alps and in Slovenia.

3. ST populations were high in the three Slovenian vineyards, moderate in the Swiss vineyard and very low in the two Italian vineyards. Insecticide applications in nurseries were efficient when applied correctly. Re-infestation from adjacent vineyards cannot be excluded, but is rare.
4. One year old grapevine wood is much less preferred for oviposition than older wood. The first contained at most 0.003 larvae per cm^2 while two year old wood included at most 100 times more. On two year old wood, the node area contained 0.18 eggs per cm^2 while the internode central portion contained about half the density. In the field, the bark of the trunk is as important an oviposition site as the cordons. Both contained about 0.1 larvae per cm^2. Commercial propagation material from all countries was never infested.
5. One year old wood contained at most 0.6 larvae per kg, while two year old canes included at most 100 times more. HWT provided a total control on one year old woods and generally a 90% reduction on two years old woods (canes or rootstock).

Mandatory insecticide treatment of nurseries is a useful measure to limit ST infestations, but is does not eliminate the vector completely. To prevent introductions not only of FD phytoplasma but also of ST into other continents, nurseries are recommended to distribute only one year old canes treated in hot water. Within Europe, regarding ST, hot water treatment is most justified for exchanges of grafted plants exposed to one season in the field.

3. Report

This report provides EUPHRESCO with an overview of the outputs of the research project. More details are available in the reports of each work package.

Objectives and Tasks

1. Provision of project management and coordination. Efficient communication and collaboration and timely delivery of deliverables is assured.
2. Study of the colonization history of ST in Europe based on microsatellite and mitochondrial markers. The observation of the genetic variability among several European populations helps in understanding the colonization patterns in Europe. .
3. Evaluation of insecticide treatment efficacy against immigration of ST adults in nurseries. Study of mortality in nurseries and immigration of adults from outside. Adults are monitored regularly before and after treatment with traps.
4. Study of ST egg distribution on propagation material. Representative samples of grapevine plants are taken regarding origin, age, pruning method and the number of eggs per plant organ are observed.
5. Evaluation of ST egg mortality due to heat treatment. The standard hot water treatment against FD phytoplasm is applied to commercial propagation material and material from heavily infested, abandoned vineyards. Egg hatching of treated and untreated material is compared.

The above objectives were all achieved completely. The stated objective *Mortality study on propagation material* of work package 5 was not pursued due to a too small number of eggs on propagation material. Therefore, the title of work package 5 was slightly adapted.

Methods and Results

1. Project Management and Coordination

Two meetings took place, one to prepare common experiments and the other to discuss results and coordinate common reporting.

No major problems were encountered so that the project was concluded with satisfaction of all partners. Considering the late beginning of the project, the objectives were obtained and the deliverables made available according to the contract.

Some propositions to improve management of EUPHRESCO projects are to make part of the funding dependent on achieving the promised objectives and to finance project coordination not nationally but directly by EUPHRESCO.

2. Colonization History of ST in Europe Based on Microsatellite and Mitochondrial Markers

To better explain the colonization history of ST in Europe, genetic characterisation of ST populations from Italy, Switzerland, Slovenia and France was done using

microsatellite and mitochondrial markers. The overall genetic diversities estimated with the nine polymorphic microsatellite loci were similar in France, Italy, Switzerland and Slovenia. Genetic differentiation between the 21 European ST populations studied here was low but significantly different from zero. Genetic and geographical distances were correlated when ST populations sampled in 2008 from Italy, Switzerland and four Slovenia were tested. Low level of population structure and significant pattern of isolation by distance suggest that the leafhopper vector of FD pytoplasma is substantially dispersed by natural spread or commercial exchanges at this geographical scale.

The analysis on mitochondrial data showed the existence of one haplotype common to all European populations tested here and three additional haplotypes, two of them were present only in the South of the Swiss Alps and in Slovenia. These results, supported by the result of the assignment test, suggest that the invasions of S. titanus in Slovenian and Swiss vineyards could have, at least partially, a common origin.

