# Open field-trials for a low-copper viticultural itinerary (P4.1)

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**Result from WG4**

GT leader : SOGRAPE VINHOS S.A.
Involved partners: PTV, ADVID, IFV, CVAN, UPC, SVBNA, EURECAT, JEAN LEON (TORRES), GREENUPORTO, SCEA (GERARD BERTAND)

# Protocol for GT4 field trials (E4.2.1)

**Result from activity 4.2. planning of viticultural itineraries and definition of GT4 experimental protocol**
**E4.2.1 - Protocol definition for field pilot-trials**

GT leader : SOGRAPE VINHOS S.A.
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# List and characterization of trial sites - GT4 COPPEREPLACE

**Selection of the plots**

Ideally, for the selection of the experimental site, some requirements should try to be met:

- The youngest vines available;
- Previous physical and chemical characterization (pH, soil organic matter% or carbon content, electrical conductivity, clay content) of soils would be relevant, if absent, these analyses need to be performed;
- Acidic soils (pH<6);
- Vineyards with at least 54 complete vine rows with 100m.

Chosen trials sites for execution of the GT4 protocol are listed below:

**PORTUGAL**

| Partner | Property | Block | Location[1] | Surface (ha) | Altitude (m) |
|---|---|---|---|---|---|
| **SOGRAPE** | Quinta do Sairrão | 57 | 41°07'24.4"N 7°24'24.0"W | 3.3049 | 590 |

[1] Geographical coordinates or link to Google Earth / Google Maps placemark

**Block characteristics**

| | |
|---|---|
| **Mode of production** *(highlight one)* | Conventional (profit only) IPM<br>**Integrated Production** Bio |
| **Grape variety** (*dominant if more than one*) | Tinta Roriz |
| **Rootstock** (*dominant if more than one*) | R110 |
| **Training system** | Bilateral Royat |
| **Vine spacing** *(row x inter-row in meters)* | 2.2 x 1.1 |
| **Year of plantation** | 1990 |
| IFT *(last 3 years)* | 1.28 |
| **Homogeneity** *(stdNDVI[1] ratio)* | 1.08 |
| **Irrigation** *(highlight one)* | Yes **No** |
| **Soil type** (*dominant if more than one*) | Slate schist |
| **Cover crops** | No **Yes-temporary** Yes-permanent |

[1] **stdNDVI ratio = stdNDVI block ÷ stdNDVI vineyard** *(std: standard deviation)*

**FRANCE**

| Partner | Property | Block | Location[1] | Surface (ha) | Altitude (m) |
|---|---|---|---|---|---|
| **SCEA CHATEAU HOSPITALET - GB** | Chateau l'Hospitalet | Aups Bas | 43°10'03.6" N 3°07'00.6"E | 1,52 ha | 120 |

[1] Geographical coordinates or link to Google Earth / Google Maps placemark

**Block characteristics**

| | |
|---|---|
| **Mode of production** *(highlight one)* | Conventional (profit only) IPM<br>Integrated Production **Bio** |
| **Grape variety (***dominant if more than one***)** | GRENACHE N |
| **Rootstock (***dominant if more than one***)** | Unknow |
| **Training system** | Gobelet |
| **Vine spacing** *(row x inter-row in meters)* | 2,5 x 1,20 |
| **Year of plantation** | 1979 |
| IFT *(last 3 years)* | 15 |
| **Homogeneity** *(stdNDVI[1] ratio)* | Unknown |
| **Irrigation** *(highlight one)* | **Yes** No |
| **Soil type (***dominant if more than one***)** | Clay |
| **Cover crops** | No **Yes-temporary** Yes-permanent |

[1] **stdNDVI ratio = stdNDVI block ÷ stdNDVI vineyard** *(std: standard deviation)*

**FRANCE**

| Partner | Property | Block | Location[1] | Surface (ha) | Altitude (m) |
|---|---|---|---|---|---|
| **SVBNA** | Château Rioublanc | "3 hectares" | 45°2'10.77"N 0°17'02.7"W | 2,8 ha | 60 m |

[1] Geographical coordinates or link to Google Earth / Google Maps placemark

**Block characteristics**

| | |
|---|---|
| **Mode of production** *(highlight one)* | Conventional (profit only) IPM<br>Integrated Production **Bio** |
| **Grape variety** (*dominant if more than one*) | Merlot |
| **Rootstock** (*dominant if more than one*) | 101 14 |
| **Training system** | 2 *lattes « médocaine »*<br>More than 3 sq. m of vegetation, high canopy (posts at 2,8 m) |
| **Vine spacing** *(row x inter-row in meters)* | 1 x 2 |
| **Year of plantation** | 2002 |
| IFT *(last 3 years)* | 4 |
| **Homogeneity** *(stdNDVI[1] ratio)* | Unknown |
| **Irrigation** *(highlight one)* | Yes **No** |
| **Soil type** (*dominant if more than one*) | Deep soil : Clay<br>Surface soil : "Boulbène" Clay Sand Silt |
| **Cover crops** | No Yes-temporary **Yes-permanent** |

[1] **stdNDVI ratio = stdNDVI block ÷ stdNDVI vineyard** *(std: standard deviation)*

## Protocol for plant protection

*Table 1 – Protection products to use and when to apply them*

| PRODUCT | France – Bordeaux | France – Narbonne | Portugal – Douro |
|---|---|---|---|
| **WHEN TO START** | 50% at 3-4 leaves (E, BBCH 14) | At budbreak (E, BBCH 14) | At visible bunches (F, BBCH 53) |
| **COPPER** | 25% of the usual copper dose. For example, if the usual dose is 300 g copper per hectare, then 25% is 75 gCu/ha.<br><br>This needs to be weighed as a function of the vegetative growth at the moment of spraying. For example, if the growth is at 25% of full leaf area, the spraying should be 75 x 0.25 = 19 g/ha.<br><br>Recommended formulations are copper sulphate or hydroxide. | | |
| **GLUCOSEI** | 25% of the usual copper dose.<br><br>For example, if the usual dose is 300 g copper per hectare, then 25% is 75 gCu/ha. As Glucosei (8%) doses 80 gCu/L, this translates as 0.94 L of Glucosei per hectare.<br><br>This needs to be weighed as a function of the vegetative growth at the moment of spraying. For example, if the growth is at 25% of full leaf area, the spraying should be 0.94 x 0.25 = 0.23 L/ha of Glucosei. | | |
| **ORANGE OIL** | To be applied with each copper treatment, **except during blossom** (H - J, BBCH 57 – 71), as it is forbidden. Maximum 6 applications during growth cycle, always leave 7 days interval between applications. If you need a second spray before 7 days have passed do not use it. Maximum dose 1,6 L/ha. Use table below for dose calculation (*for more information see document Protocole_GP2021_Bio_Diff in the COPPEREPLACE Intranet, Technical Management – GT4*): | | |

*Table 2- Doses of application for orange oil*

| Solution application in the field (L/ha) | Product dose (L/ha) | Product concentration |
|---|---|---|
| 80 | **0,48** | *0,60%* |
| 90 | **0,54** | *0,60%* |
| 100 | **0,6** | *0,60%* |
| 110 | **0,66** | *0,60%* |
| 120 | **0,72** | *0,60%* |
| 130 | **0,78** | *0,60%* |
| 140 | **0,84** | *0,60%* |
| 150 | **0,9** | *0,60%* |
| 160 | **0,96** | *0,60%* |
| 170 | **1,02** | *0,60%* |
| 180 | **1,08** | *0,60%* |
| 190 | **1,14** | *0,60%* |
| 200 | **1,2** | *0,60%* |
| 210 | **1,26** | *0,60%* |
| 220 | **1,32** | *0,60%* |
| 230 | **1,38** | *0,60%* |
| 240 | **1,44** | *0,60%* |
| 250 | **1,5** | *0,60%* |
| 260 | **1,56** | *0,60%* |
| *270* | *1,6* | *0,59%* |
| *280* | *1,6* | *0,57%* |
| *290* | *1,6* | *0,55%* |
| *310* | *1,6* | *0,52%* |

Start the experimentation at the beginning of the season. If possible, make a pulverization test with hydrosensitive paper to verify the quality of the application before treatment. Make sure the hydrosensitive paper is well packaged and conserved before use to avoid the problems reported in E3.1.1. Quality of pulverization should be evaluated by placing small pieces of water-sensitive paper in the targets intended to spray (for example leaves, bunches, internal leaves) along a small row of grapevines. The assessment of the spraying quality should be done by verifying the homogeneity of the drops' dispersion on water-sensitive paper and by counting a minimum number of 70 drops per square centimetre. To facilitate measurements, application DropLeaf may be used.

For each treatment, be careful to be up to date with all the green pruning, cover crop management. It could be important for the efficiency of the treatment.

**Renew treatment when the following conditions are met:**

1. Every 8 to 10 days. If no rainfall is expected, this may be extended up to 10- 12 days (maximum persistence at low pressure);
   a. During the high growth rate period renew after 6 days (only If rainfall or higher disease pressure is predicted)
2. If rainfall over 3 days is equal or higher than 15 mm
3. If rainfall since last treatment is equal or higher than 20 mm.

All trial modalities should be renewed at the same time. If products other than copper are used, renew treatment according to each product's persistence and observed (and recorded) disease pressure.

**Copper product reference:**

Each trial site manager may choose which copper formulation to be used (it's advised 1 product during the whole campaign), according to each site's usual practice.
If several products are to be used, better alternate formulations over consecutive dates.
In all cases, the copper dose for all trial modalities in each date of treatment must follow Table 1 prescriptions. For better copper efficiency, copper sulphate or hydroxide formulations are recommended.

**Dose:**

Prior to the spraying an assessment of the average vegetation development must be made using EPPO Guide 18-23715 - "Standard Measurement Procedure in High Growing Crop Trials" (as described in E3.1.1) and recorded. The target dose rate of spraying products must be adapted to the observed vegetation volume according to the above referred assessment. Each partner decides and records for each treatment the application dose to fulfil the experimentation protocol.

**Other disease or pest management:**

Additional phytosanitary treatments for pests and diseases other than downy mildew should be done at the same time, using the same products on all modalities, according to usual practices at each trial site.

The CTL modality (Figure 1) should NOT be treated against downy mildew **only**. Accordingly, when planning protection against other pests and diseases, be mindful of products also having certifications against downy mildew!! Those should not be used in this modality.

## Experimental design

The field experimental design should follow the schematic representation described in figure 1. Considering only complete vine rows with at least 100m, 9 of them should be treated with the New Treatment Protocol (NTP) alternated with 9 vine rows that receive the Usual Treatment Protocol (UTP). This scheme should be repeated three times, making a total of 54 vine rows under experiment in each field trial.

A small area without any treatment for mildew should be considered to be used as Control of the field trial (CTL). For this purpose, and to ensure that these won't become points of dispersion of infection, uncompleted rows may be selected in the border or corners of the plot (figure 1).

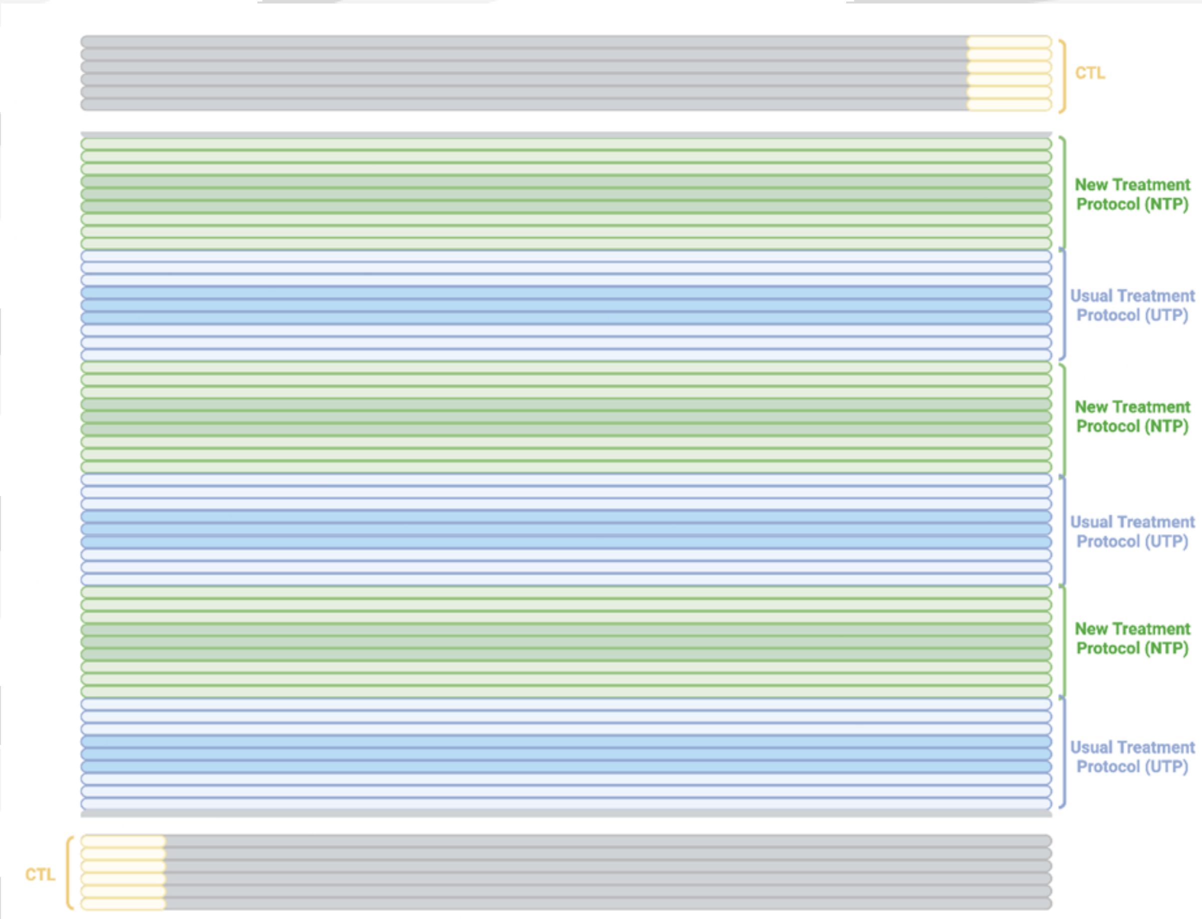



*Figure 1 - Schematic representation of the field experimental design*

## 1. Sampling strategy

| | France - Bordeaux | France - Narbonne | Portugal - Douro |
|---|---|---|---|
| **SAMPLES** | **FOR COPPER ANALYSES -** Same protocol all sites | | |

For the analysis of copper, samples should be collected in the three middle rows of each treatment, as shown in figure 2. In each one of these rows, three composite samples should be withdrawn per vine row, consisting of three different sampling points separated by 25 m, starting from the middle of the row, as described in figure 2. In the CTL area (for more details please see section 2.4), three composite samples should be collected. Thus, a total of 21 composite (soil, leaves and bunches) samples should be collected per plot (9 NTP+9UTP+3CTL).

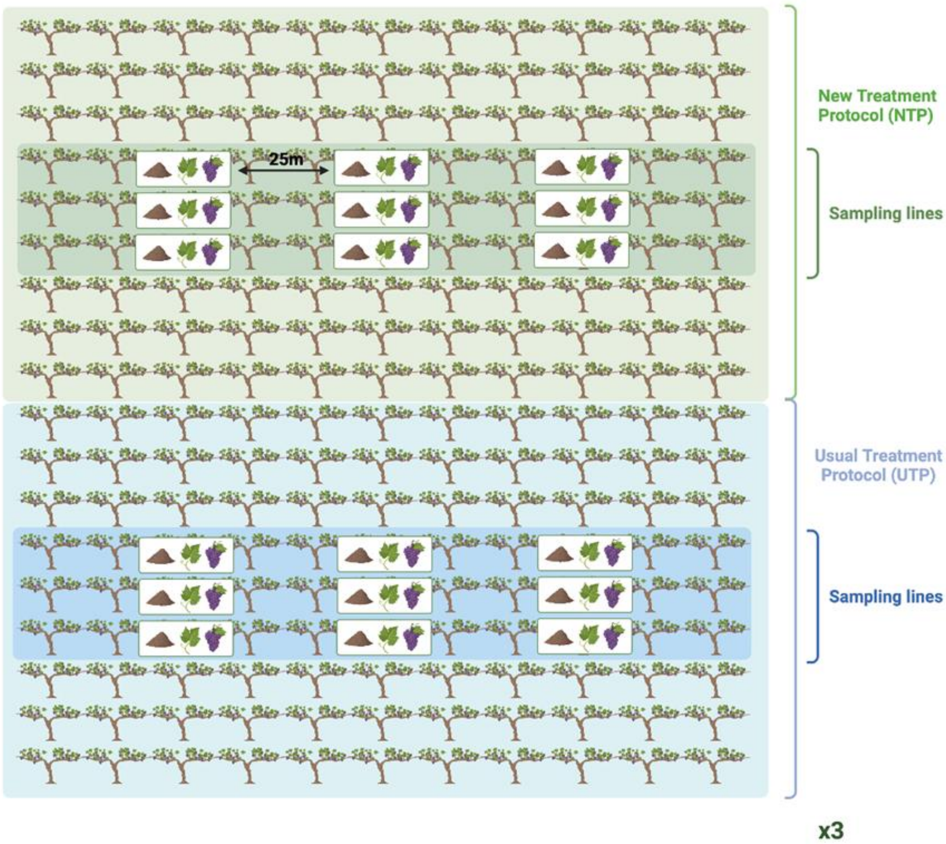



*Figure 2 - Schematic representation of the field experimental design, except control areas (CTL).*

## 2. Sampling protocol and samples pre-treatment

**Material required**

- 2mm mesh sieve
- weighing scale
- shovel
- plastic bags
- oven
- trays
- labels
- styrofoam boxes
- blender
- freezer

### 2.1. Soil samples

A composite soil sample should be collected per vine row, consisting of three different sampling points separated by 25 m, starting from the middle of the row, as described in figure 2.

