European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes

PP 1/268 (1)

Efficacy evaluation of plant protection products
Evaluation biologique des produits phytosanitaires

Study of unintentional effects of plant protection products on fermentation processes and characteristics of wine

Specific scope

This standard describes the conduct of trials for the evaluation of unintentional effects of plant protection products, in particular fungicides and insecticides, on fermentation processes during wine making and on the characteristics of wine. If necessary, this standard can also be adapted for distillation.

Specific approval and amendment

First approved in 2010–09.

Trials described in this standard are normally required for a product containing an active substance known to have caused unintentional effects, or chemically similar to one known to cause unintentional effects on fermentation processes and characteristics of wine. These trials are particularly needed in the case of fungicidal activity being present in the residues at harvest. The EPPO Standard PP 1/243 *Effects of plant protection products on transformation processes* provides general guidance on the need for data on possible adverse effects of plant protection products on processes for the transformation of harvested crops.

1. Experimental conditions in the field

1.1 Selection of crop and cultivar

The trial should be performed in the field on commonly grown *Vitis vinifera* crops (VITVI), of both white and red varieties. The grape varieties should be representative of the region where the trials are conducted. The selected vineyard should be at least 5 years old.

1.2 Trial conditions

The trial should be set up in the field in major wine-growing regions of the trial country. Sites should be chosen where the Potential Alcoholometric Titer (PAT) of the wine matches or exceeds the minimum standards for 'quality wine psr'[1] of the region. In the case of distillation trials, the PAT should be at least 7.5% in volume.

The trial should form part of a series carried out in different regions with distinct environmental conditions. A minimum of six trials is required. It is recommended that the tests be conducted for at least 2 years in viticultural regions which differ in climate and particularly in the sugar content of the grape at harvest. At least 4 of the trials should be carried out in wine-growing zones, relevant to the country where the product is intended for registration. In the case of red wine, malolactic fermentation (MLF) should be sought where technically possible. The trials should include red and white (e.g. 4 red and 2 white), as well as early and late grape varieties.

Cultural conditions (e.g. soil type, fertilization, pruning) should be uniform for all plots of the trial and should conform to local agricultural practice.

1.3 Design and layout of the trial

Treatments: test product(s) and reference product(s), arranged in a suitable statistical design. No untreated plot is required.

Plot size (net): should allow harvesting at least 60 kg of grapes per treatment sufficient to produce 40 L of grape juice. A net plot consists of a single row for sampling. If spray drift to neighbouring plots cannot be avoided (e.g. by the use of a spray tunnel), there should be guard rows on each side of the

[1] psr = produced in specified regions

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net plot. Where air-assisted sprayers are used, the number of guard rows (or separation distance between plots) should reflect this.

Replicates: at least 3.

For further information on trial design, see EPPO Standard PP 1/152 *Design and analysis of efficacy evaluation trials.*

2. Application of treatments in the field

2.1 Test product(s)

The product(s) under investigation should be the named formulated product(s) (see EPPO Standard PP 1/181 *Conduct and reporting of efficacy evaluation trials, including good experimental practice*).

2.2 Reference product(s)

The reference product should be a product known to be satisfactory in practice under the conditions of the area of intended use. In general, mode of action, time of application and method of application should be as close as possible to those of the test product. If this is not possible, reference product and test product should be applied according to their specified use.

2.3 Mode of application

Applications should comply with good standard practice.

2.3.1 Type of application

The type of application should be as specified for the intended use.

2.3.2 Type of equipment

Application(s) should be made with suitable equipment providing an even distribution of product on the whole plot or accurate directional application where appropriate (e.g. grey mould or berry moth control). Factors such as operating pressure, nozzle type or volume rate should be chosen in relation to the intended use.

2.3.3 Time and frequency of application

The number of applications and the date of each application should be as specified for the intended use. The BBCH growth stage of the crop at each date of application should be recorded.

2.3.4 Doses and volumes

The product should always be applied at the maximum dosage specified for the intended use.

Full details on doses and volumes are given in EPPO Standard PP 1/239 *Dose expression for plant protection products.* In summary, the dosage applied should normally be expressed in kg (or L) of formulated product per ha and volume of water per ha should also be recorded for sprays. It may also be useful to record the dose in g of active substance per ha. In certain circumstances, the dose may be expressed as a concentration (e.g. % or g hL^{-1}), ideally combined with a volume (L ha^{-1}) appropriate to specific use.

Deviations from the intended dosage should be noted.

2.3.5 Data on other plant protection products

If other plant protection products (or any biocontrol agents) have to be used, they should be applied uniformly to all plots, separately from the test product and reference product. Possible interference with these should be avoided. In the case of wine trials, crop maintenance products should not be applied within 1 month of harvest. Protection should be made to ensure that the severity of grey mould (*Botryotinia fuckeliana,* BOTRCI) and other diseases or pests do not affect the trials.