3. Insecticide Treatment Efficacy against Immigration of ST Adults in Nurseries

The commonly applied strategy for suppressing the vector is use of insecticides in vineyards and nurseries. The objective of the work package was to evaluate the risk of immigration of ST adults in nurseries from the nearby vineyards and the effectiveness of insecticide treatments for reducing a ST population in nurseries.

Three experiments were conducted in Slovenian, two in Italian and one in Swiss plots. A common protocol containing experimental methods was adopted. It was based on using yellow sticky traps posted at 4 distances in nurseries and nearby vineyards on three parallel lines (replicates), perpendicular to the plot border. Monitoring started at the latest nymphal stages (normally on the middle of June) and finished in October. Insecticide treatments were applied in nurseries, according to national practices.

ST populations were high in the three Slovenian vineyards, moderate in the Swiss vineyard and very low in the two Italian vineyards. The use of insecticide in nurseries was, except in one case, sufficient to practically prevent ST infestation. In some cases, a slight gradient was observed in nurseries close to infested vineyards.

4. ST Egg Distribution on Propagation Material

Laboratory trials were performed on different grapevine wood material coming from commercial nurseries located in different European wine growing areas (Italy, France, Switzerland, Slovenia) and from untreated vineyards of Latium (Central Italy) and Veneto (Northern Italy) regions.
The grapevine wood material was collected during the winter pruning period and then stored in climatic chambers at 4° C. The rearing cages were kept in climatic chambers set at 24°C, 70-75 % RH and 16:8 photoperiod. Number of test replicates and observation periods were different depending on the laboratory conditions of the scientific institutions involved in the project. The presence and density of ST eggs in the grapevine bark were indirectly evaluated through the almost daily searching, counting and removing of the newly hatched larvae within the rearing cages. Larval hatching sites were observed on the internode central portion and on the node area.

Specific field surveys were performed in two Latium vineyards to improve the knowledge about the egg distribution on the different woody parts of the vine plant. In May-June, in each vineyard, larval hatching was observed by means of ST larval traps placed on the trunk, the cordon, the canes or the buds.

Observations in the laboratory of untreated grapevine wood confirmed that the bark of two or more year old canes is the preferred site for ST egg laying. However, ST larvae emerged also from one-year old cane samples collected in one untreated vineyard and in one abandoned rootstock plot. The node area of the cane contained much more eggs than the internode central portion, particularly in the two-year old wood.
The high presence of ST eggs in the bark of two-year old canes was also observed in the field. The bark of the trunk was in these trials a very important site for egg laying as well.

No ST larvae were found on wood of the commercial material coming from the nurseries, irrespective of the country of origin and the kind of material, suggesting good phytosanitary practices of the European nursery industry. These practices are necessary, as our observations show the presence of ST eggs on untreated grapevine wood.

5. ST Egg Mortality due to Heat Treatment

ST can be spread as egg laid on propagation material. As hot water treatment (HWT) becomes more common to eliminate FD phytoplama, its effect was studied on the mortality of ST eggs. Various kinds of woods cut in winter 2008 in infested vineyards of France, Italy and Switzerland were sent to ACW and stored in a cold chamber. In March 2009, half of the material was treated according to standard procedures (50°C - 45 minutes) in a HWT unit. Treated and untreated woods were then placed in individual insect-proof cages in a greenhouse and egg hatching was regularly evaluated. Most of ST larvae emerged from untreated two years old woods but some hatching occurred also on one year old untreated material. This confirmed the ability of the leafhopper to lay its eggs on wood material that could be used for propagation and stressed the importance of our study. HWT prevented hatching totally on one year old wood but failed to achieve the same result on two years old wood even though an important reduction of egg hatching was observed.

One year old wood used for cuttings and rootstocks presents a very low risk of hosting eggs. If submitted to HWT, the risk of ST spread on one year old material can be rated as practically nil. Subsequently, the grafted plants in the nursery field should be protected against ST in order to avoid new egg laying and FD transmission.

