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REPORT
PO14 039 PO1

EVALUATION OF THE EFFICACY OF PB001 ON PROCESSING AND QUALITY OF WINES IN PORTUGAL

Trial ID: PO14 039 PO1

Protocol ID: PO14 039

Location: PORTUGAL

SPONSOR :

Alberto Botella Cayuelas

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1 PROCESSING PART

1.1 GENERAL TRIAL INFORMATION

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1.2 ASSESSMENTS ON THE DELIVERED GRAPE BEFORE PROCESSING

Following the field part conducted in Portugal on red grapes (Arinto), a Botrytis cinerea assessment (% of attack of bunches) and an evaluation of the Potential Alcohol content (TAP) were conducted on the harvested grape samples (harvest conducted on September 30th 2014).

This control was done on berries sampling taken in each replicate received.

Treatment		5 PB001	6 SERENADE MAX
% of grey mould	Rep 1	3	4
	Rep 2	2	3
	Rep 3	4	3
	Rep 4	3	2
	average	3	3
TAP (% vol)	Rep 1	9,1	9,0
	Rep 2	9,0	9,3
	Rep 3	8,8	9,2
	Rep 4	9,2	8,8
	average	9	9,1

Legend:
TAP: Potential Alcoholic strength (% volume)

Grey mould and potential alcohol degree - conclusion:
No significant grey mould infection was observed on the harvested bunches before processing.
The TAP value of all grape samples was quite low but satisfactory according to the CEB143 method criteria.
All grape samples were acceptable regarding the criteria of the CEB143.

1.3 COMPLEMENTARY ASSESSMENTS PER TREATMENT BEFORE PROCESSING

Harvest date: 30/09/14
Receipt date: 02/10/14

Treatment	5 PB001	6 SERENADE MAX
Grapes weight with packaging (kg)	62,14	58,00
Weight's packaging empty (kg)	7,72	7,72
Specimen weight (kg)	54,42	51,28
Must weight (kg)	52,69	49,60
Stalk weight (kg)	1,52	1,51
Lost weight (kg)	0,21	0,17

1.4 MUST ANALYSIS

Note: the treatment n°2 was the reference treatment

Treatment	5 PB001		6 SERENADE MAX		Acceptable difference
	value	≠	value	≠	
Density	1066	-2	1068		NA
Temperature must (°C)	20,5	0	20,5		NA
Sugar (g/l)	149	-8	157		NA
TAP (corrected to 20°C)	9	-0,3	9,3		0,3
Total acidity (g/l H2SO4)	6,80	0	6,80		0,2
pH	3,23	0,07	3,16		0,2
Total SO2 (mg/l)	253	80	173		10
Assimilable N (mg/l)	30	-42	72		50

Legend:
≠: Difference with the standard reference corresponding value
NA: no acceptable difference determined for this parameter

Must analysis - conclusion:
There was no significant difference between the PB 001 treatment and the SERENADE MAX reference except for the total SO2 of the PB 001 which was higher than the reference. This difference was without incidence because this was the result of an addition of sulphur dioxide.

1.5 FERMENTATION PROCESS

1.5.1 FERMENTATION PROCESS - DESCRIPTION

At the receipt, the grapes were weighed, crushed, stemmed and sulfited at 8 g/hl.
A juice sample was done to analyse the must. Grapes were put in little vat to make the alcoholic fermentation. To start the alcoholic fermentation, a yeast inoculation was done at 20 g/hl with *Saccharomyces cerevisae* 522 D.
Every day during the alcoholic fermentation, the cap was punched and a pumping over was done. Densities and temperatures were measured every day.
Once the fermentation was finished, the free-run juice was drained away and the pomace was pressed. The press wine obtained was mixed with the free-run juice and put in a full vat to make the malolactic fermentation.
The evolution of the malolactic fermentation was followed by paper chromatography. When the malolactic fermentation was finished, the wine was sulfited at 7 g/hl.
Few days later, the wine clarified was racked.

About one month later, the wine was bottled and the free SO2 was adjusted around 15-25 mg/l.