3. Assessments, recordings and measurements before processing

3.1 Meteorological and edaphic data

3.1.1 Meteorological data

On the days before and after application (e.g. 7 days before and 7 days after), meteorological data should be recorded which are likely to affect the behaviour of the plant protection product. This normally includes data on precipitation and temperature.

All data should preferably be recorded on the trial site, but may be obtained from a nearby meteorological station. Its location and distance from a trial site should be noted.

Throughout the trial period, extreme weather conditions, such as severe or prolonged drought, heavy rain, early frosts, hail etc., which are likely to influence the results, should also be reported. All data concerning irrigation should be recorded as appropriate.

3.1.2 Edaphic data

Not needed.

3.2 Direct effects on the crop

The crop should be examined for the presence of phytotoxic effects. In addition, any positive effects should be noted. The type and extent of such effects on the crop should be recorded. The absence of any effects should also be recorded. Unintended effects on the crop should be accurately described (discoloration, necrosis, deformation etc.). For further details, see EPPO Standard PP 1/135 *Phytotoxicity assessment which contains sections on individual crops.*

3.3 Assessment of bunch diseases

A visual scoring of BOTRCI, incidence and severity should be performed at or shortly before the forecasted date of harvest as given in EPPO standard PP 1/17 *Botryotinia fuckeliana* on grapevine. Other diseases affecting bunches, e.g. powdery mildew *(Erysiphe necator* UNCINE), downy mildew *(Plasmopara viticola* PLASVI, *Coniella diplodiella* CONLDI), secondary bunch rots (*Penicillium*, *Aspergillus*, sour bunch rots etc.), may also be assessed.

3.4 Maturity assessment

Grapes should be harvested during the general harvesting period of the particular variety in the region. An assessment of total sugar content (g/L), PAT (% ethanol), pH and Total Acidity (TA, g L^{-1} H_2SO_4) can be made a few days before the forecasted date of harvest on approximately 100 bunches per plot and 2 berries per bunch (i.e. a total of 200 berries per plot) according to standard procedures.

3.5 Quantitative and qualitative recording of yield

Not needed.

4. Processing

The standard experimental vinification procedure is 'minivinification'.

4.1 Sampling

A sufficient quantity of grapes should be harvested in each plot for processing trials[2] A minimum of 60 kg of grapes per treatment should be harvested. Unless this is a desired feature, only healthy grape bunches with less than 10% *B. fuckeliana* severity should be harvested. The samples should be transferred to the processing laboratory without delay.

4.2 Minivinification

The grape samples from the 3 replicates are bulked before processing to make 1 sample per treatment. It may be appropriate in some cases to collect a second sample to be used as a back-up in case of vinification failures. Vinification should be made according to the local practice and, in the case of red wine, should include a maceration step. It is recommended that yeast is added to initiate alcoholic fermentation, unless the objective is to evaluate the effects on spontaneous fermentation. Evaluating spontaneous fermentation is useful because it may show the direct effects of plant protection products on naturally occurring yeast. When yeast is added, the name of the strain, brand and lot number should be recorded. When it is necessary, sugar may be added in order to obtain a comparable PAT among treatments. The PAT should fulfil the requirements for quality wine psr.

If MLF is desirable, the must may be seeded with a malolactic bacterial strain. The name of the strain, brand and lot number should be recorded.

The wines from the different treatments should be treated similarly during the post- fermentation phase ('clarification', sulphite or cold treatment). The wines should be bottled after filtration and stored for 15 months at 10–15°C for organoleptic evaluation.

[2] The larger the fermentation tank is, the more the fermentation process may be controlled. This increases the likelihood of obtaining evaluable results.

5. Assessments, recordings and measurements during processing

5.1 Assessments on fermentation parameters

5.1.1 Density and temperature of the must

Density and temperature of the must should be recorded daily during the alcoholic fermentation phase according to standard procedures.

5.1.2 Fermentation kinetics

The start and end time of the alcoholic and malolactic fermentation processes should be recorded.