Conclusions

The study of the genetic structure of several populations from France, Italy, Slovenia and Switzerland showed low levels of genetic variability and suggest substantial dispersal abilities of ST, naturally or on propagation material. Current practices of treating nurseries against ST adults reduce the risk of ST egg infestations on propagation material, but cannot exclude it completely. The insecticide application is itself efficient when applied properly, but re-infestation from adjacent vineyards, especially if they are close and heavily infested is possible. One year old wood is much less preferred by ST females for egg laying than two year and older old wood.

Spreading ST by one year old canes is possible, but very unlikely. Hot water treatment as used against FD phytoplasma infection is very efficient in reducing ST egg hatching but does not provide 100% control.

For intercontinental exchanges, to assure practically 100% absence of ST eggs, nurseries are recommended to distribute only one year old canes treated in hot water. Within Europe (plant passport), this level of security does not seem necessary as other paths of spread are possible. Hot water treatment is justified for exchanges of grafted plants exposed to one season in the field.

Dissemination activities

WP1: A summary article of all four work packages is planned. It could be translated from English to French, Italian and Slovenian and published in a national journal aimed at phytosanitary practitioners.
A joint submission of four papers from each work package is considered. The EPPO Bulletin could be the appropriate journal.
WP2: A manuscript is in preparation for the journal Molecular Ecology.
WP3: Results will be presented 17th December 2009 at the Phytosanitary Symposium, organized by the Phytosanitary administration of the Republic of Slovenia. An article for the Acta Agriculturae Slovenica is in preparation.
WP4: Oral presentation given: Bagnoli B., Gargani E., 2009 - Survey on Scaphoideus titanus egg distribution on grapevine. IOBC/wprs Bulletin, in press. IOBC/WPRS European Meeting of the Working Group "Integrated Protection and Production in Viticulture" 1st – 4th November 2009, Staufen im Breisgau, Germany.
Oral presentation submitted: Bagnoli B., Gargani E., Ferretti L., Gentili A., Pasquini G., Frosinini R., Tirinnanzi L. - Distribution of Scaphoideus titanus eggs on grapevine; Forte V., Rizzini F.M., Dalla Cia L., Patriarca E., Rainato A., Borgo M., Angelini E. - Scaphoideus titanus egg hatching rates from grapevine propagation material. COST Action FA0807 “Integrated Management of Phytoplasma Epidemics in Different Crop Systems” for the first meeting in Sitegs, Barcellona, Spain, 31 January - 2 February 2010.
WP5: Oral presentation given: Linder Ch., Schaub L. 2009. Effectiveness of hot water treatments against the eggs of Scaphoideus titanus Ball. IOBC Bulletin In press. IOBC/WPRS European Meeting of the Working Group "Integrated Protection and Production in Viticulture" 1st – 4th November 2009, Staufen im Breisgau, Germany.
A publication is planned in Revue suisse de Viticulture Arboriculture et Horticulture.

Recommendations for future work

These first results of the study of the genetic variability of ST in Europe could be competed by the analysis of supplementary populations coming from Italy (Lombardy and Veneto), Slovenia and other ST infested European country (Portugal, Croatia, Hungary, Serbia and Romania) and from Native American regions. We thus will be able to explain the colonization history of this insect in all of Europe and to trace introduction pathways to its origin.

The results of this project suggest going deeper into some aspects of the biology and dispersal behaviour of ST.

- ST oviposition preferences including the morphological characteristics of the bark of one-year old canes from varieties with different period of wood maturation;
- ST oviposition preference affected by grafting procedures on finished plants in the nursery.
- ST dispersal in nurseries
- Importance of abandoned vineyards as ST reservoirs.

Recent preliminary observations in Switzerland indicate that up to 50% of the ST population is hosted by other plants than grapevine, such as weeds in the vineyard and adjacent habitats. These findings have to be confirmed in other wine producing regions of Europe. If they are, this would have great implications on ST population dynamics and FD epidemiology and open many questions regarding their interaction.

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- Switzerland - Federal Office of Agriculture (FOAG)

Appendix

Work package reports including more details, acknowledgements, literature references.