At each sampling point three subsamples should be collected: one in the vine line and the other two on each side, at a distance of about 50 cm from the vine line, as described in figure 3 and detailed in figure 4. Soil subsamples from the 5 cm top layer should be collected into plastic bags, using a shovel, after removing the vegetation covering the soil. At each sampling point a total of about 1kg of soil should be collected.

Soils collected in the same row are to be mixed in the same plastic bag. Each complete composite soil sample per row should have about 3kg. Although such an amount of soil is not required for analysis, it is important to obtain a representative sample.

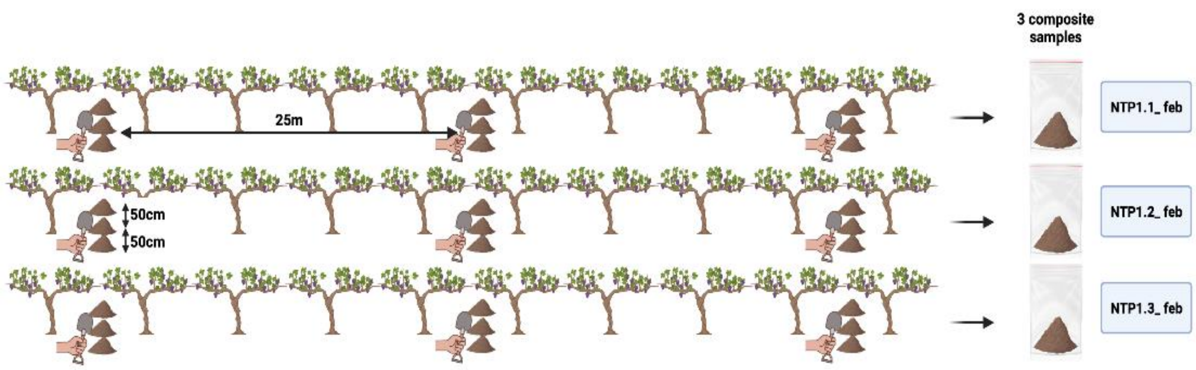



*Figure 3 - Soil sampling design, consisting of one composite sample per vine row. Each composite sample consists of samples collected at three different points separated by 25 m, and each point in three subsamples.*


*Figure 4 - Illustration of soil subsampling at each sampling point in the vine row.*

**Number of samples:** A total of 21 composite soil samples should be analyzed per field trial and sampling period.

**Pre-treatment:** Each composite soil sample should be truly mixed, air dried (inside a sheltered space, distributed in trays or even open plastic bags), periodically turning off the soil with a shovel for uniform drying. To accelerate the drying process, break down the size of larger aggregates. This drying process should be carried until the soil is visibly dried and ready to then be sieved through a 2mm mesh size sieve (larger aggregates should be crushed lightly by hand). Afterwards, 500gr of each sieved composite soil sample should be weighed, placed in plastic bags and labelled to be sent to GreenUPorto.

**Labelling:** The labels of samples should include:
The initial of the partner responsible, the code of the composite sample and the month of sampling, as exemplified below for Sogrape (February sampling campaign):

- SO_CTL1.1_feb, SO_CTL1.2_feb, SO_CTL1.3_feb
- SO_NTP1.1_ feb, SO_NTP1.2_feb, SO_NTP1.3_feb, SO_NTP2.1_ feb, SO_NTP2.2_ feb, SO_NTP2.3_ feb, SO_NTP3.1_ feb, SO_NTP3.2_ feb, SO_NTP3.3_ feb
- SO_UTP1.1_feb, SO_UTP1.2_feb, SO_UTP1.3_feb, SO_UTP2.1_feb, SO_UTP2.2_feb, SO_UTP2.3_feb, SO_UTP3.1_feb, SO_UTP3.2_feb, SO_UTP3.3_feb

**Sampling periods**: Before the phytosanitary treatment (ideally in February or March) and at the end of the application period.

### 2.2. Vine leaf samples

**Number of samples:** A total of 21 composite samples of leaves, per sampling period should be collected at the same sampling points described in the experimental design (figure 1 and 3). Ten leaves (leaf blade + petiole, cut with the help of scissors) per sampling point should be collected as described in figure 2. Thus, a total of 30 leaves per vine row, corresponding to a composite sample, should be joined in the same tray.

**Pre-treatment:** Each composite sample should be dried at 60ºC for 4 days, wrapped in aluminium foil and stored in plastic bags.

**Sampling periods:** The first sampling period should occur when the first bunches are visible, and the second sampling at the end of phytosanitary treatments.

**Labelling:** Follow the example described for soil samples.

### 2.3 Grapes samples

**Number of samples**: A total of 21 composite samples of grapes should be collected at the same sampling points described in the experimental design (figure 2 and 3). Three grape bunches per sampling point should be collected (cut with the help of scissors) as described in figure 2 Thus, a total of 9 bunches per vine row, corresponding to a composite sample, should be joined in the same box or tray.

**Pre-treatment:** The bunches composite samples should be de-stemmed. All grape berries of each composite sample are to be homogenized using a blender, and an aliquot of 1L should be transferred into a plastic bottle (e.g., empty mineral water bottles of 1.5L). Bottles should be then frozen at -20ºC (without closing the lids fully). Samples should be then sent, properly refrigerated, in a styrofoam box to GreenUPorto.

**Sampling periods:** At harvest.

**Labelling:** Follow the example described for soil samples.

### 2.4 Sampling in the control area (CTL)

Depending on the vine rows available without any treatment, the same sampling approach described above (for soil, leaves and bunches) should be followed. Nevertheless, the sampling design needs to be adapted to each case.

## 3. Soil trays experiment (only at Portugal site)

**Principle:** The aim of this protocol is to get a more precise quantification of the input of copper per soil surface area, in the lines of vines, both under usual and new treatment protocols. For this purpose, trays with an artificial soil and with a given surface area, prepared in the lab, should placed in the line of vines. For each line two composite samples of 4 trays each should be obtained and analyzed for total copper content, at two different periods. One composite sample should be withdrawn from the field 24h after the first treatment application and the second at the end of the treatment protocol. The experimental design should follow the one decided for soil sampling.

### 1.1. Selection of the plots

Ideally, for the selection of the experimental site, vineyards with at least 54 complete vine rows with 100m should be selected (the same as the previous protocol for soils, leaves and grapes sampling).

### 1.2. Material required

- Aluminium trays (33X219x127mm; 650mL volume)
- Artificial OECD soil (OECD 222)
- Plastic bags
- Labels

### 1.3. Field experimental design

The field experimental design should follow the schematic representation described in figure 1. Considering only complete vine rows with 100m, 9 of them should be treated with the new treatment protocol (NTP) alternated with 9 vine rows that receive the usual treatment protocol (UTP). This scheme should be repeated three times, making a total of 54 vine rows under experiment as shown in figure 2.

When possible, a small area without any treatment should be used as control (CTL) of the field trial. For this purpose, and to ensure that these won't become points of dispersion of infection, uncompleted rows may be selected in the border or corners of the plot (figure 1).

### 1.4. Procedure

In two different points in each vine line (figure 5) (the same selected for soil, leaves and grapes sampling) four aluminium foil trays with a surface area of 0.027m$^2$ should be placed side by side at the soil surface, precisely in the line, as exemplified in the figure 6. The trays should be filled with an artificial soil, prepared according to a standard OECD protocol, and composed of 70% sand (<2mm), 20% kaolin clay and 10% sphagnum peat.

The trays should be placed before the phytosanitary treatment and, at each point, two of them should be collected 24h after the treatment, to let the applied copper-based compound to move from the vines to the soil under gravity. The soil of the four trays of each row should be joined to obtain a composite soil sample per line, as described in figure 3.

The other two trays of each point should be left during the entire application period and collected after the treatment protocol has been completed, following the same methodology as described before.

Although such an amount of soil is not required for analysis, it is important to obtain a representative sample.

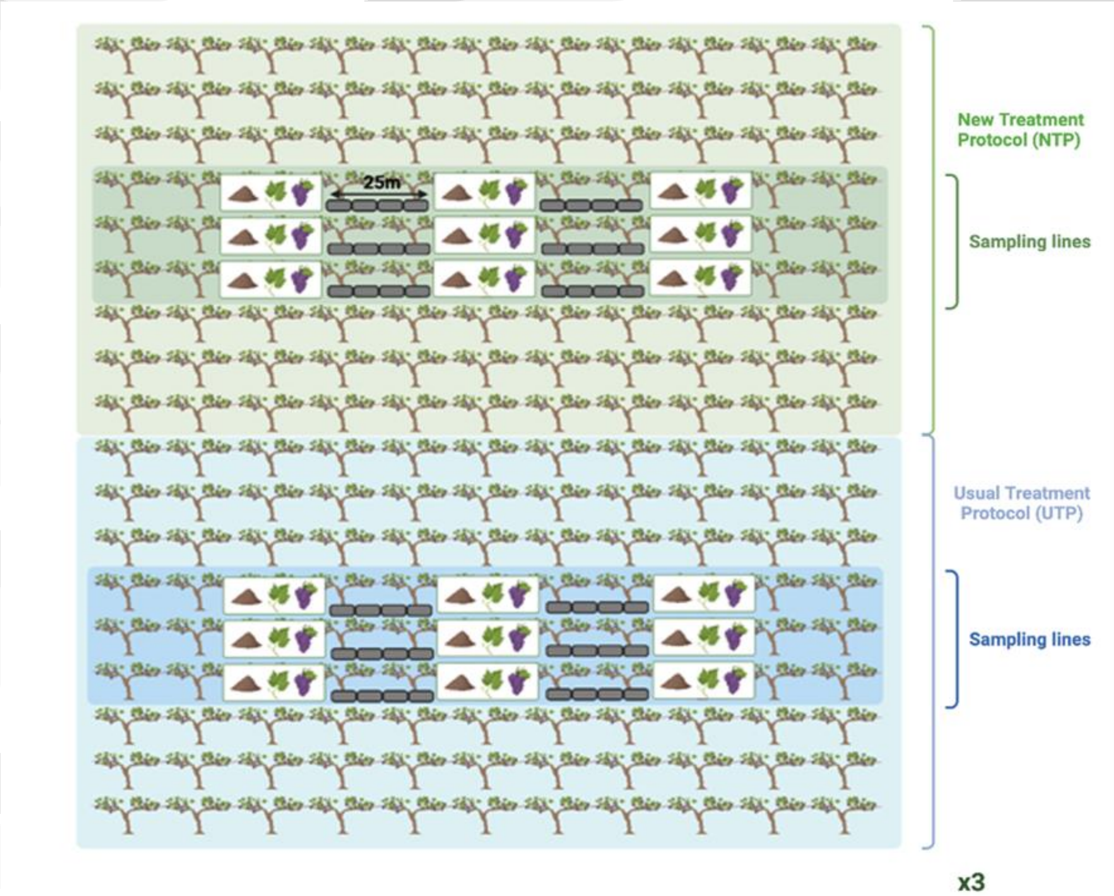



*Figure 5 - Representation of points where trays should be placed in each vine row. The same vine rows for sampling of soil, leaves and grapes.*

**Control area**: Three composite artificial soil samples should be obtained in the CTL area, for a total of 12 trays (both after 24h and at the end of the applications). This area represents possible atmospheric transport of copper during sprayings. Thus, a total of 21 composite artificial soil samples from trays are obtained per plot (9 NTP+9UTP+3CTL), both after 24h and at the end of applications.


*Figure 6 - Soil sampling design, consisting of one composite sample per vine row. Each composite sample consists of four trays per line, collected at two different points, after 24h of the first application and at the end of the applications.*

## 2. Data collection and organization

*Table 3 - Soil analyses*

| Parameter | France - Bordeaux | France - Narbonne | Portugal - Douro |
|---|---|---|---|
| pH($H_2O$)<br>pH (KCl)<br>% Organic matter or organic C<br>Copper (total)<br>Texture<br>Soil type | Same analysis all sites (if not available from the last 3 years, to be done before bud-break) | | |

**DISEASE EVALUATION**

| | France - Bordeaux | France - Narbonne | Portugal - Douro |
|---|---|---|---|
| **OBSERVATIONS** | **FOR DISEASE EVALUATION -** Same protocol all sites | | |

Using the experimental design (figure 1) and description in "1. Experimental design", on the 3 interior rows of each repetition, 3 plots of 20 vines should be selected (more, if not enough bunches).

The plots should be identified to be observed at 3 phenological stages: **50% flowering**, **starting bunch closure**, **50% veraison.**

If sanitary pressure rises quickly after budbreak, assessments must be anticipated to avoid losing information. A careful evaluation of the evolution of disease pressure should be made continuously. If there is an imminent risk of severe crop loss, observations should be made at a moment when there still is valid information, even if that means not respecting the above listed three observation moments. In this case, the phenological stage of the observation should be recorded using the **Phenological Scale Reference** included in the end of this document (Use BBCH code).

On each plot, the assessment should be done on **100 leaves and 100 bunches** - frequency and intensity of downy mildew (GRAPE ASSESS application[1] should be trialled and used for the duration, if it helps). Pictures (2-3 per observation moment) representative of the average sanitary state of grapevines should be made to have a visual timeline of the development of sanitary pressure.

<u>(3 repetitions x NTP x 3 plots) + (3 repetitions x UTP x 3 plots) + (1 CTL x 3 plots) x (100 Leaves + 100 Bunches)</u>

## <u>Monitoring</u>

The table below shows some examples of performance criteria (Agronomic, Environmental, Economic, Social) that should be collected along the experimental trial. These indicators required are related to the tested treatments.