5.2 Analytical assessment of the grape juice (must)

The grape must from the different treatments should be analysed for the following parameters according to standard methods:

- pH
- TA
- Turbidity (white and rosé wines only)
- PAT by refractometry
- Sulphite concentration
- Potassium concentration (optional)
- Nitrogen concentration (total or ammonium)

5.3 Analytical assessment of the bottled wine

The bottled wines made from the different treatments should be analysed for the following parameters according to standard methods:

- Reducing sugars (chemical analysis)
- Ethanol concentration
- pH
- TA and volatile acidity (VA)
- Sulphite concentration
- Optical Density 420, 520 and 620 (optional)

5.4 Organoleptic assessment of bottled wine

Assessment of wine quality is made by a panel of at least 10 qualified jurors (OIV, 2009). Tests are performed 1–2 months after bottling (young wine) and again 1 year later. Each wine is given an olfactory and a gustative quantitative rating, as well as an overall quality assessment. Wine made from grapes treated with the test product is compared to that made from grapes treated with the reference product. The test is set up in such a way that the same wine is presented twice to the jury (triangle test) AFNOR (2007).

An alternative test (Four-step assessment) is proposed in Appendix 1.

6. Results

The results should be reported in a systematic format and the report should include an analysis and evaluation. Original (raw)

data should be available. Statistical analysis on maturity assessments should use appropriate methods, which should be indicated.

Appendix 2 defines and standardizes elements which could be included in a final report for the registration process.

References

AFNOR (2007) Analyse sensorielle – Méthodologie essai triangulaire V-09 013 Octobre 2007. (in French).

OIV (2009) Compendium of International Methods of Analysis of Wine and Musts, (Vol. 1& 2 and updates) http://news.reseau-concept.net.

Council Regulation (EC) No 479/2008 on the common organisation of the market in wine, amending Regulations (EC) No 1493/1999, (EC) No 1782/2003, (EC) No 1290/2005, (EC) No 3/2008 and repealing Regulations (EEC) No 2392/86 and (EC) No 1493/1999. Official Journal of the European Union L 148, 6 June 2008, pp. 1-61.

OEPP/EPPO (2005) EPPO Standard PP 1/242 Taint tests. *Bulletin OEPP/EPPO Bulletin* **35,** 573–579. (The collection of PP1 standards is also available at http://pp1.eppo.org/list.php.)

OEPP/EPPO (2005) EPPO Standard PP 1/243 Effects of plant protection products on transformation processes. *Bulletin OEPP/EPPO Bulletin* **35,** 581–582. (The collection of PP1 standards is also available at http://pp1.eppo.org/list.php.)

Appendix – 1 Four-step assessment

The wine tasting is carried out as a simultaneous test. At least 10 qualified jurors are required. The test is to be set up in such a way that the same wine is presented twice to the jury (a random non-constant order should be used).

Smell and taste are assessed and scored, as follows:

1 = without flaw/imperfection
2 = mild flaw/imperfection
3 = moderate flaw/imperfection
4 = major flaw/imperfection

Off-flavours are to be described wherever possible.

The results of the olfactory and gustative testing in the four-step assessment may be presented in a table such as the following:

	Reference				Test product			
	Smell		Taste		Smell		Taste	
Juror	1. Rep	2. Rep	1. Rep	2. Rep	1. Rep	2. Rep	1. Rep	2. Rep
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								

Appendix – 2 Trial report

Definition and standardization of elements which could be included in a final report of the trials for the registration process

1. Trial conditions

- Aim of the trial
- Description of the trial site (vineyard): (identity of the trial site, grape variety, age of the grapevine, grapevine density, training system)
- Products compared
- Treatments with other plant protection products (herbicides, fungicides, acaricides, insecticides etc.)
- Treatments with test product(s) and reference product(s) (product and dose/ha)
- Date of harvest, date received at the processing laboratory[3] sanitary conditions of the samples

2. Maturity assessment before and during harvest

The following parameters can be reported:

- % of BOTRCI and if relevant other diseases affecting bunches
- total sugar content (g L^{-1})
- PAT (% ethanol)
- pH
- total acidity (g L^{-1} H_2SO_4)
- Sample size and number of days before harvest should be noted and analysis of variance should be performed for the above parameters.

State whether the trial was carried out according to the EPPO Standard PP 1/268 *Study of unintentional effects of plant protection products on fermentation processes and characteristics of wine*.

3. Grape must analysis

- Description of wine processing for red, white or rosé
- SO_2 addition (g hL^{-1}) (if required, depending on the sanitary conditions)

The following parameters should be analyzed and reported after bulking the grape samples from the three replicates into one sample per treatment and before enrichment:

- Turbidity Nephelometric Turbidity Units (NTU)*
- Sugars g L^{-1}
- pH
- Total acidity (in g L^{-1}) as H_2SO_4
- Total SO_2 mg L^{-1}

[3] Testing Facility or Organization The testing facility or organization which performs the tests should be identified and it should be clearly stated whether the trials are performed by an official or officially recognized testing facility or organization (see EPPO Standard PP 1/181 *Conduct and reporting of efficacy evaluation trials*, including good experimental practice). Tests carried out by official or officially recognized organizations are valid studies for registration authorities as well for processors.