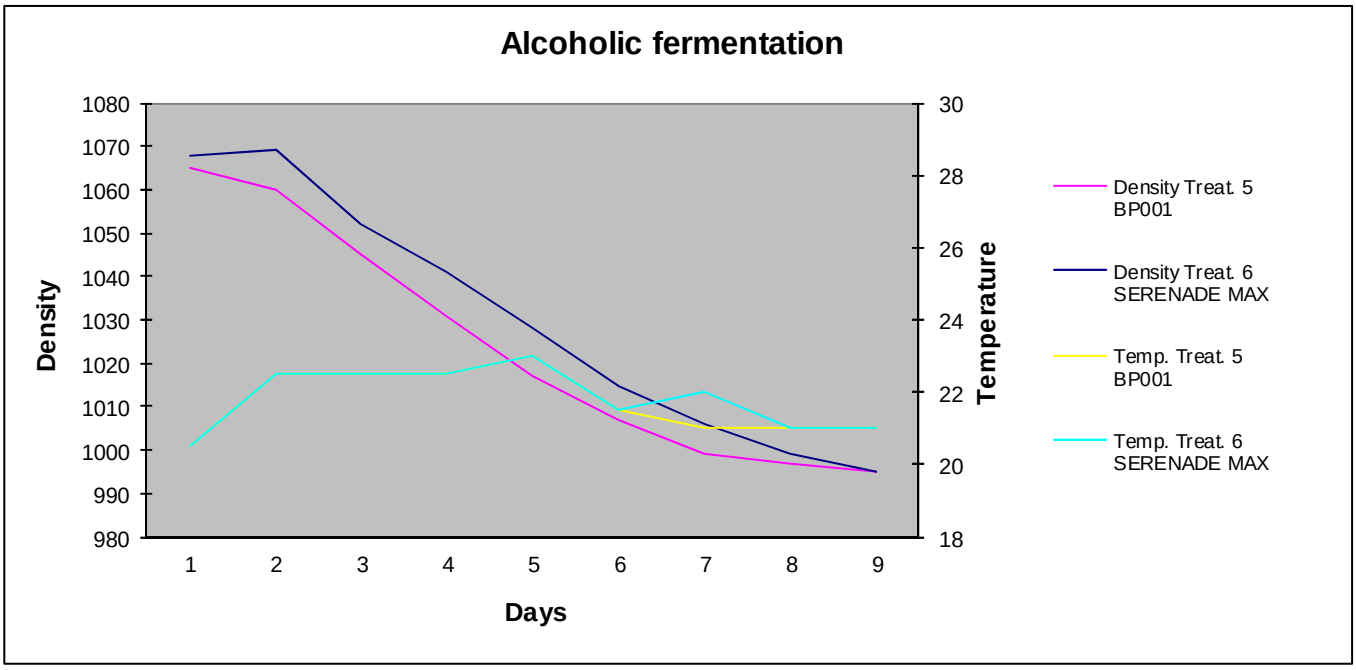
1.5.2 VINIFICATION TASKS

OPERATIONS	DATE	COMMENTS
Received	02/10/2014	
Yeasts inoculation	03/10/2014	Levuline CER522D BATCH N°15700450730621M
Starting of the alcoholic fermentation	04/10/2014	
Cap punching	From 03/10/14 to 09/10/14	
Ammonium phosphate supplement	none	
Pressing	10/10/2014	
End of the alcoholic fermentation	11/10/2014	
First Racking of	13/10/2014	
Lactic bacteria inoculation	25/11/2014	
Starting of the malolactic fermentation	03/12/2014	
End of the malolactic fermentation	12/12/2014	
Second Racking of	15/12/2014	
Bottling	13/01/2015	
Wine analyses	14/01/2014	
Wine tasting	19/02/2015	

1.5.3 FERMENTATION PROCESS - KINETICS

Days	Density Treat. 5 BP001	Density Treat. 6 SERENADE MAX	Temp. Treat. 5 BP001	Temp. Treat. 6 SERENADE MAX
1	1065	1068	20,5	20,5
2	1060	1069	22,5	22,5
3	1045	1052	22,5	22,5
4	1031	1041	22,5	22,5
5	1017	1028	23	23
6	1007	1015	21,5	21,5
7	999	1006	21	22
8	997	999	21	21
9	995	995	21	21

Treatment	5 BP001	6 SERENADE MAX
Latent period of the alcoholic fermentation	1 day	1 day
Alcoholic fermentation duration	8 days	8 days



Alcoholic fermentation - conclusion:
No difference on the temperature evolution recorded during the alcoholic fermentation was detected between the treatments.
The alcoholic fermentation of both treatments was finished at the same time.
Concerning the fermenting duration, there were no significant difference between the SERENADE MAX reference and the BP001 treatment.