[1] https://play.google.com/store/apps/details?id=com.lemursoftware.grapeassess&hl=en&gl=US

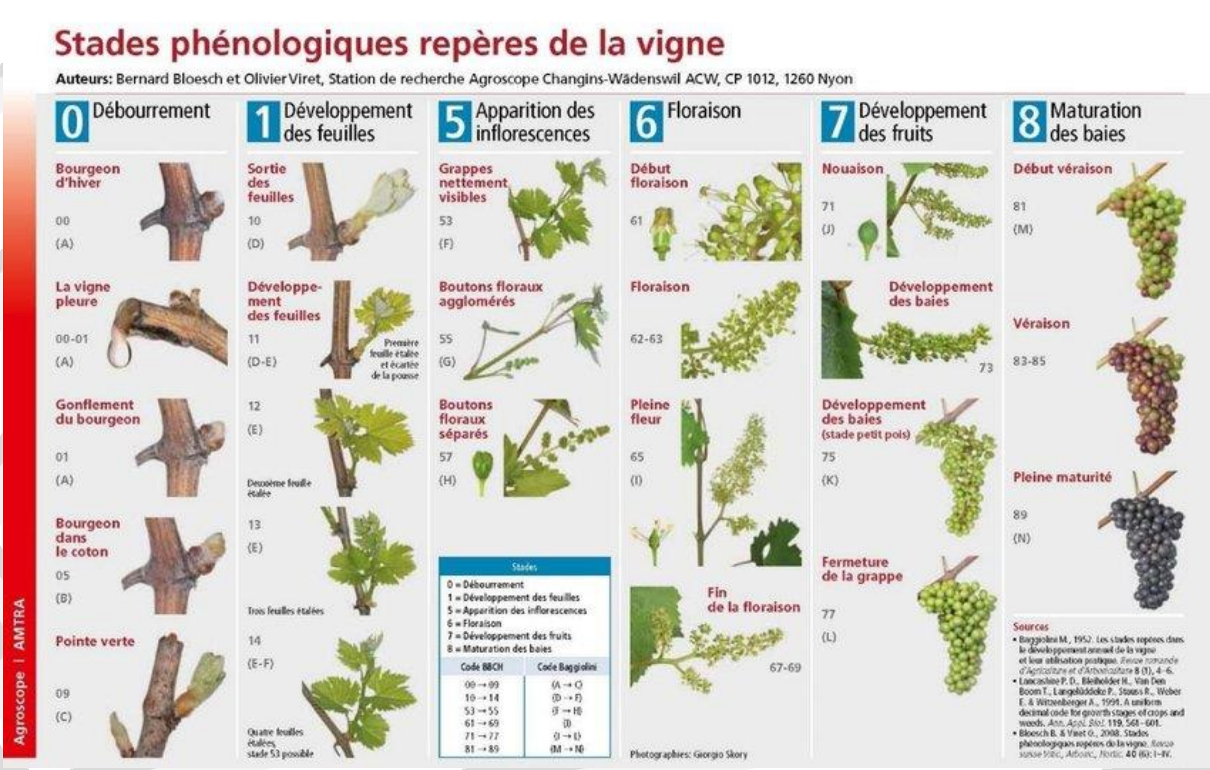
*Table 4 - Performance criteria*

| Agronomic | Environmental | Economic | Social |
|---|---|---|---|
| Yield (kg/ha)<br><br>Canopy (Leaf area) | Cover crop management operations (nr, seeds dose and cost, destination of cover crop residues)<br><br>Type of fuel (electricity; gasoline; gas)<br><br>Fuel related to copper treatments and total (quantity)<br><br>Water related to copper treatments and total (L)<br><br>Tractor use (hours of labor)<br><br>Green Interventions - Leaf trimming, Leaf thinning, Shoot thinning, Shoot positioning, bunch pruning (mechanical or not, nr) | Income (€/kg)<br><br>Tractor use related to copper treatments (€/h)<br><br>Investments related to copper treatments (€)<br><br>Fuel related to copper treatments and total (€)<br><br>Water related to copper treatments and total (€)<br><br>External service acquisition (€)<br><br>Human resources (€)<br><br>Product cost (fungicides, herbicides, fertilizers?) (€) | Human resources (h)<br><br>Human resources (nr)<br><br>Type - permanent, temporary (nr)<br><br>Gender (nr)<br><br>Schooling (level) |

## Calendar planning

| BBCH phenological scale | | | COPPEREPLACE PROTOCOL |
|---|---|---|---|
| **0: Sprouting/Bud development** | 0 | Dormancy | |
| | 1 | Beginning of bud swelling: buds begin to expand inside the bud scales | Soil analysis (if necessary)<br>Spraying test with hydrosensitive paper |
| | 3 | End of bud swelling: buds swollen, but not green | |
| | 5 | "Wool stage": brown wool clearly visible | First soil sampling |
| | 7 | Beginning of bud burst: green shoot tips just visible | |
| | 9 | Bud burst: green shoot tips clearly visible | |
| | 11 | First leaf unfolded and spread away from shoot | Start of spraying period - France |
| **1: Leaf development** | 12 | 2nd leaves unfolded | |
| | 13 | 3rd leaves unfolded | |
| | 1 . | Stages continuous till ... | |
| | 19 | 9 or more leaves unfolded | Laying of soil trays - Portugal<br>(half to recover after first spraying) |
| | 53 | Inflorescences clearly visible | Start of spraying period - Portugal<br>First leaf sampling |
| **5: Inflorescence emerge** | 55 | Inflorescences swelling, flowers closely pressed together | |
| | 57 | Inflorescences fully developed; flowers separating | |
| | 60 | First flowerhoods detached from the receptacle | |
| **6: Flowering** | 61 | Beginning of flowering: 10% of flowerhoods fallen | |
| | 62 | 20% of flowerhoods fallen | |
| | 63 | Early flowering: 30% of flowerhoods fallen | |
| | 64 | 40% of flowerhoods fallen | |
| | 65 | Full flowering: 50% of flowerhoods fallen | First observation for disease evaluation |
| | 66 | 60% of flowerhoods fallen | |
| | 67 | 70% of flowerhoods fallen | |
| | 68 | 80% of flowerhoods fallen | |
| | 69 | End of flowering | |
| | 71 | Fruit set: young fruits begin to swell, remains of flowers lost | |
| **7: Development of fruits** | 73 | Berries groat-sized, bunches begin to hang | |
| | 75 | Berries pea-sized, bunches hang | |
| | 77 | Berries beginning to touch | Second observation for disease evaluation |
| | 79 | Majority of berries touching | |
| | 81 | Beginning of ripening: berries begin to develop variety-specific colour | |
| **8: Ripening of berries** | 83 | Berries developing colour | Third observation for disease evaluation |
| | 85 | Softening of berries | |
| | 89 | Berries ripe for harvest | Grape berry sampling<br>Second soil sampling<br>Second leaf sampling |
| | 91 | After harvest; end of wood maturation | Recovery of soil trays - Portugal<br>Submission of data for evaluation of performance criteria |
| **9: Senescence** | 92 | Beginning of leaf discolouration | |
| | 93 | Beginning of leaf-fall | |
| | 95 | 50% of leaves fallen | |
| | 97 | End of leaf-fall | |
| | 99 | Harvested product | |

## Phenological scale reference




## Glossary

**Composite sample**: Mix of samples from the three sampling points collected in each row.

**Sampling point**: Each one of the three sampling points in a vine row, 25 m apart from each other. In each one of these sampling points, it is expected to collect about 1 kg of soil, 10 leaves and 3 bunches of grapes.

**Subsample**: Each one of the three soil samples collected in each point, separated by 50 cm.

# Pilot trial execution Report (E4.3.1)

**<u>Result from activity 4.3. Execution report of integrated pilot trials</u>**
**<u>E4.3.1. Execution Report</u>**

GT leader : SOGRAPE VINHOS S.A.
Involved partners: ADVID, IFV, UPC, SVBNA, EURECAT, GREENUPORTO, SCEA (GERARD BERTAND)

## 1. Introduction

Action 4.3 was critical for the project as it tested the alternatives and methodologies studied in and chosen from GT1, GT2 and GT3 in real field conditions, under a strict field experimental design, observation and control protocol as defined in E4.2.1. The strategy of multiple field trials, repeated in three locations with very different soil and climate conditions, in the same year, was devised as a way to counter the limitation caused by the funding program of having just one year to perform field trials. The field trials aimed at assessing the ability of the new alternatives and methodologies in reducing the input of copper to soils and grapes, in parallel with its efficiency to combat downy mildew. However, out of the three locations, only one presented environmental disease pressure conditions compatible with the experimental objectives. In this way, results reported in this document do not possess the required statistical robustness we tried to achieve.

## 2. Methodology

Field trials methodology and samples (soil, grapes and leaves) collection and pre-treatment is described in deliverable **E4.2.1 - Protocol for GT4 field trials.**

### a. Timeline

| | March | April | May | June | July | August | September | October | November | December |
|---|---|---|---|---|---|---|---|---|---|---|
| *Phenological stage* | Budbreak | First leaves | Flowering | Fruit set | Veraison | Maturation | Harvest | | | |
| *Sampling* | Soil sampling kits sent for all locations. First soil samplings in Douro. | First soil samplings in Bordeaux and Narbonne. | Soil tray experiment setup in Douro. First leaf samplings in all locations | | All soil and leaf samples received by GREENUPORTO | | Second round of soil and leaf samples. Sampling of berries | | All samples received | |
| *Downy mildew pressure* | | | Bordeaux | Bordeaux | Bordeaux | | | | | |
| *Spraying tests* | | | | Bordeaux Narbonne Douro | Bordeaux | | | | | |
| *Protection treatments* | No treatments | Bordeaux: in UTP Narbonne: 1 in UTP and NTP | Bordeaux - 3 Narbonne - 1 Douro - 2 | Bordeaux - 5 Narbonne - 3 | Bordeaux - 1 (same in UTP and NTP because of hail) | | | | | |
| *Production observations* | | | | | | | At all sites | | | |
| *Sample analysis* | | | | | | | | | X | X |

## b. Trial sites characterization

### Site 1 - Château Rioublanc, Bordeaux, FRANCE, managed by SVBNA

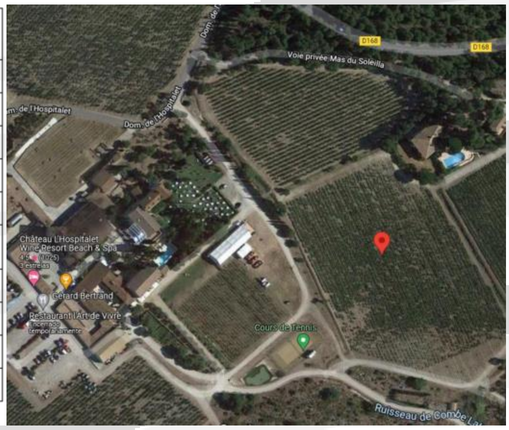
| | |
|---|---|
| **Mode of production** *(highlight one)* | Conventional (profit only) IPM Integrated Production **Bio** |
| **Grape variety** (*dominant if more than one*) | Merlot |
| **Rootstock** (*dominant if more than one*) | 101 14 |
| **Training system** | 2 *lattes « médocaine »* More than 3 sq. m of vegetation, high canopy (posts at 2,8 m) |
| **Vine spacing** *(row x inter-row in meters)* | 1 x 2 |
| **Year of plantation** | 2002 |
| IFT *(last 3 years)* | 4 |
| **Homogeneity** *(stdNDVI[1] ratio)* | Unknown |
| **Irrigation** *(highlight one)* | Yes **No** |
| **Soil type** (*dominant if more than one*) | Deep soil : Clay<br>Surface soil : "Boulbène" Clay Sand Silt |
| **Cover crops** | No Yes-temporary **Yes-permanent** |

[1] **stdNDVI ratio = stdNDVI block ÷ stdNDVI vineyard** *(std: standard deviation)*


### Site 2 - Château l'Hospitalet, Narbonne, FRANCE, managed by SCEA GB

| | |
|---|---|
| **Mode of production** *(highlight one)* | Conventional (profit only) IPM Integrated Production **Bio** |
| **Grape variety** (*dominant if more than one*) | GRENACHE N |
| **Rootstock** (*dominant if more than one*) | Unknow |
| **Training system** | Gobelet |
| **Vine spacing** *(row x inter-row in meters)* | 2,5 x 1,20 |
| **Year of plantation** | 1979 |
| IFT *(last 3 years)* | 15 |
| **Homogeneity** *(stdNDVI[1] ratio)* | Unknown |
| **Irrigation** *(highlight one)* | **Yes** No |
| **Soil type** (*dominant if more than one*) | Clay |
| **Cover crops** | No **Yes-temporary** Yes-permanent |

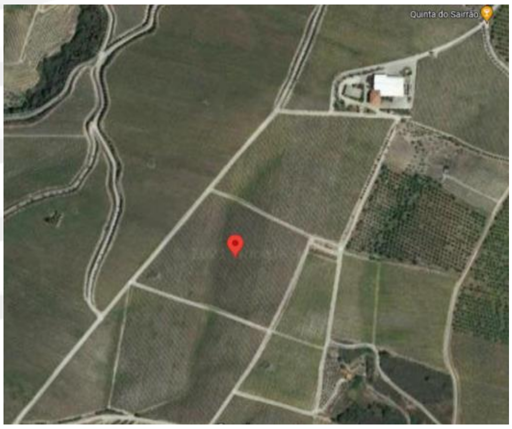
[1] **stdNDVI ratio = stdNDVI block ÷ stdNDVI vineyard** *(std: standard deviation)*




### Site 3 - Quinta do Sairrão, Douro, PORTUGAL, managed by SOGRAPE

| | |
|---|---|
| **Mode of production** *(highlight one)* | Conventional (profit only) IPM **Integrated Production** Bio |
| **Grape variety** (*dominant if more than one*) | Tinta Roriz |
| **Rootstock** (*dominant if more than one*) | R110 |
| **Training system** | Bilateral Royat |
| **Vine spacing** *(row x inter-row in meters)* | 2.2 x 1.1 |
| **Year of plantation** | 1990 |
| IFT *(last 3 years)* | 1.28 |
| **Homogeneity** *(stdNDVI[1] ratio)* | 1.08 |
| **Irrigation** *(highlight one)* | Yes **No** |
| **Soil type** (*dominant if more than one*) | Slate schist |
| **Cover crops** | No **Yes-temporary** Yes-permanent |

[1] **stdNDVI ratio = stdNDVI block ÷ stdNDVI vineyard** *(std: standard deviation)*



## c. Execution of trial modalities

At each site, three modalities were established according to the experimental design detailed in E4.2.1 (figures 1, 4 and 5), comprising the following protection modalities against downy mildew:

1. CTL - non-treated control: a small area without any treatment against downy mildew.
2. UTP - Usual treatment protocol: in this area the usual protection strategy against downy mildew at each site was applied.
3. NTP - New treatment protocol (table 1): in this area the low-copper protection strategy against downy mildew developed by COPPEREPLACE project was applied.

*Table 1 - Detailed NTP modality (according to E4.2.1)*

| PRODUCT | France – Bordeaux | France – Narbonne | Portugal – Douro |
|---|---|---|---|
| WHEN TO START | 50% at 3-4 leaves (E, BBCH 14) | At budbreak (E, BBCH 14) | At visible bunches (F, BBCH 53) |
| COPPER | 25% of the usual copper dose. For example, if the usual dose is 300 g copper per hectare, then 25% is 75 gCu/ha.<br><br>This needs to be weighed as a function of the vegetative growth at the moment of spraying. For example, if the growth is at 25% of full leaf area, the spraying should be 75 x 0.25 = 19 g/ha.<br><br>Recommended formulations are copper sulphate or hydroxide. | | |
| GLUCOSEI | 25% of the usual copper dose.<br><br>For example, if the usual dose is 300 g copper per hectare, then 25% is 75 gCu/ha. As Glucosei (8%) doses 80 gCu/L, this translates as 0.94 L of Glucosei per hectare.<br><br>This needs to be weighed as a function of the vegetative growth at the moment of spraying. For example, if the growth is at 25% of full leaf area, the spraying should be 0.94 x 0.25 = 0.23 L/ha of Glucosei. | | |
| ORANGE OIL | To be applied with each copper treatment, **except during blossom** (H - J, BBCH 57 – 71), as it is forbidden. Maximum 6 applications during growth cycle, always leave 7 days interval between applications. If you need a second spray before 7 days have passed do not use it. Maximum dose 1,6 L/ha. Use table below for dose calculation (*for more information see document Protocole_GP2021_Bio_Diff in the COPPEREPLACE Intranet, Technical Management – GT4*): | | |

In all sites and for each of the three modalities, protection strategies against pests and diseases other than downy mildew were maintained according to the usual practice at each site.

## Site 1 - Château Rioublanc, Bordeaux, FRANCE, managed by SVBNA

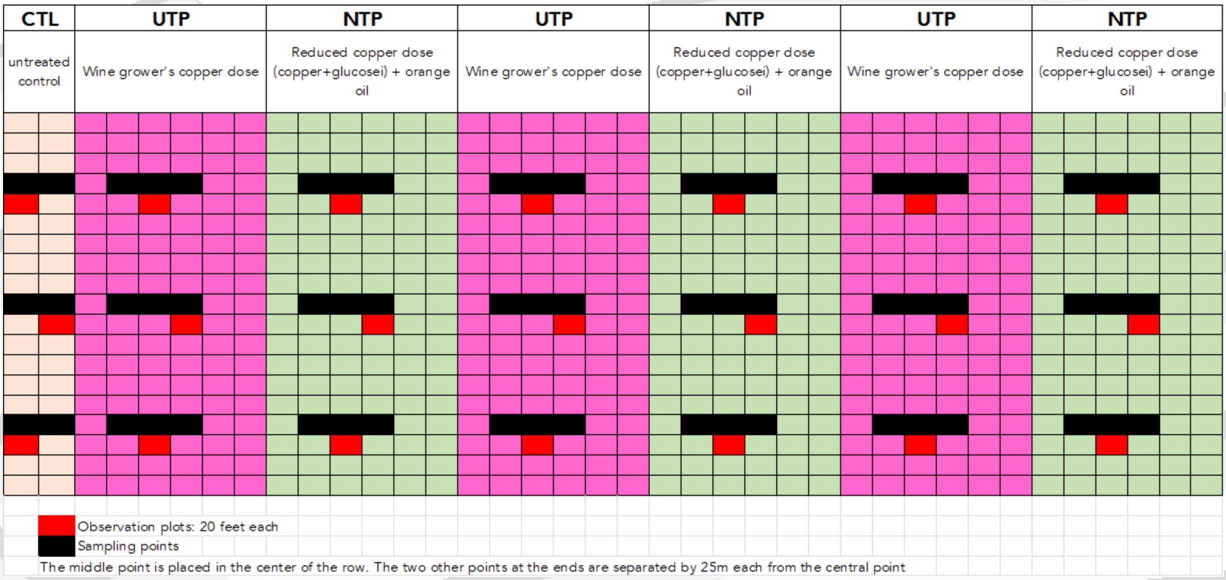



*Figure 1 - Experimental design at the Château Rioublanc*

Details of dates and doses of applied treatments at this site are detailed in table 2. A total of 10 and 9 treatments were made respectively for UTP and NTP modalities.