- K mg L^{-1}
- Assimilable N mg L^{-1} **
- N-ammonium mg L^{-1} **

*Measured on settled must and of comparable turbidity for white and rosé wines only

**A choice of assessment should be made

If an enrichment was necessary, indicate the dose of sugar added and the intended increase in the degree of alcohol.

Note:

- compare maturity (grape ripeness) status between treatments (samples) and reference product
- decide whether a DAP (diammonium phosphate) addition for must was necessary

4. Minivinification

4.1 Wine processing for: white, red, rosé

Duration, frequency and doses of products of the following operations should be indicated when relevant.

- Destemming
- Crushing
- SO_2 addition (SO_2 in g L^{-1})
- Settling (duration in hours)
- Yeast addition (quantity, name of the strain, brand and lot number)
- Diammonium phosphate (DAP) addition (quantity in g hL^{-1} and note the stage of fermentation)
- Aeration of must (note the stage of fermentation)
- Remontage (*délestage* 'rack and return') (frequency)
- Pigeage (frequency)
- Enrichment (degree of alcohol)
- Duration of maceration (days)
- Bacteria inoculation
  - Procedure used
  - Name of the strain, brand and lot number
- Temperature at:
  - Clarification settling
  - Maceration
  - Alcoholic fermentation
  - MLF
  - Stabilization
- First SO_2 addition (in g L^{-1}) after fermentation
- Number of racking
- Type of filtration
- Desired free SO_2 when bottling (SO_2 in mg L^{-1})
- Date of bottling
- Storage temperature for bottles
- Remarks, other practices

4.2 Fermentation process

If there are small temperature differences between the various treatments, indicate the following:

- temperature of must (°C) at the beginning of the fermentation
- the range (minimum and maximum) of temperatures during the fermentation.

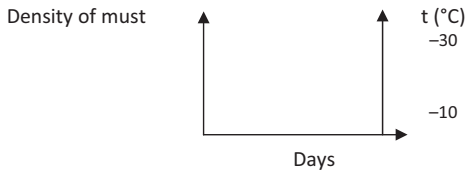

Fig. 1 Fermentation kinetics.

Figure 1 should show all the fermentation curves of the trial.

The summary of beginning and duration of the fermentation in days for all treatments are shown in Table 1.

Table 1 Fermentation Stages

Time to the start of fermentation (1)
Duration of alcoholic fermentation (2)
Time to the start of spontaneous MLF (1)
Duration of spontaneous MLF (3)
Time to the start of the inoculated MLF
Duration of the inoculated MLF

(1) Calculated at 'Time 0': starting at settling for white and steeping (maceration) for red varieties.
(2) Time between start of alcoholic fermentation and consumption of sugar (<2 g L^{-1}).
(3) Time between formation of lactic acid and the end of MLF.
Note: Compare the kinetics of the various treatments at the minivinification. Include results of the microvinification (when performed).

4.3 Analysis after bottling

Analysis of the wines after bottling is shown in Table 2.

Table 2 Analysis of the wines after bottling

Parameters	Units
Residual sugar	g L^{-1}
Ethanol	% volume
pH	
Total acidity	g L^{-1} H_2SO_4
Volatile acidity	g L^{-1} H_2SO_4
Free or available SO_2	mg L^{-1}
Total SO_2	mg L^{-1}
OD 420 (1)	
OD 520 (1)	
OD 620 (1)	
Colour intensity	OD 420 + OD 520 + OD 620
Tonality (nuance in colour)	OD 420 OD 520
IPT (total phenol index)	d 280 = OD 280 × 100 (2)

(1) Optical density (OD) unit for 1 cm cuvette for red and rosé; OD 420 only for white wine (OIV, 2009)
(2) The wine is diluted in distilled water (1/100), the optical density is measured at 280 nm.
Note: Compare wines of all treatments; consider the results of must analysis.

4.4 Organoleptic assessment

4.4.1 Tasting of the young wine

- Number of jurors.
- Description of triangle test, results by level of significance, number of correct identifications.
- Description of wine(s) smell, aroma, flavour.
- Description of wine(s) by explaining the differences.

An alternative test (Four-step assessment) is proposed in Appendix 1.

4.4.2 Wine tasting after 1 year's storage

The results are presented in an additional report.

Conclude whether the results of wine tasting after storage correspond to those of the young wines as described in the main report.

5. General conclusion

Summarize the following results:

- Fermentation kinetics
- Wine analysis
- Wine tasting

Explain whether the application of the test products has changed any of the parameters and draw conclusions.

Finally, estimate whether further assessments are essential, necessary or preferable.