1.6 WINES ANALYSIS

Treatment	5 BP001		6 SERENADE MAX		≠ acceptable
	value	≠	value	≠	
Reducing sugar (g/l)	1,73	-0,25	1,98		1
TAV (% by volume)	9,35	-1,01	10,36		0,3
pH	3,4	0,04	3,36		0,2
Total acidity (g/l of H2SO4)	4,44	-0,03	4,47		0,2
Volatile acidity (g/l of H2SO4)	0,65	0,14	0,51		0,1
Free SO2 (mg/l)	20	-5	25		10
Total SO2 (mg/l)	94	7	87		10
DO 420	0,221	-25 %	0,296		10%
DO 520	0,360	-29 %	0,510		10%
DO 620	0,072	-20 %	0,090		10%
IC colour intensity	0,653	-27 %	0,896		10%
Nuance DO420/DO520	0,61	6 %	0,58		10%
I.P.T index total polyphenols	36	-18 %	44		10%

Legend:
≠: Difference with the standard reference corresponding value
TAV: Alcoholic strength (% volume)

Wine analysis - conclusion:

The volatile acidity of the BP 001 was a bit higher than the SERENADE MAX reference.
A lot of other parameters were lower for the BP 001 treatment than for the SERENADE MAX reference:

- The TAV
- The DO 420
- The DO 520
- The DO 620
- The colour intensity
- The I.P.T (index total polyphenols).

All these values had a significant difference between the BP 001 and the SERANADE MAX reference and indicated a less maturity of the BP 001 treatment.

Concerning all the other parameters (reducing sugar, pH, total acidity, free and total SO2), there was no difference between the BP 001 treatment and the SERENADE MAX reference.

1.7 WINE TASTING

1.7.1 WINE TASTING METHOD

Wine testing has been carried out according to the French Guidelines CEB 143 following the normalized testing method AFNOR V09-013 using black INAO glasses.
The jury was gathering 10 experienced tasters. The temperature of all samples was the same (8°C).
The taint testing compared the treatment per pair. Three test frames of samples were done as followed ABB, BAA, AAB, BBA, ABA, and BAB… Three glasses of the code test frame were presented to each member of the jury; the forced selected choice glass was input on each taint testing sheet.
The following observations were assessed: raw number of good answers per test frame.
All results were delivered to statistical analyses with the following significant level (α = 0, 05; significant level P95% or α = 0, 01 highly significant P99%).
The intensity of the eventual detected difference is assessed according to a 3 levels scale (minor, medium, major); the type of difference was asked to be qualified, first when smelling (intensity, bouquet), secondly in the mouth (acidity, smoothness, length, bouquet).

1.7.2 ONE MONTH BOTTLED TAINT TESTING RESULTS

Test 1: comparison between the BP001 treatment and the SERENADE MAX reference.

Test number	Total jury testers	date	Exact answers	Wrong answers	Significant Exact answers required P95%	Significant Exact answers required P99%
Test 1	10	19/02/2015	6	4	7	8

There was no difference between the BP001 treatment and the SERENADE MAX reference.

2 CONCLUSION

TRIAL SUMMARY
This selectivity trial was set up in Portugal on red grapes (Arinto).
The no intentional effect on wine making of BP001 product was compared to the no intentional effect of SERENADE MAX used as a reference product.

VALIDITY
The maturity parameters recorded at harvest and the degree of *Botrytis cinerea* attack on bunches before the process was in compliance with the criteria of the guideline CEB143.

RESULTS

Analysis on grapes before processing:
At harvest, grape samples of each plot were analysed for their sugar and the total acidity. The percentage of grey mould was noted.

Must analysis - conclusion:
There was no difference between the BP001 product and the SERENADE MAX for all the must analysis. The total SO2 of the BP001 was a bit higher than the reference but without incidence.

Alcoholic fermentation:
There was no difference between the two treatments on alcoholic fermentation kinetic.