*Table 2 - Details of applied treatments at Château Rioublanc*

| | | | UTP | | NTP | | | |
|---|---|---|---|---|---|---|---|---|
| TREATMENT | DATE | PRODUCT | APPLIED RATE OF PRODUCT (mL or g/ha) | CORRESPONDING METAL COPPER DOSE (g/ha) | APPLIED RATE OF PRODUCT (mL or g/ha) | Applied dose of GLUCOSEI (Labicuper) (mL/ha) | Applied volume of SWEET ORANGE ESSENTIAL OIL (mL) | CORRESPONDING METAL COPPER DOSE (g/ha) |
| 1 | 22/04/2022 | BOUILLIE BORDELAISE RSR DISPERSS NC (20% cuivre) | 900 | 180 | 0 | 0 | 0 | 0 |
| 2 | 05/05/2022 | BOUILLIE BORDELAISE RSR DISPERSS NC (20% cuivre) | 750 | 150 | 188 | 470 | 112,8 | 75,2 |
| 3 | 17/05/2022 | BOUILLIE BORDELAISE RSR DISPERSS NC (20% cuivre) | 1500 | 300 | 560 | 1400 | 900 | 224 |
| 4 | 31/05/2022 | BOUILLIE BORDELAISE RSR DISPERSS (20% cuivre) | 1500 | 300 | 375 | 937,5 | FLORAISON | 150 |
| 5 | 06/06/2022 | KOCIDE 35 DF (35% hydroxide de cuivre) | 1400 | 490 | 350 | 1540 | 1600 | 245,5 |
| 6 | 16/06/2022 | KOCIDE 35 DF (35% hydroxide de cuivre) | 1250 | 437,5 | 315 | 1375 | 1600 | 220,25 |
| 7 | 21/06/2022 | KOCIDE 35 DF (35% hydroxide de cuivre) | 1100 | 385 | 275 | 1250 | 1600 | 196,25 |
| 8 | 23/06/2022 | COPREN HI BIO WG (200g/kg) | 2200 | 440 | 550 | 1375 | 1600 | 220 |
| 9 | 29/06/2022 | BOUILLIE BORDELAISE RSR DISPERSS NC (20% cuivre) | 2250 | 450 | 560 | 1375 | 1600 | 222 |
| 10 | 19/07/2022 | BOUILLIE BORDELAISE RSR DISPERSS NC (20% cuivre) | 1800 | 360 | 1800 | 0 | 0 | 360 |
| | | TOTAL COPPER METAL (kg/ha) | | 3,49 | | | | 1,91 |

Sweet Orange essential oil was used in all NTP treatments except at flowering. In the last NTP treatment (19/07/22) it was used the same products and doses as UTP to attempt to salvage remaining bunches after hail event. The differential application of copper metal in the two modalities meant a significant 45% reduction in the total cumulative copper metal application per hectare (3.5 kg/ha for UTP and 1.9 kg/ha for NTP) (figure 2) at the end of the season.

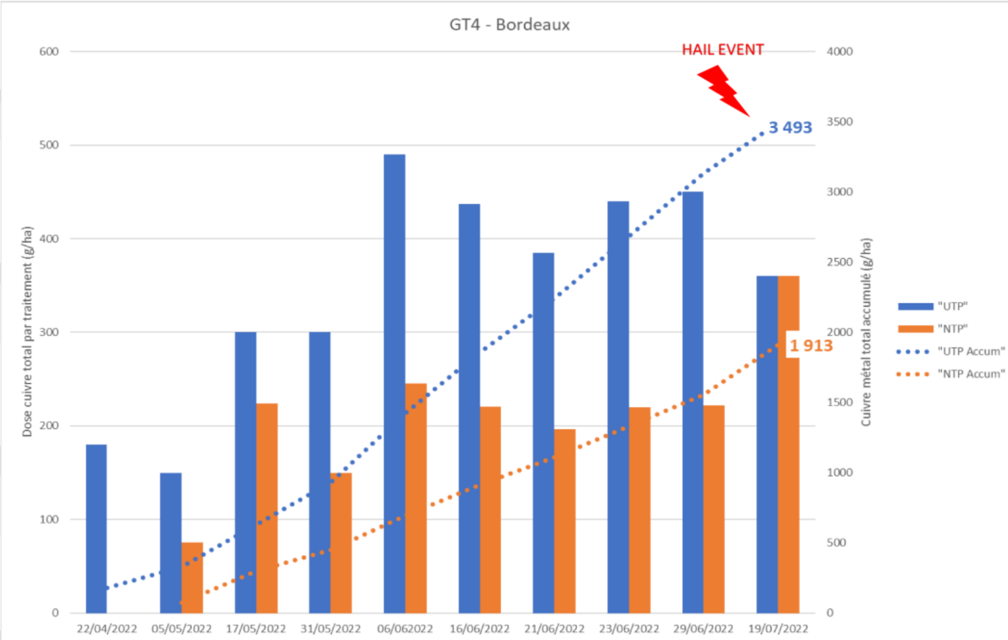



*Figure 2 - Cumulative application of copper metal in Château Rioublanc (UTP - usual treatment protocol; NTP - new treatment protocol)*

Treatments were made using protective product recovery panels (figure 3) to avoid spray drift. Because of a severe hail event in late June, at the last reported treatment date (July 19th), the NTP modality was treated with the same products and doses as UTP in an attempt to save production from a rampant downy mildew infection that followed.

*Figure 3 - Sprayer with product recovery panels*

## Site 2 - Château l’Hospitalet, Narbonne, FRANCE, managed by SCEA GB

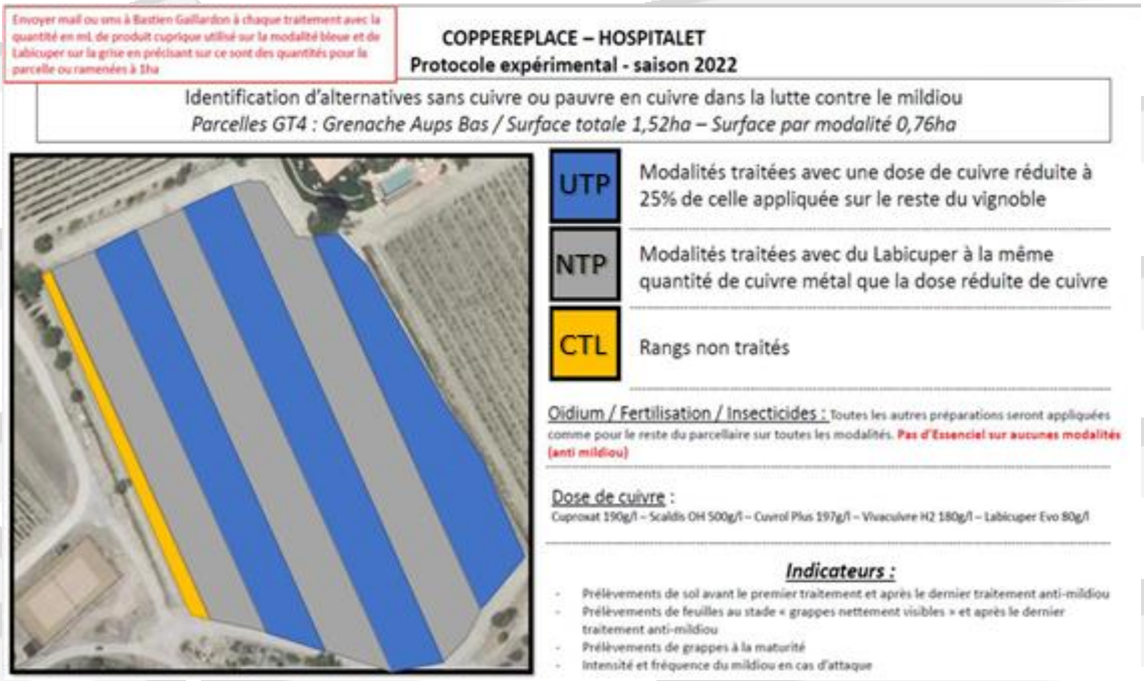



*Figure 4 - Experimental design at Château l’Hospitalet*

A total of 5 preventive treatments were made, the protection period effectively ending by the end of June for absence of downy mildew pressure (table 3).

*Table 3 - Details of applied treatments at Château l’Hospitalet*

| | UTP | NTP | | |
|---|---|---|---|---|
| DATES | MODALITE BLEU | MODALITE GRISE | NOMBRE BROUETTE | |
| | | | | |
| 29/04/2022 | 0,25 L/ha cuproxat soit 0,19L | 0,59L/ha labicuper soit 0,45L | 80L par modalité | |
| | 1,52l de flosul + 1,52 kg de citrothiol | | | |
| 18/05/2022 | 0,125L/Ha cuproxat 0,096 l | 0,29 L/Ha labicuper 0,22 l | 80L par modalité | |
| | 2,2 l flosul + 2,2 kg citrothiol / modilate | | | |
| 08/06/2022 | 0,125L/Ha cuproxat 0,096 l | 0,29 L/Ha labicuper 0,22 l | 80L par modalité | |
| | 2,2 l flosul + 2,2 kg citrothiol / modilate | | | |
| 17/06/2022 | 0,125L/Ha cuproxat 0,096 l | 0,29 L/Ha labicuper 0,22 l | 80L par modalité | |
| | 2,2 l flosul + 2,2 kg citrothiol / modilate | | | |
| 29/06/2022 | 0,075l/ha cuproxat 0,05l | 0,17l/ha labicuper 0,12l | 80L par modalité | |
| | 2,2 l flosul + 2,2 kg citrothiol / modilate | | | |

Because of a lapse in protocol interpretation, a similar amount of copper metal was applied in both modalities: 25% of the usual dose (table 4). According to the protocol (see E4.2.1), only NTP should have received this lower dose. UTP should have received the full dose usually applied at the site.

*Table 4 - Copper metal quantities for both modalities in the trial*

| | UTP | UTP | UTP | NTP | NTP | NTP |
|---|---|---|---|---|---|---|
| Date | Product | Dose / ha | Dose Copper Metal g / ha | Product | Dose / ha | Dose Copper Metal / ha |
| 29/04/2022 | CUPROXAT | 0,250 | 47,50 | LABICUPER | 0,590 | 47,20 |
| 18/05/2022 | CUPROXAT | 0,125 | 23,75 | LABICUPER | 0,290 | 23,20 |
| 08/06/2022 | CUPROXAT | 0,125 | 23,75 | LABICUPER | 0,290 | 23,20 |
| 17/06/2022 | CUPROXAT | 0,125 | 23,75 | LABICUPER | 0,290 | 23,20 |
| 29/06/2022 | CUPROXAT | 0,075 | 14,25 | LABICUPER | 0,170 | 13,60 |
| **TOTAL** | | | **133,00** | | | **130,40** |

## Site 3 - Quinta do Sairrão, Douro, PORTUGAL, managed by SOGRAPE


*Figure 5 - Experimental design at Quinta do Sairrão*

A total of 2 preventive treatments were made (table 5), the protection period effectively ending by the end of May due to absence of downy mildew pressure.

*Table 5 - Details of applied treatments at the Quinta do Sairrão*

| Date | Phenological stage | Decision trigger | % of full vegetation | Type of treatment | Used product(s) | Dose(s) of active substance(s) ( per ha) | Field application rate (L/ha) | Comments / observations |
|---|---|---|---|---|---|---|---|---|
| 10/05/2022 | G - BBCH 55 | IBERMETEO/Boletim ADVID | 25 | NTP (New treatment protocol) | Vitra 40 Micro + GLUCOSEI +Prev-Am Plus | 187,5g + 940 mL + 600 mL | 100 | Sprayer Rocha CRONOS 400 Lts - Nozzles- 1 x 1 (Yellow) |
| 10/05/2022 | G - BBCH 55 | IBERMETEO/Boletim ADVID | 25 | Integrated Production | Profiler + Thiovit Jet | 250g + 500g | 100 | Sprayer Rocha CRONOS 400 Lts - Nozzles- 1 x 1 (Yellow) |
| 30/05/2022 | I - BBCH 61-62-63 | IBERMETEO/Boletim ADVID | 75 | NTP (New treatment protocol) | Vitra 40 Micro + GLUCOSEI | 562g + 2800ml | 300 | Sprayer Rocha CRONOS 400 Lts - Nozzles- 2 x 2 (Yellow) |
| 30/05/2022 | I - BBCH 61-62-63 | IBERMETEO/Boletim ADVID | 75 | Integrated Production | Douro | 90 mL | 300 | Sprayer Rocha CRONOS 400 Lts - Nozzles- 2 x 2 (Yellow) |

In UTP, unlike the usual practice at the site, because of very low disease pressure, no copper was used, the preventive treatments used active substances authorised in certified Integrated Production (PRODI) grape production: fluopicolide + fosetyl aluminium (Profiler) against downy mildew. A usual practice in PRODI, these treatments were applied simultaneously with powdery mildew control using sulphur (Thiovit Jet) and penconazole (Douro).

In NTP, the total copper metal dose applied was 0.599 kg/ha: 0.150 kg/ha in the first spraying and 0.449 kg/ha in the second.

### d. **Sampling and observations**

## Sprayer calibration

At all three trial sites, spraying machines (figure 6) were calibrated by running dummy spray tests with water and hydrosensitive paper placed in the vineyard trellises.

*Figure 6 - Spraying equipment used at Quinta do Sairrão*

At Château Rioublanc, two sprayers were tested (figure 7). The TEC sprayer had an open, side-by-side configuration, brown nozzles on the bottom and yellow nozzles on the top, using 5-bar spraying pressure. Fans were replaced after the first trial with larger ones, of which one with 8 blades. The DUGHES sprayer had a confined configuration and used 3-bar spraying pressure (ideal for nozzles). It was used until June.

*Figure 7 - Spraying equipments at Château Rioublanc (left: TEC, right: DUGHES)*

## Soil sampling and analysis

At all three sites, soils were sampled before treatments started and again after the last treatment of the campaign was executed (figure 8), according to protocol (see E4.2.1 for details). All soil samples were well mixed, air dried and sieved before being sent to GREENUPORTO.

Soil characterisation was performed by the determination of physical and chemical parameters: pH and electrical conductivity (EC), performed by GREENUPORTO; total organic carbon (TOC) and texture, performed by University of Vigo (UVigo). The pH of soil samples was determined in both soil:water and soil:KCl (1M) suspensions (1:5 v/v), as described in ISO/FDIS 10390:2020. Electric conductivity (EC) was measured in the supernatant of the soil:water suspension used for pH. TOC was measured by elemental analysis and texture was determined by the pipette method after wet sieving.

For the analysis of total content of Cu, soil samples were air-dried, sieved at 2mm, milled and then digested with *Aqua Regia*. In addition, an extraction with calcium chloride (0.01M) was performed, in order to get an estimation of the available Cu content. The total and available Cu content (dry weight basis) were both determined by Atomic Absorption Spectroscopy (AAS).

Results have been subjected to statistical analysis, and for each sampling period and for each trial site, an one-way ANOVA and a multiple comparisons test (Tukey's test) was then performed, allowing for the comparison between control and treated areas and between the two different treatment protocols.

*Figure 8 - Bag with soil sample (left) and sampling soil at Château l'Hospitalet (right)*

According to protocol (E4.2.1), at Quinta do Sairrão, a tray experiment, for assessing the input of copper to soil by each phytosanitary treatment individually and by all the treatment campaign, was set up between GREENUPORTO and SOGRAPE. An artificial soil was prepared and placed inside trays under vine rows for its later analysis for total and available Cu contents, as the result of Cu contamination from trial treatments (figure 9). The artificial soil was made by combining 10% of sphagnum peat, 20% kaolin clay, 70% dried sand and calcium carbonate to obtain a pH of 6.0 ± 0.5, according to OECD 222. A total of 72 trays per each treatment were placed before the first phytosanitary treatment, 36 being removed after the first one (11/05/2022) and the remaining trays collected after the last phytosanitary treatment (31/08/2022). For more details please see the protocol (E4.2.1). Artificial soil placed on the trays was air-dried, sieved at 2mm, milled and then digested with *Aqua Regia* or extracted with calcium chloride, as described previously for soil samples. The Cu content (dry weight basis) was determined by Atomic Absorption Spectroscopy (AAS). Results were subjected to statistical analysis, and for each sampling period an one-way ANOVA and a multiple comparisons test (Tukey's test) was then performed, allowing for the comparison between control and treated areas and between the two different treatment protocols.

*Figure 9 - Setting up the soil tray experiment at Quinta do Sairrão*

## Leaf sampling

At all three trial sites, the first sampling campaign of leaves occurred when the first bunches were visible (figure 10), and again at the end of the treatment campaign. Leaves were dried for 4 days at 60°C, wrapped in aluminium foil, placed in plastic bags and sent to GREENUPORTO where samples were prepared to send for analyses.

*Figure 10 - Sampling leaves at Château Rioublanc (left and center) and conservation bag for sampled leaves (right)*

Dried leaves were milled and then digested with $HNO_3$ and $H_2O_2$ in a heating block. The Cu content (dry weight basis) was determined by inductively coupled plasma mass spectrometry (ICP-MS). Results were subjected to statistical analysis and, for each sampling period and for each trial site, an one-way ANOVA and a multiple comparisons test (Tukey's test) was then performed, allowing for the comparison between control and treated areas and between the two different treatment protocols.

## Grape berry sampling

At all three trial sites, immediately before harvest, grape berries were sampled and homogenised with a blender (figure 11). A one-litre aliquot of each sample was placed in a plastic bottle, frozen and sent to GREENUPORTO. Homogenised grapes were then prepared in aliquots and freeze-dried, for its ensuing digestion with $HNO_3$ and $H_2O_2$ in a heating block. The Cu content (based on grape dry weight) was determined by Inductively coupled plasma mass spectrometry (ICP-MS).

*Figure 11 - Preparation of berry samples: separation of berries from stems (left), homogeneization in a blender (center), samples ready for freezing (right)*

### e. Deviations to protocol

At Château Rioublanc, a severe hail event in late June forced the usage of the same treatment (UTP products and dosages) on both UTP and NTP modalities for the last (July) spraying, ending the differential treatments trial at the end of June. This was required due to the need to reduce the damage inflicted upon the plants and to help them recover for the next season.

At Château l'Hospitalet, the UTP modality used 25% of the usual copper dosage. In the NTP modality, the equivalent dosage of copper metal was applied using only gluconate (Labicuper), the active substance of Glucosei. This deviation, however, had no impact on downy mildew control as no disease pressure was observed at this trial site.

## 3. Results

### a. Sprayer calibration with hydrosensitive paper

#### Site 1 - Château Rioublanc, Bordeaux, FRANCE, managed by SVBNA

Two tests were made. The first, on June 10th (table 6), had papers placed on the upper face of leaves. The second one, on July 12th, had papers folded and stapled to leaves to observe application for both upper and lower faces.

*Table 6 - Data from sprayer test on June 10th at Château Rioublanc (leaf upper face)*

| Sprayer | Plant | Paper n. | Position | Depth | >70 drops/cm$^2$ | <70 drops/cm$^2$ | Heterogeneous |
|---|---|---|---|---|---|---|---|
| DUGHES | 1 | A11 | Top left | Shallow | X | | |
| | | A12 | Top center | Deep | X | | |
| | | A13 | Top right | Very deep | | | X (> 70 drops/cm2) |
| | | A14 | Bottom left | Shallow | | X | |
| | | A15 | Bottom center | Deep | | X | |
| | | A16 | Bottom right | Very deep | | X | |
| | 2 | A21 | Top left | Shallow | X | | |
| | | A22 | Top center | Deep | | X | |
| | | A23 | Top right | Very deep | | | X ( > 70 drops/cm2) |
| | | A24 | Bottom left | Shallow | X | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | A25 | Bottom center | Deep | | | X ( > 70 drops/cm2) |
| | | A26 | Bottom right | Very deep | | X | |
| | 3 | A31 | Top left | Shallow | | X | |
| | | A32 | Top center | Deep | | X | |
| | | A33 | Top right | Very deep | X | | |
| | | A34 | Bottom left | Shallow | | X | |
| | | A35 | Bottom center | Deep | | X | |
| | | A36 | Bottom right | Very deep | X | | |
| TEC | 1 | B11 | Top left | Shallow | X | | |
| | | B12 | Top center | Deep | | X | |
| | | B13 | Top right | Very deep | | X | |
| | | B14 | Bottom left | Shallow | | X | |
| | | B15 | Bottom center | Deep | | | X ( > 70 drops/cm2) |
| | | B16 | Bottom right | Very deep | | X | |
| | 2 | B21 | Top left | Shallow | X | | |
| | | B22 | Top center | Deep | X | | |
| | | B23 | Top right | Very deep | | X | |
| | | B24 | Bottom left | Shallow | | X | |
| | | B25 | Bottom center | Deep | | X | |
| | | B26 | Bottom right | Very deep | | | X ( > 70 drops/cm2) |
| | 3 | B31 | Top left | Shallow | | X | |
| | | B32 | Top center | Deep | X | | |
| | | B33 | Top right | Very deep | | X | |
| | | B34 | Bottom left | Shallow | | X | |
| | | B35 | Bottom center | Deep | | X | |
| | | B36 | Bottom right | Very deep | | X | |

Examples of droplet distribution are shown in figure 12.

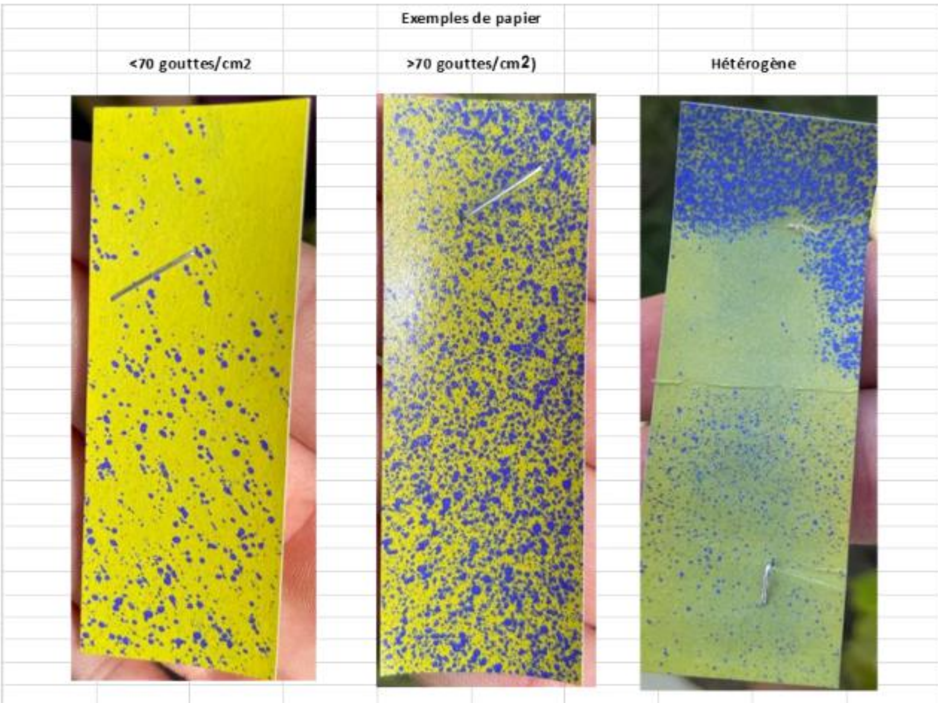



*Figure 12 - Hydrosensitive paper results of sprayer trial at Château Rioublanc on June 10th.*

Results of the first test with the two available sprayers led to a change from a confined configuration sprayer using 3-bar optimal pressure to a face-to-face configuration using 5-bar optimal pressure. This change improved the spraying performance (tables 7 and 8).

*Table 7 - Data from sprayer test on July 12th at Château Rioublanc (leaf upper face)*

| Sprayer | Plant | Paper n. | Position | Depth | >70 drops/cm² | <70 drops/cm² |
|---|---|---|---|---|---|---|
| TEC | 1 | A11 | Top left | Shallow | | X |
| | | A12 | Top center | Deep | | X |
| | | A13 | Top right | Very deep | X | |
| | | A14 | Bottom left | Shallow | X | |
| | | A15 | Bottom center | Deep | X | |
| | | A16 | Bottom right | Very deep | X | |
| | 2 | A21 | Top left | Shallow | X | |
| | | A22 | Top center | Deep | | X |
| | | A23 | Top right | Very deep | | X |
| | | A24 | Bottom left | Shallow | | X |
| | | A25 | Bottom center | Deep | | X |
| | | A26 | Bottom right | Very deep | | X |
| | 3 | A31 | Top left | Shallow | X | |
| | | A32 | Top center | Deep | | X |
| | | A33 | Top right | Very deep | X | |
| | | A34 | Bottom left | Shallow | X | |
| | | A35 | Bottom center | Deep | | X |
| | | A36 | Bottom right | Very deep | | X |

*Table 8 - Data from sprayer test on July 12th at Château Rioublanc (leaf lower face)*

| Sprayer | Plant | Paper n. | Position | Depth | >70 drops/cm2) | <70 drops/cm2 |
|---|---|---|---|---|---|---|
| TEC | 1 | A11 | Top left | Shallow | | X |
| | | A12 | Top center | Deep | | X |
| | | A13 | Top right | Very deep | | X |
| | | A14 | Bottom left | Shallow | X | |
| | | A15 | Bottom center | Deep | | X |
| | | A16 | Bottom right | Very deep | | X |
| | 2 | A21 | Top left | Shallow | | X |
| | | A22 | Top center | Deep | | X |
| | | A23 | Top right | Very deep | X | |
| | | A24 | Bottom left | Shallow | | X |
| | | A25 | Bottom center | Deep | | X |
| | | A26 | Bottom right | Very deep | | X |
| | 3 | A31 | Top left | Shallow | | X |
| | | A32 | Top center | Deep | | X |
| | | A33 | Top right | Very deep | | X |
| | | A34 | Bottom left | Shallow | | X |
| | | A35 | Bottom center | Deep | | X |
| | | A36 | Bottom right | Very deep | | X |

Figure 13 shows examples of droplet distribution obtained at this second test. The test revealed a much better distribution of the spraying with a higher number of samples achieving higher droplet per square centimetre count at both faces, all depths and positions.

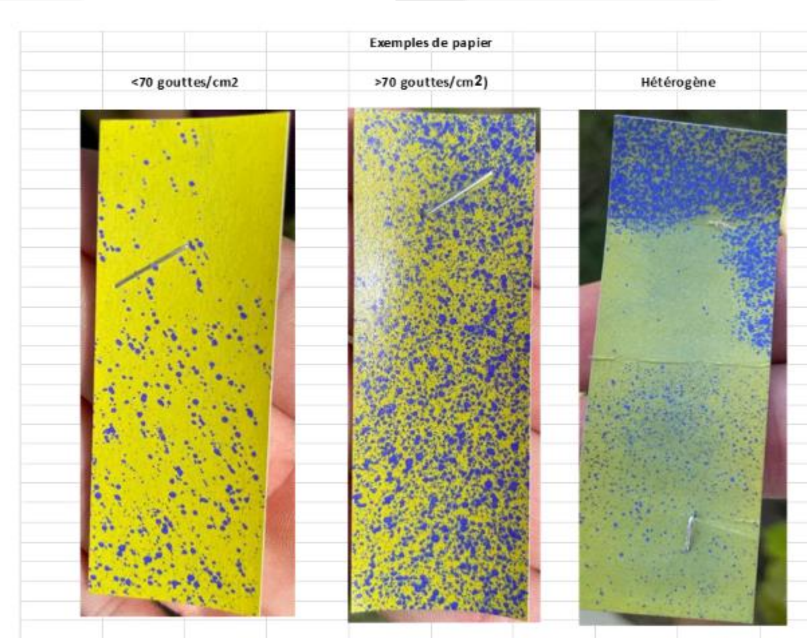



*Figure 13 - Hydrosensitive paper results of sprayer trial at Bordeaux site on July 12th.*

## Site 2 - Château l’Hospitalet, Narbonne, FRANCE, managed by SCEA GB

The analysis of the hydrosensitive papers at Château l’Hospitalet (figure 14) revealed a high level of heterogeneity in the spraying at this trial site.

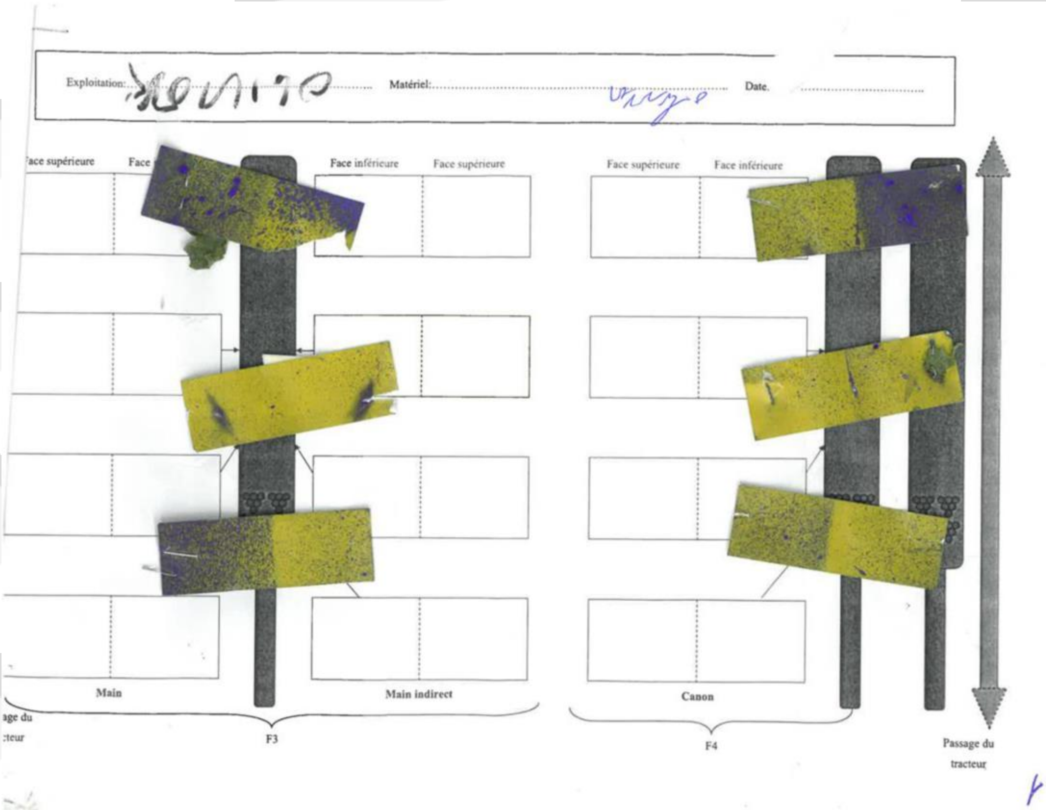



*Figure 14 - Hydrosensitive paper examples at Château l’Hospitalet*

## Site 3 - Quinta do Sairrão, Douro, PORTUGAL, managed by SOGRAPE

The analysis of the hydrosensitive papers (table 9) and data from the DropLeaf application (figure 15) revealed adequate drop density but some level of heterogeneity, mainly in the lower face of leaves.

*Table 9 - Data from sprayer test at Quinta do Sairrão*

| Vine nr | Position | Depth | > 70 drops/cm2 | < 70 drops/cm2 | Heterogenity |
|---|---|---|---|---|---|
| 1 | Lower Face | Exterior | | X | Lower area |
| | Upper Face | Interior | | X | |
| | Bunch | interior | | X | |
| 2 | Lower Face | Exterior | | X | |
| | Upper Face | Interior | | X | Lower area |
| | Bunch | interior | | X | |
| 3 | Lower Face | Exterior | | | Impossible to assess |
| | Upper Face | Interior | | | Impossible to assess |
| | Bunch | interior | | | Impossible to assess |

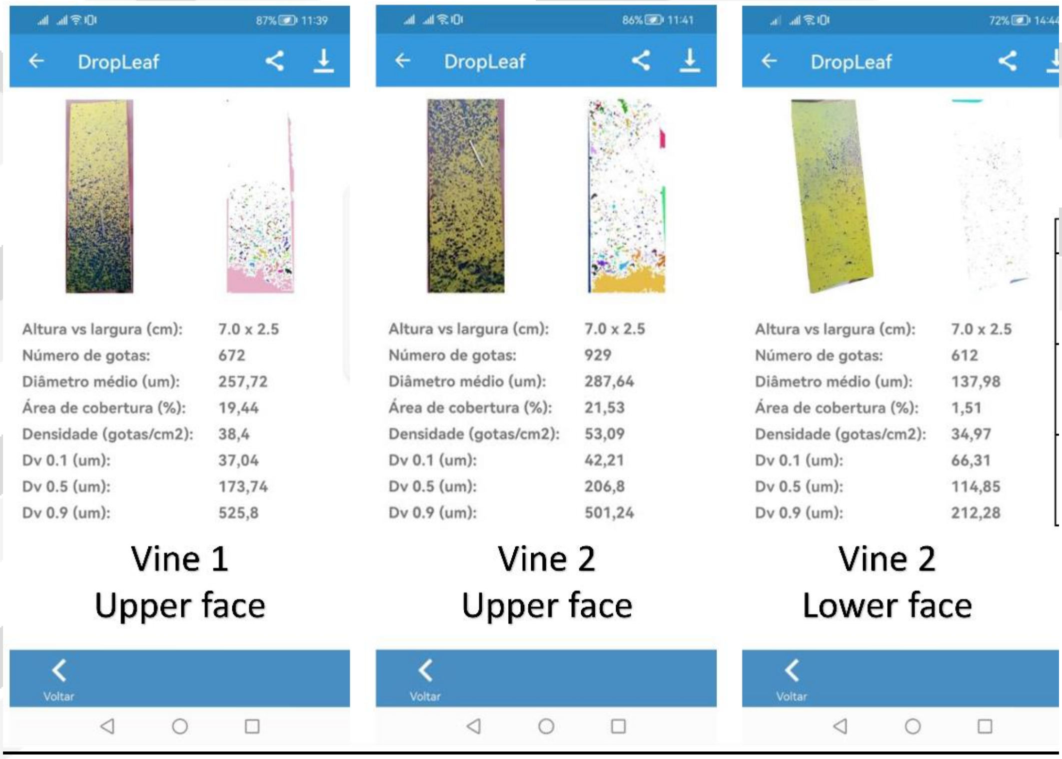



*Figure 15 - Hydrosensitive paper examples and results at Douro trial site*

## b. Copper levels in soils, leaves and grape berries

### i. Soil characterization

Regarding general soil physico-chemical parameters, of the three trial sites, Château l'Hospitalet had the highest soil pH and electrical conductivity, followed by Château Rioublanc, whereas Quinta do Sairrão showed the lowest pH and EC values. TOC content was higher at Quinta do Sairrão, and similar in the other two experimental sites (table 10).