Wine analysis - conclusion:
There were significant differences on wine analysis on the TAV, the colour parameters and the I.P.T (index total polyphenols) who were lower for the BP 001 treatment than for the SERENADE MAX treatment. The volatile acidity was a little bit higher for the BP 001 treatment than for the SERENADE MAX treatment but without incidence.
For the other parameters (reducing sugar, pH, total acidity, free and total SO2) there was no difference between the BP 001 treatment and the SERENADE MAX reference.

Wine tasting – conclusion:
No difference was noted on wine tasting.

LABORATORY INVESTIGATOR : GEOFFROY Angélique
SIGNATURE:
DATE: 27/02/2015

3 APPENDIX

3.1 PROTOCOL

ANADIAG PORTUGAL

Evaluation the efficacy of PB001 against Botrytis, phytotoxicity and side effects on yield and quality of wine processing on grape in Portugal

Protocol ID:PO 14 039 Location:Portugal Trial Year:2014
Trial ID:PO 14 039 PO2 By:Sandra Rodrigues
Project ID:PO 14 039 Study Director:Marlene Oliveira
Sponsor Contact:Alberto Botella Cayuelas

Trt No.	Type	Treatment Name	Description	Rate	Rate Unit	Appl Code	Appl Description
1	CHK	Untreated Check	not treated				
2	FUNG	PB001		5	ml/l	ABC	7-14 days between applications
3	FUNG	PB001		8	ml/l	ABC	7-14 days between applications
4	FUNG	PB001		12	ml/l	ABC	7-14 days between applications
5	FUNG	PB001		15	ml/l	ABC	7-14 days between applications
6	FUNG	SERENADE MAX		4	kg/ha	ABC	7-14 days between applications

Replications: 4, Untreated treatments: 1, Conduct under GLP/GEP: Yes (GEP with no protection), Design: Randomized Complete Block (RCB), Treatment units: Treated 'Plot' experimental unit size, Dry Form. Unit: %, Treated 'Plot' experimental unit size Width: 4,8 meters, Treated 'Plot' experimental unit size Length: 11 meters, Application volume: 500 L/ha, Mix size: 12 liters, Format definitions: G-All7.def, G-All7.frm

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Trial Establishment Guidelines

Project ID:PO 14 039 **Developer:**PROBELTE
Revision Number:1,0 **Revision Status:**I Interim
Revision Date:6/8/2014 **Issue Date:**24/7/2014
Site Type:FIELD field
Treated Plot Width:4,8 m **Country:**PRT Portugal
Treated Plot Length:11 m **Climate Zone:**EPOMED EPPO Mediterranean
Treated Plot Area:52,8 m2 **Experimental Unit:**1 PLOT plot
Replications:4 **Tillage Type:**CONTIL conventional-till
Study Design:RACOBL Randomized Complete Block (RCB)

Keywords: PROBELTE/PB001/BOTRYTIS/GRAPEVINE

Trial ID	Responsible	Number of Trials	Site Requirements
PO 14 039	Anadiag Portugal	2	PT

Total Trials:2
Conduct Under GEP: Yes **Officially Recognized Organization:**019

No.	Guideline	Description
1.	PP 1/242(1)	Taint tests
2.	PP 1/135(3)	Phytotoxicity assessment
3.	PP 1/17(3)	Botryotinia fuckeliana on grapevine
4.	PP 1/152(4)	Design and analysis of efficacy evaluation trials
5.	PP 1/181(4)	Conduct and reporting of efficacy evaluation trials including good experimental

Objectives:
1 - Evaluate the efficacy of PB001 against *Botrytis* on grapevine.
2 - Compare the efficacy of PB001 against *Botrytis* with national standards.
3 - Evaluate the possible unintended adverse effects of PB001 on the crop and on the non target organisms.
4- Compare PB001 with standard reference product

Crop Description

Crop 1:VITVI Vitis vinifera European grape
BBCH Scale:BGRA

Target Pest Description

Pest 1 Type:D **Code:**BOTRSP Botrytis sp.
Common Name: Botrytis sp.

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Application Directions:
APPLICATION TYPE: Foliar application (atomizer) in accordance with local practices.

WATER VOLUME : 500 - 1000 L/HA according to vegetation size
The application should be performed up to run-off.