*Table 10. Results of pH, electrical conductivity, total organic carbon, presented as mean values ± standard deviation for soil samples collected at the three trial sites. Texture classes are also presented.*

| Trial sites | pH | | Electrical conductivity ($\mu S\ cm^{-1}$) | Total organic carbon (%) | Texture |
|---|---|---|---|---|---|
| | $H_2O$ | KCl | | | |
| **Château Rioublanc** | 7.69 ± 0.16 | 6.59 ± 0.28 | 124.7 ± 26.0 | 1.3±0.2 | Sandy Loam |
| **Château l'Hospitalet** | 9.00 ± 0.11 | 8.20 ± 0.05 | 228.4 ± 34.2 | 1.2±0.2 | Loam |
| **Quinta do Sairrão** | 6.97 ± 0.20 | 6.05 ± 0.21 | 123.6 ± 32.1 | 2.1±0.5 | Loam |

### ii. Levels of Cu in soils

Results for total and available content of Cu in soil samples, collected at the three trial sites, are presented in table 11. ANOVA results for the total Cu content are plotted in figure 16.

*Table 11. Total and available Cu in soil samples, presented as mean values ± standard deviation, for the two sampling periods, and the three trial sites.*

| Trial sites | Sampling period | Trial modality | Total Cu (mg kg$^{-1}$) | Available Cu (mg kg$^{-1}$) |
|---|---|---|---|---|
| Château Rioublanc | Before 1st spraying | CTL | 79.5 ± 5.8 | 4.47 ± 0.02 |
| | | UTP | 96.0 ± 9.1 | 3.91 ± 0.58 |
| | | NTP | 126 ± 67 | 4.58 ± 0.80 |
| | End of treatments | CTL | 136 ± 10 | 3.88 ± 0.11 |
| | | UTP | 104 ± 10 | 3.29 ± 0.34 |
| | | NTP | 113 ± 11 | 3.69 ± 0.39 |
| Château l'Hospitalet | Before 1st spraying | CTL | 103 ± 28 | 2.69 ± 0.15 |
| | | UTP | 107 ± 14 | 2.78 ± 0.43 |
| | | NTP | 107 ± 16 | 2.53 ± 0.16 |
| | End of treatments | CTL | 91.6 ± 15.2 | 3.49 ± 0.16 |
| | | UTP | 96.2 ± 13 | 3.71 ± 0.29 |
| | | NTP | 99.7 ± 7.7 | 3.76 ± 0.43 |
| Quinta do Sairrão | Before 1st spraying | CTL 1 | 63.6 ± 3.7 | 3.78 ± 0.25 |
| | | CTL 2 | 79.0 ± 6.1 | 3.70 ± 0.88 |
| | | UTP | 68.6 ± 20.7 | 3.06 ± 0.42 |
| | | NTP | 57.0 ± 8.5 | 2.99 ± 0.32 |
| | End of treatments | CTL 1 | 60.0 ± 2.8 | 2.55 ± 0.65 |
| | | CTL 2 | 69.6 ± 11.0 | 1.92 ± 0.20 |
| | | UTP | 72.0 ± 22.0 | 4.18 ± 0.52 |
| | | NTP | 60.3 ± 22.3 | 3.18 ± 1.41 |

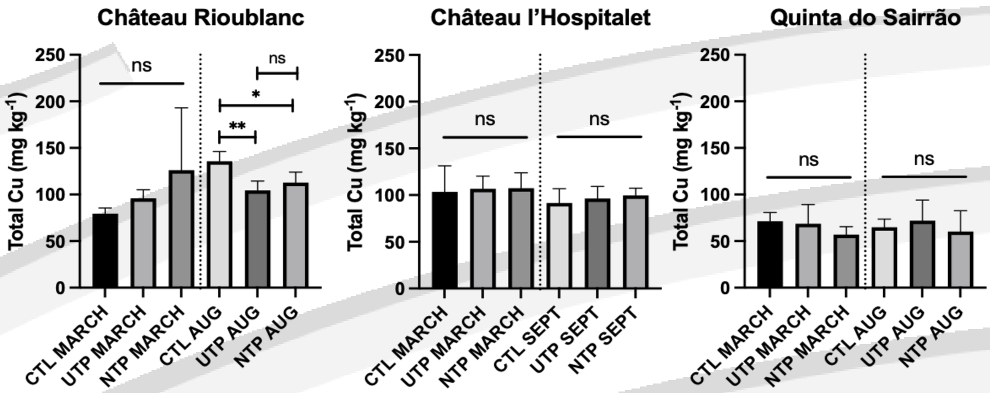



*Figure 16. Total Cu (mg/kg) for soil samples (dry mass), for the three trial sites and both sampling periods. ns stands for non-significant differences, whilst *represents significant differences between results when p<0.05 and ** when p<0.01. CTL - not-treated, UTP - usual protocol, NTP - new protocol.*

Mean values of total Cu in soils for each set of three sampled rows are presented in figure 17 for Château Rioublanc, figure18 for Château l'Hospitalet and in figure 19 for Quinta do Sairrão.

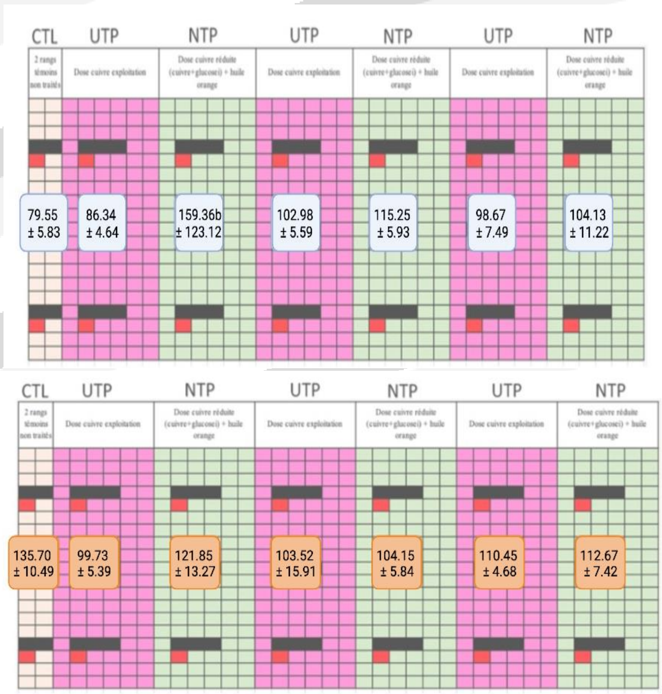



*Figure 17. Total Cu values (mg/kg) in soils presented as mean values and standard deviation, for each set of three sampled rows, for Château Rioublanc. Left values (blue squares) refer to the first sampling period and right values refer to the second sampling (orange squares).*

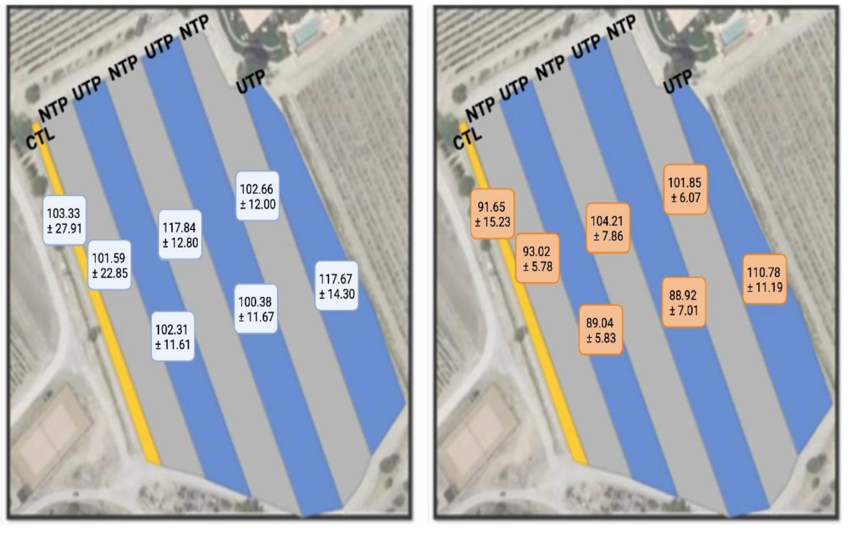



*Figure 18. Total Cu values (mg/kg) in soils presented as mean values and standard deviation, for each set of three sampled rows, for Château l'Hospitalet. Left-side values (blue squares) refer to the first sampling period and right-side values refer to the second sampling (orange squares).*

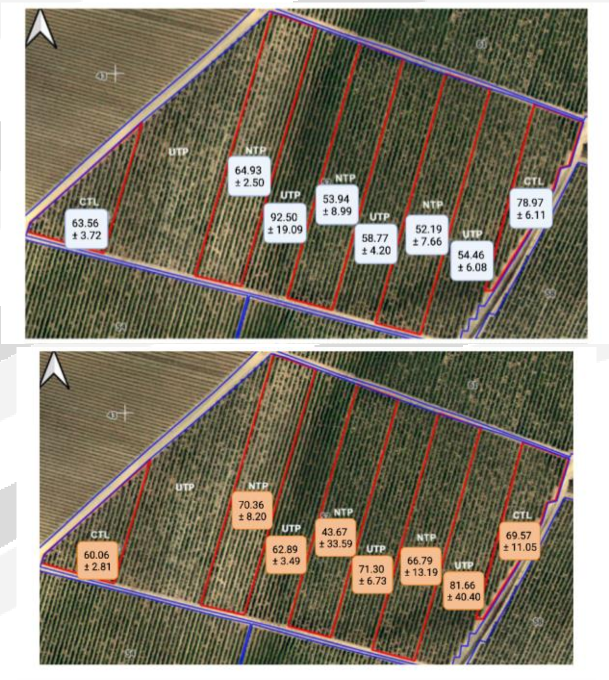



*Figure 19. Total Cu values (mg/kg) in soils presented as mean values and standard deviation, for each set of three sampled rows, for Quinta do Sairrão. Left values (blue squares) refer to the first sampling period and right values refer to the second sampling (orange squares).*

ANOVA results for the available Cu content are plotted in figure 20 for all three sites.

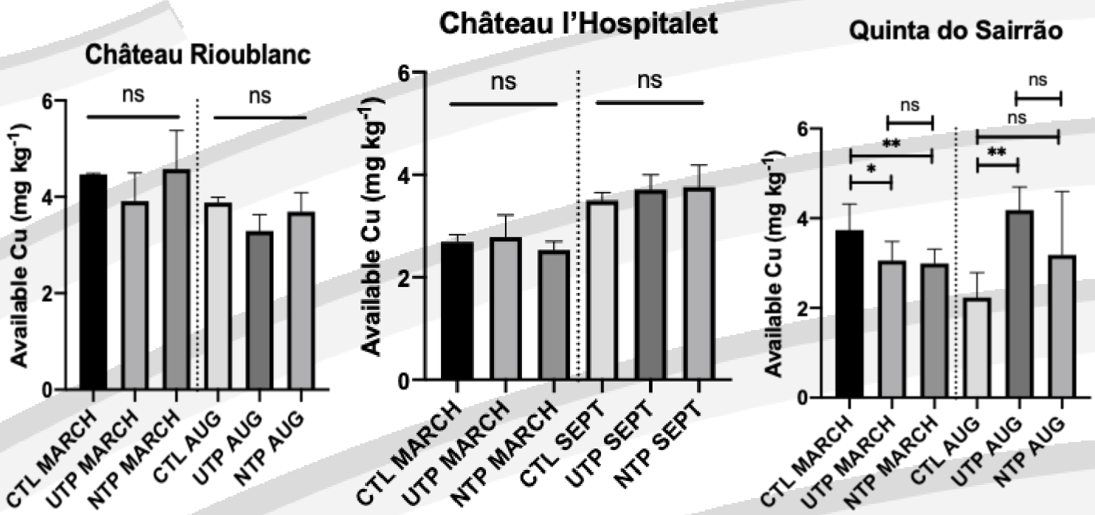



*Figure 20. Available Cu (mg/kg) for soil samples (dry mass), for the three trial sites and both sampling periods. ns stands for non-significant differences, whilst *represents significant differences between results when p<0.05 and ** when p<0.01. CTL - non-treated, UTP - usual protocol, NTP - new protocol.*

Mean values of available Cu in soils for each set of three sampled rows are presented in figure 21 for Château Rioublanc, figure 22 for Château l'Hospitalet and in figure 23 for Quinta do Sairrão.

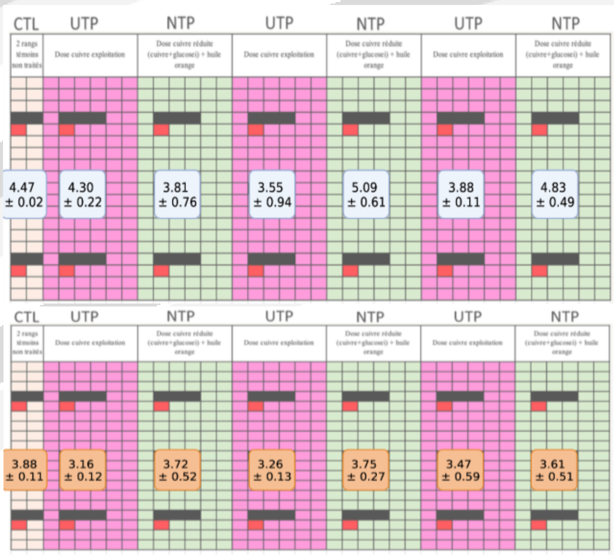



*Figure 21. Available Cu values (mg/kg) in soils presented as mean values and standard deviation, for each set of three sampled rows, for Château Rioublanc. Left-side values (blue squares) refer to the first sampling period and right-side values refer to the second sampling (orange squares).*


*Figure 22. Available Cu values (mg/kg) in soils presented as mean values and standard deviation, for each set of three sampled rows, for Château l'Hospitalet. Left-side values (blue squares) refer to the first sampling period and right-side values refer to the second sampling (orange squares).*

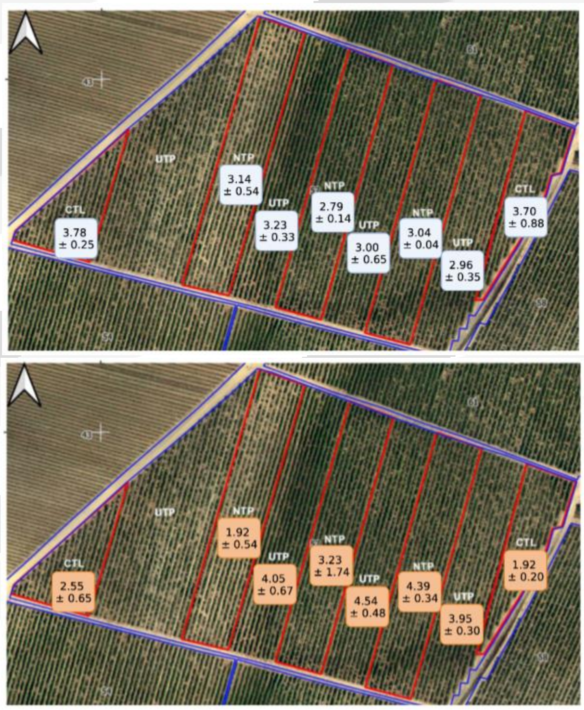



*Figure 23. Available Cu values (mg/kg) in soils presented as mean values and standard deviation, for each set of three sampled rows, for Quinta do Sairrão. Left-side values (blue squares) refer to the first sampling period and right-side values refer to the second sampling (orange squares).*

### iii. Soil trays experiment – Douro, Portugal

Regarding total and available Cu content in the tray's experiment artificial soil, exposed to phytosanitary treatments executed at Quinta do Sairrão, results are presented in table 12.

*Table 12. Total and available Cu content (mg/kg) in artificial soil samples placed on trays at Quinta do Sairrão, presented as mean values ± standard deviation.*

| Sampling period | Trial modality | Total Cu (mg kg$^{-1}$) | Available Cu (mg kg$^{-1}$) |
|---|---|---|---|
| After 1st spraying | CTL 1 | 6.72 ± 1.39 | 3.86 ± 0.46 |
| | CTL 2 | 4.31 ± 1.49 | 3.38 ± 1.26 |
| | UTP | 5.05 ± 0.29 | 3.54 ± 0.48 |
| | NTP | 5.04 ± 0.22 | 3.83 ± 0.77 |
| End of treatments | CTL 1 | 115 ± 7 | 4.80 ± 0.25 |
| | CTL 2 | 3.23± 1.36 | 2.76 ± 1.33 |
| | UTP | 7.56 ± 4.42 | 4.31 ± 0.51 |
| | NTP | 6.79 ± 6.72 | 3.63 ± 0.80 |

ANOVA results for total and available copper content in the artificial soil exposed in the field are plotted in figure 24.

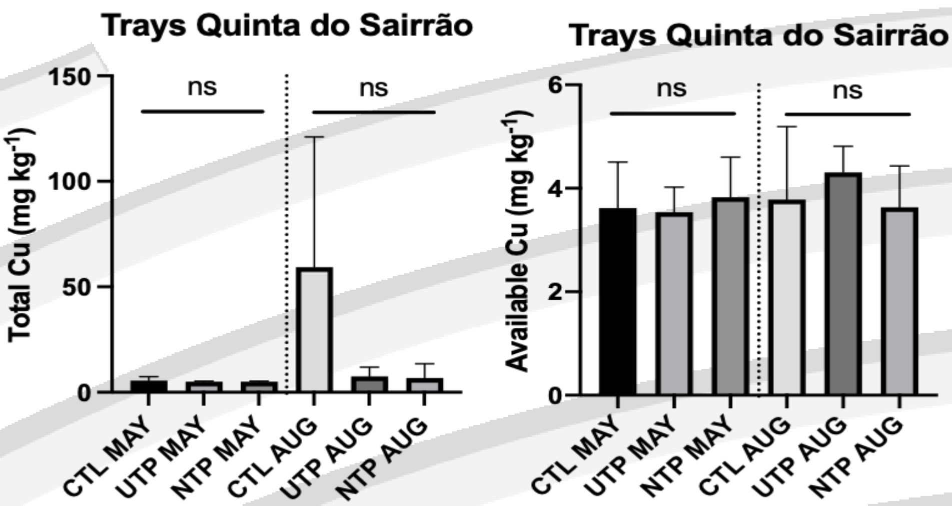



*Figure 22. Total (left) and available (right) Cu in the artificial soil samples placed on trays at Quinta do Sairrão for both sampling periods. ns stands for non-significant differences. CTL - non-treated, UTP - usual protocol, NTP - new protocol.*

No significant increase in the total content of Cu in the soil was observed for NTP when compared to CTL, after the first spraying. The same was observed for the UTP, but this was, in fact, expected as no copper-based products were used this year at Quinta do Sairrão because of low downy mildew pressure. However, at the end of the phytosanitary treatments a remarkable increase especially at CTL 1, was recorded, likely caused by cross contamination.

### iv. Levels of Cu in leaves

The results of total Cu content in the leaves for both sampling campaigns are presented in table 13. The first sampling campaign occurred in May, after the first bunches were visible, whereas the second sampling campaign occurred in August at Château Rioublanc and Quinta do Sairrão and September at Château l'Hospitalet. The values include both Cu accumulated in leaves tissues and adsorbed at the surface, although the latter is expected to be higher.

*Table 13. Total Cu in leaves determined by ICP-MS and presented as mean values ± standard deviation.*

| Trial sites | Sampling period | Trial modality | Total Cu (mg kg$^{-1}$) | |
|---|---|---|---|---|
| | | | Mean | STD |
| Château Rioublanc | 1st sampling | CTL | 23.52 | 3.95 |
| | | UTP | 220.9 | 22.4 |
| | | NTP | 137.9 | 31.9 |
| | End of treatments | CTL | 46.68 | 3.89 |
| | | UTP | 289.01 | 42.79 |
| | | NTP | 197.33 | 52.56 |
| Château l'Hospitalet | 1st sampling | CTL | 10.98 | 1.25 |
| | | UTP | 116.44 | 38.05 |
| | | NTP | 28.65 | 6.91 |
| | End of treatments | CTL | 269.15 | 34.17 |
| | | UTP | 305.40 | 42.47 |
| | | NTP | 322.68 | 17.64 |
| Quinta do Sairrão | 1st sampling | CTL 1 | 14.36 | 3.04 |
| | | CTL 2 | 20.22 | 1.88 |
| | | UTP | 21.27 | 4.17 |
| | | NTP | 86.60 | 41.12 |
| | End of treatments | CTL 1 | 88.25 | 20.80 |
| | | CTL 2 | 7.74 | 2.36 |
| | | UTP | 82.59 | 13.73 |
| | | NTP | 52.22 | 15.02 |

The respective ANOVA results are plotted in figure 25.

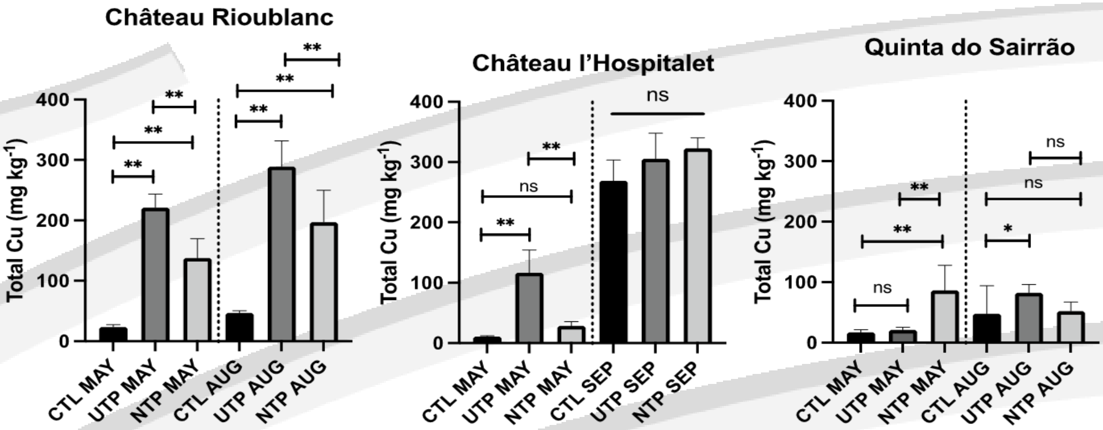



*Figure 25. Total Cu (mg/kg) as for leaves dry mass, determined by ICP-MS, for the three trial sites and both sampling periods. ns stands for non-significant differences, whilst *represents significant differences between results when p<0.05 and ** when p<0.01. CTL - non-treated, UTP - usual protocol, NTP - new protocol.*

Mean values of Cu in leaves for each set of three sampled rows are presented in order to allow a better visualisation of on-site value distribution.

In Château Rioublanc (figure 26), for both sampling periods, total amounts of Cu found in leaves were significantly higher in the usual treatment protocol, when compared to the new treatment protocol.

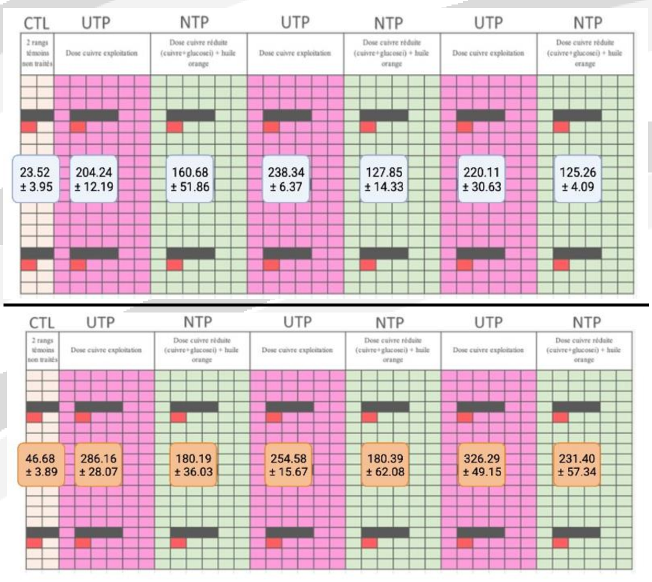



*Figure 26. Cu values (mg/kg) in leaves presented as mean values and standard deviation, for each set of three sampled rows, for Château Rioublanc. Left-side values (blue squares) refer to the first sampling period and right-side values refer to the second sampling (orange squares).*

The same tendency was observed for the first sampling period at Château l'Hospitalet (figure 27), however, Cu levels for leaves collected at the end of all treatments, were similarly high across all treatments, and even for the control area, with no significant differences among these, due to the application of the same concentration of Cu in both treatments.

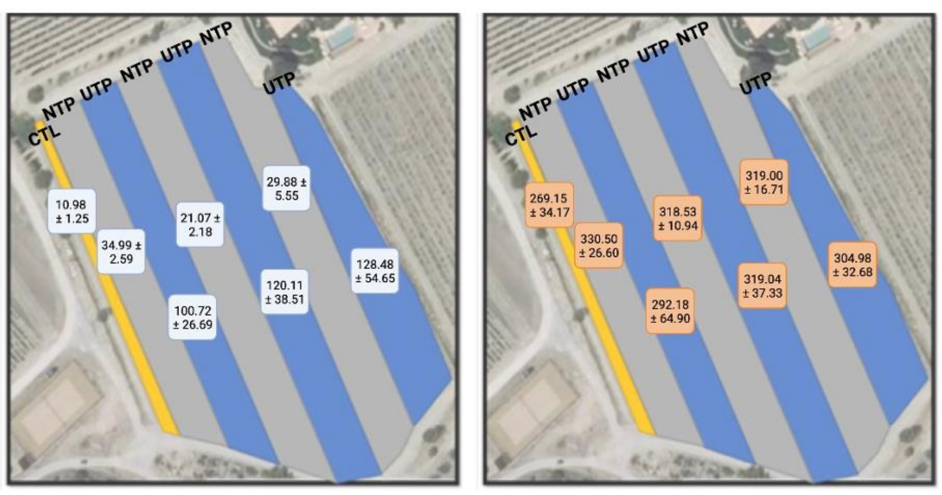



*Figure 27. Cu values in leaves presented as mean values and standard deviation, for each set of three sampled rows, for Château l'Hospitalet. Left-side values (blue squares) refer to the first sampling period and right-side values refer to the second sampling (orange squares).*

For Quinta do Sairrão (Figure 28), after the first application, Cu levels on leaves from NTP sites were significantly higher than for those from the UTP, which is consistent with the fact that UTP did not use any copper for absence of downy mildew pressure. However, the opposite was observed for the last sampling period, again possibly resulting from cross-contamination between modalities. The abnormally high values recorded in one of the CTL blocks is considered an outlier and may have resulted from cross-contamination from using copper-based protection at an adjacent block outside the trial.


*Figure 28. Cu values in leaves presented as mean values and standard deviation, for each set of three sampled rows, for Quinta do Sairrão. Left-side values (blue squares) refer to the first sampling period and right-side values refer to the second sampling (orange squares).*

## v. Levels of Cu in grape berries

Grape samples were freeze-dried before analysis (figure 29), and thus, results are expressed on a dry mass basis.

*Figure 29. Preparation of grape berries homogenates for copper determination in GREENUPORTO lab.*

The results for the total Cu content in grape berries at harvest for the 3 trial sites are presented in table 14. It was not possible to obtain grape samples from the control area in Château Rioublanc due to downy mildew infection causing total grape loss.

*Table 14. Total Cu content in grapes determined by ICP-MS and presented as mean values ± standard deviation.*

| Trial sites | Trial modality | Total Cu (mg kg$^{-1}$) | |
|---|---|---|---|
| | | Mean | STD |
| Château Rioublanc | UTP | 13.5 | 1.4 |
| | NTP | 13.7 | 1.7 |
| Château l'Hospitalet | CTL | 10.6 | 2.0 |
| | UTP | 10.4 | 1.2 |
| | NTP | 10.9 | 1.1 |
| Quinta do Sairrão | CTL 1 | 5.41 | 1.31 |
| | CTL 2 | 3.75 | 0.46 |
| | UTP | 6.03 | 2.33 |
| | NTP | 4.28 | 0.51 |

Results have been subjected to statistical analysis, and for Château l'Hospitalet and Quinta do Sairrão, an one-way ANOVA and a multiple comparisons test (Tukey's test) was performed, allowing for the comparison between control and treated areas and between the two different treatment protocols. For Château Rioublanc a t-Test was performed to compare the two treatments. Results are plotted in figure 30. No statistically significant differences were observed.

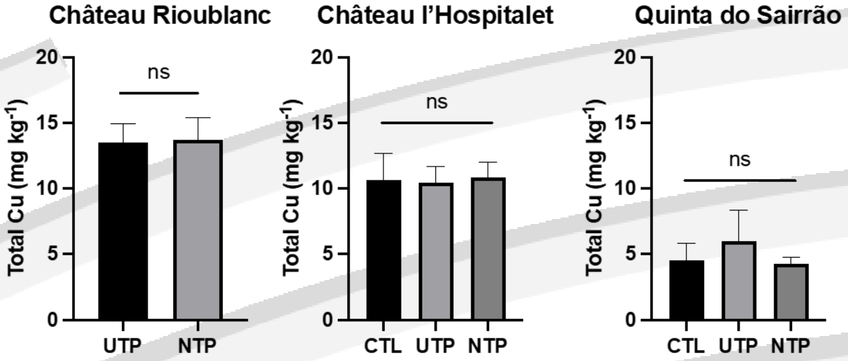


*Figure 30. Total Cu (mg/kg) for grapes, expressed as dry mass, for the three trial sites. ns stands for non-significant differences. CTL - non-treated, UTP - usual protocol, NTP - new protocol.*

Mean values of Cu in grapes for each set of three sampled rows are presented in figure 31 for Château Rioublanc, figure 32 for Château l'Hospitalet and in figure 33 for Quinta do Sairrão.

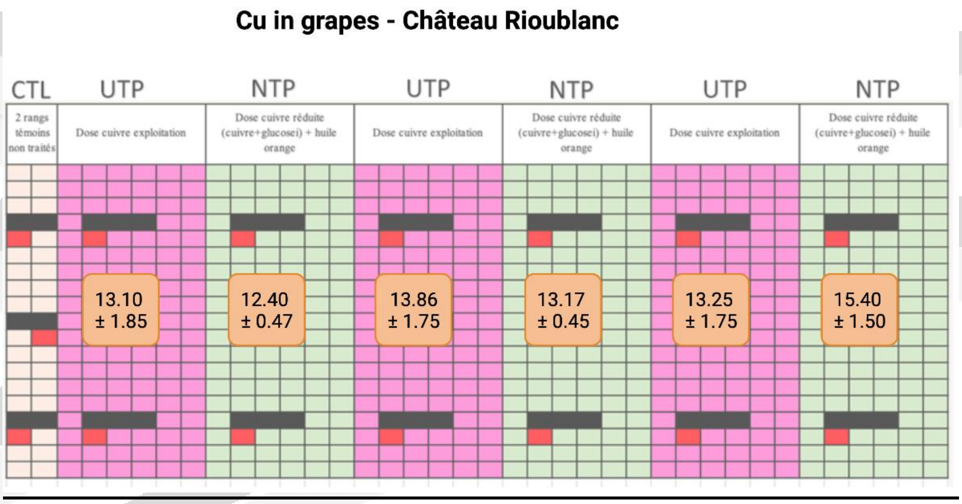


*Figure 31. Cu values (mg/kg) in grapes at harvest presented as mean values and standard deviation, for each set of three sampled rows, for Château Rioublanc.*

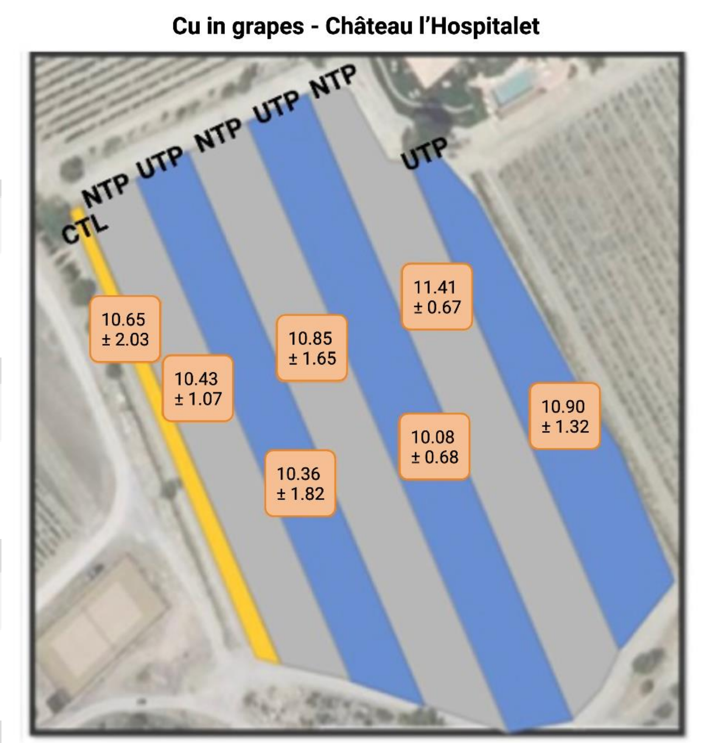



*Figure 32. Cu values (mg/kg) in grapes at harvest presented as mean values and standard deviation, for each set of three sampled rows, for Château l'Hospitalet.*