Number of applications: 3

APPLICATION TIMING :
Applications must be performed in a 7-14 days interval and start in preventive conditions.
Applications BBCH:71-85

Crop Stage At Each Application

	A
Crop 1 Code, BBCH Scale:	VITVI BGRA
Stage Scale Used:	BBCH
Stage Minimum, Percent:	71
Stage Maximum, Percent:	85

Pest Stage At Each Application

	A
Pest 1 Code, Disc., Scale:	BOTRSP D

Geographic Area/Environmental Considerations:
PLOT SIZE:
min. 20 plants per plot or sufficient to yield at least 15 kg of grapes per plot (total per treatment 60kg)

4 REPLICATES

UNTREATED PLOT : required
Randomized complete block design

CROP DESTRUCTION:
According to national regulation in the different countries

Cropping Considerations:
The trial should be set up in the field. The vineyard should be homogeneous in cultivar, age, plant width, training system, rootstock and general cultivation and health status. Cultural conditions (e.g. soil type, fertilization) should be uniform for all plots of the trial and should conform with local agricultural practice.

The trial should be carried out in a climatically and topographically homogeneous environment.

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Data to Collect:

Collect weather data from 7 days before the first application to the end of the trial (at least minimum temperature, maximum temperature and rainfalls). Any parameter which is likely to affect the development of the crop or the pest and the action of the plant protection product should be recorded.
The other treatments applied on the trial field (maintenance) have to be recorded.

1. APPLICATIONS
For each application following data must be reported :
- Date, time of the day, temperature (°C), wind (m/s), clouds cover (%), Water volume (l/ha) and BBCH stage of target crop
- Remaining volume (l) for each treatment and actual dose rate applied

2. OTHER DATAS TO COLLECT
Meteorological datas like temperature (°C), irrigation (type and amount)
Extreme weather conditions (prolonged drought, heavy rain, late frost hail etc.)which are likel to influence the results, should be reported.
Maintenance plant protection products and fertilizers applyed on the trial site.

3- EFFICACY
Assessment timing: Weekly assessments from 4 weeks before harvest until harvest (4 assessments)
- Sample type and size: 100 bunches per plot
- Assessment type:
Evaluate the bunch area covered by the disease on 100 bunches per plot (Pest severity)
Count the number of damaged bunches on 100 bunches per plot (Pest incidence)

4- PHYTOTOXICITY
Assess the symptom severity and describe the type of symptoms observed

Assessment on bunches and leaves:
- 50 leaves per plot
- 25 bunches per plot

Assessment timing:
Before Applications B and C
* EVALUATE % of leaf area attacked per leaf (severity),on 50 leaves per plot
* EVALUATE the percent of bunch attacked per bunch (severity) on 25 bunches per plot

At Harvest:
* EVALUATE the % of bunch area attacked by Botrytis (severity)

5- EFFECTS ON NON TARGET ORGANISMS AND ON OTHER PESTS
At each assesment date, any effects on benefical entomofauna must be recorded.
Any observed effects, positive or negative, on the incidence of other pests should de recorded.

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Statistical Analysis:

STUDENT NEWMAN and KEULS statistical test to compare treatments.

3.2 GEP AGREEMENT

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Section Laboratoires

Convention BPE n° 4611 – Annexe 1

PORTEE DE LA DEMANDE D'EVALUATION DE CONFORMITE RELATIVE A L'AGREMENT POUR REALISER DES ESSAIS OFFICIELLEMENT RECONNUS

ORGANISME *(Entité juridique)*	**Sarl de Laulan** Domaine de Laulan 47120 DURAS
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Convention n°	**4611**
Dossier n°	**G-0520**
Référentiel	LAB BPE Ref 02

La portée de l'évaluation de conformité aux principes de Bonnes Pratiques d'Expérimentation demandée par l'organisme ci-dessus désigné et que le COFRAC accepte d'évaluer est définie comme suit :

Portée de l'Agrément demandée :

UNITE(S)	SECTEUR(S) D'ACTIVITE
Unité centrale Domaine de Laulan 47120 DURAS	Grandes cultures Vigne Cultures légumières, plantes aromatiques, médicinales, condimentaires et à parfum Cultures fruitières et arboriculture Processus de transformation : - *vinification*

LAB BPE FORM 13 – Rév. 00 – Mars 2007 Page 1/2