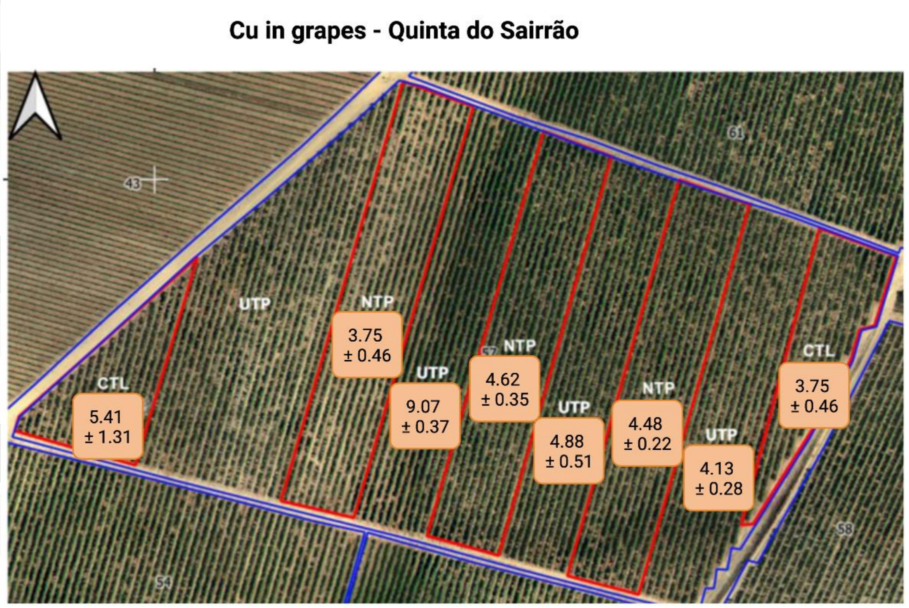



*Figure 33. Cu values (mg/kg) in grapes at harvest, presented as mean values and standard deviation, for each set of three sampled rows, for Quinta do Sairrão.*

### c. Meteorological observations

## Site 1 - Château Rioublanc, Bordeaux, FRANCE, managed by SVBNA

Regarding the meteorological conditions registered between January and September of 2022 at Château Rioublanc (figure 34), relatively low rainfall levels kept conditions less favourable than usual for downy mildew until mid-May, increasing the disease risk henceforth. Strong rainfall values by late June together with a severe hail event were observed at the trial site.

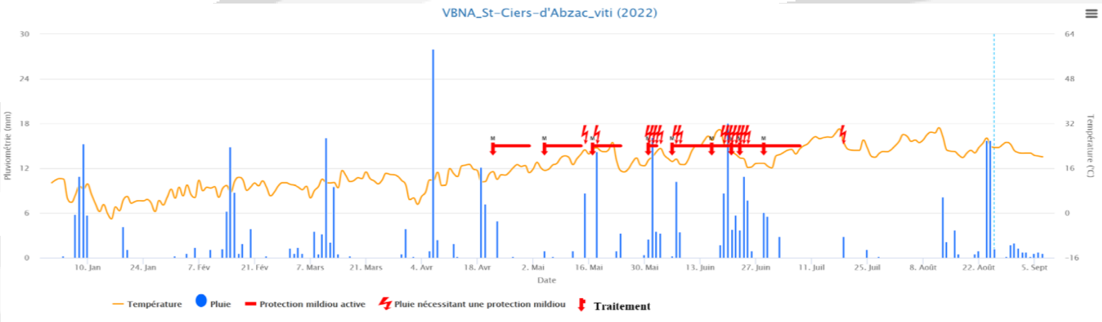



*Figure 34. Thermopluviogram at Château Rioublanc, indicating placement (red arrow) and duration (red line) of protective treatments and rainfall amounts (red spark) requiring protection against fungal disease.*

## Site 2 - Château l'Hospitalet, Narbonne, FRANCE, managed by SCEA GB

Very low rainfall values (369 mm until September 21st) meant no favourable downy mildew conditions during the whole season at Château l'Hospitalet (figure 35).

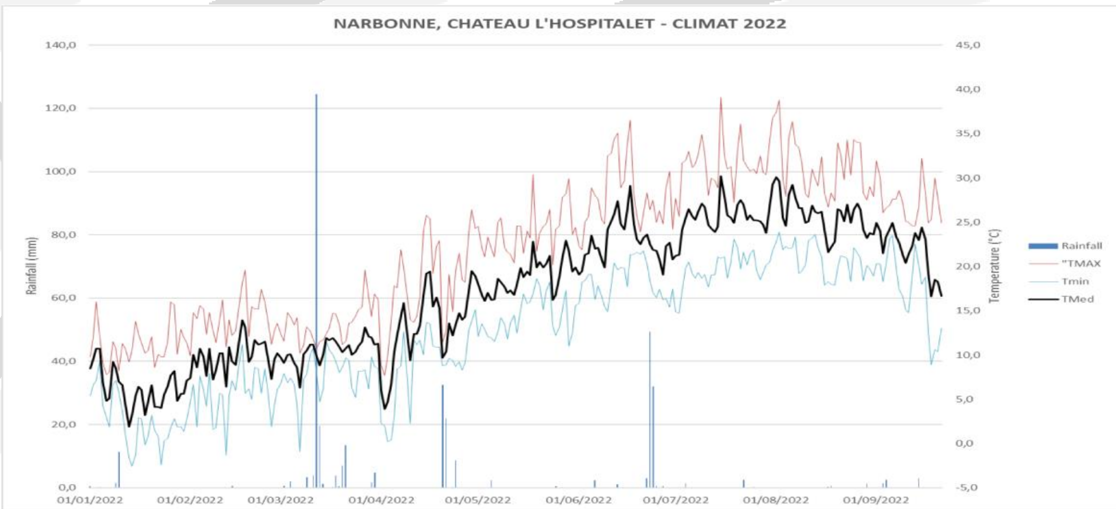



*Figure 35. Thermopluviogram at Château l'Hospitalet.*

## Site 3 - Quinta do Sairrão, Douro, PORTUGAL, managed by SOGRAPE

Extremely low rainfall values (246 mm until September 21st) meant no favourable downy mildew conditions during the whole season at Quinta do Sairrão (figure 36).

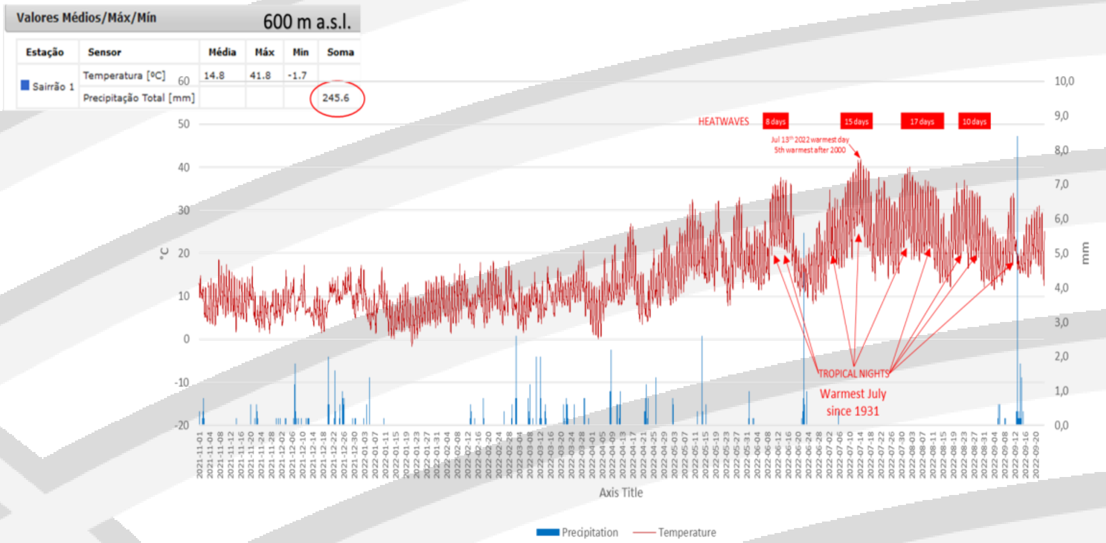



*Figure 36. Thermopluviogram at Quinta do Sairrão (red line - temperature, blue bars - rainfall) showing dates and duration of heat waves (red rectangles).*

### d. Downy mildew assessment

## Site 1 - Château Rioublanc, Bordeaux, FRANCE, managed by SVBNA

At Château Rioublanc, 3 observations were made for downy mildew presence in both leaves and bunches at 3 phenological stages: flowering (June 2nd), bunch closure (June 27th) and veraison (July 18th) (table 15).

Downy mildew pressure was observed at the CTL modality right from the first observations, even if with low intensity. Pressure progressed during the cycle, data showing a statistically better bunch protection performance of NTP over UTP at bunch closure (figure 37). Least square means (LS means)[2], i.e. the means of least squares of data in each modality clearly show differences between modalities with very low probability of happening by chance, meaning those differences are statistically likely to result from different protection levels of trial modalities.

[2] In opposition to observed means that are directly calculated from raw data, least square means (LS means) are estimated from a linear model such as an ANOVA obtained from the raw data. The conclusions withdrawn from both means are similar in terms of differences between treatments for the variables under analysis.

*Table 15. Downy mildew symptoms at Château Rioublanc at bunch closure phenological stage (values indicate infection intensity - % of infected organ - and frequency - % infected organs over total observed. Yellow cell headers: leaves; purple cell headers: bunches). Green arrow indicates better performance of NTP over UTP, red spark indicates incidence of hail event.*

LEAVES

FLOWERING

| GT4 | TNT | New Treatment Protocol | Usual Treatment Protocol |
|---|---|---|---|
| Moy tot INTENSITIES (%) | 0.44 | 0.43 | 0.39 |
| Moy tot FREQUENCIES (%) | 0.00 | 7.11 | 10.22 |

BUNCH CLOSURE

| GT4 | TNT | New Treatment Protocol | Usual Treatment Protocol |
|---|---|---|---|
| Moy tot INTENSITIES (%) | 13.45 | 2.23 | 3.39 |
| Moy tot FREQUENCIES (%) | 61.00 | 19.33 | 25.11 |

VERAISON

| GT4 | TNT | New Treatment Protocol | Usual Treatment Protocol |
|---|---|---|---|
| Moy tot INTENSITIES (%) | 31.63 | 8.12 | 6.17 |
| Moy tot FREQUENCIES (%) | 81.67 | 34.44 | 23.44 |

BUNCHES

FLOWERING

| GT4 | TNT | New Treatment Protocol | | | Usual Treatment Protocol | | |
|---|---|---|---|---|---|---|---|
| Moy block INTENSITIES (%) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Moy block FREQUENCIES (%) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

BUNCH CLOSURE

| GT4 | TNT | New Treatment Protocol | Usual Treatment Protocol |
|---|---|---|---|
| Moy tot INTENSITIES (%) | 70.49 | 0.51 | 6.92 |
| Moy tot FREQUENCIES (%) | 88.67 | 4.78 | 24.89 |

VERAISON — HAIL EVENT

| GT4 | TNT | New Treatment Protocol | Usual Treatment Protocol |
|---|---|---|---|
| Moy tot INTENSITIES (%) | 99.81 | 97.14 | 65.72 |
| Moy tot FREQUENCIES (%) | 100.00 | 100.00 | 100.00 |


*Figure 37. Statistical significance (LS means) analysis of differences between modalities in leaves and bunches at bunch closure phenological stage for Château Rioublanc. (ANOVA - Tukey HSD, Fischer LSD, Neuman-Keuls SNK tests; Var1 - frequency of infected leaves; Var2 - intensity of infection; different letters atop bars mean statistically different results, same letters mean results are not statistically different.)*

However, the hail event of late June reversed the situation, making the infection more frequent and intense in the NTP modality by veraison time with near total production loss, while revealing a higher level of protection from UTP that avoided total production loss.

### Site 2 - Château l'Hospitalet, Narbonne, FRANCE, managed by SCEA GB

At this site, downy mildew symptoms were not found at any of the three trial modalities in all occasions during which observations were made. This confirms the absence of pressure due to very dry and unfavourable meteorological conditions for fungal infections.

### Site 3 - Quinta do Sairrão, Douro, PORTUGAL, managed by SOGRAPE

At this site, downy mildew symptoms were found only on leaves at flowering time for the untreated control. Those symptoms disappeared later in the season confirming the absence of disease pressure due to extremely dry and unfavourable meteorological conditions for fungal infections.

## e. Production evaluations

### Site 1 - Château Rioublanc, Bordeaux, FRANCE, managed by SVBNA

Faced with the damage caused by the hail event of June 20th and ensuing downy mildew outbreak, the yield estimation protocol was adapted. By means of visual counting, the average number of healthy clusters per plant for each modality was estimated. Bunch samples were weighed, by modality, to estimate the average weight of a healthy bunch. The harvest weight obtained by modality was estimated by multiplication as detailed in table 16.

*Table 16. Yield estimation per trial modality at Château Rioublanc.*

| Trial modality | Healthy bunches per plant | Plants per row | Rows per modality | Average bunch weight (kg) | Estimated harvest weight (kg) | Estimated yield (kg/plant) |
|---|---|---|---|---|---|---|
| **CTL** | 0.0 | 66 | 1 | 0.000 | 0 | 0.00 |
| **UTP** | 5.0 | 66 | 18 | 0.076 | 451 | 0.38 |
| **NTP** | 0.1 | 66 | 18 | 0.046 | 8 | 0.01 |

The damaging effect of the hail event and ensuing downy mildew outbreak is obvious from the comparison of yield values across the three trial modalities. Also, quite obviously, even if until bunch closure the NTP modality achieved comparable and even better results than UTP, after the hail strike only UTP managed to somewhat protect the remaining bunches.

## Site 2 - Château l'Hospitalet, Narbonne, FRANCE, managed by SCEA GB

As no downy mildew pressure was present during the growth cycle, even the untreated control (CTL) presented a good yield performance (table 17). No significant differences were found in between the two treatment protocols.

*Table 17. Yield observed at Château l'Hospitalet.*

| Trial modality | Yield (kg/plant) |
|---|---|
| **CTL** | 2.61 ± 0.06 |
| **UTP** | 2.86 ± 0.44 |
| **NTP** | 2.84 ± 0.21 |

## Site 3 - Quinta do Sairrão, Douro, PORTUGAL, managed by SOGRAPE

As no downy mildew pressure was present during the growth cycle, even the untreated control (CTL) presented a good yield performance (table 18). No significant differences were found in between the two treatment protocols.

*Table 18 - Comparison of yield components across modalities in Quinta do Sairrão*

| Modality | Bunch weight (g) | Yield per vine (g) | Count of bunches |
|---|---|---|---|
| CTL | 214.3 ± 41.6 | 2 265 ± 484 | 10.55 ± 0.21 |
| NTP | 171.4 ± 4.6 | 1 426 ± 191 | 8.32 ± 1.00 |
| UTP | 169.0 ± 50.8 | 1 526 ± 583 | 8.93 ± 1.10 |

Because trial conditions at the three sites did not allow for replicated estimates, we did not perform a statistical analysis of yield data at harvest.

## 4. Conclusions

Differences in results observed at Château l' Hospitalet (Narbonne) and Quinta do Sairrão (Douro) cannot be attributed to differences in the phytosanitary protocols used. In fact, the absence of environmental conditions favourable for downy mildew infections rendered the trials in these two sites useless for the objectives of this GT. In this way, the only usable results are those obtained from the trial at Château Rioublanc (Bordeaux ).

First of all, this GT4 trial confirmed the great disparity that exists between wine regions in the south-west zone of Europe, and the importance of testing the solutions developed in different regions, as well as in different years. These differences have manifested themselves at the level of downy mildew pressure, this fungal disease being the target of tested treatments. In the trial year, only at Château Rioublanc (Bordeaux, southwest France) did grapevines come under strong downy mildew pressure. Douro Valley (northeast Portugal) and Narbonne (southeast France) were not affected by this disease in the trial year. This is not, however, the usual historical pattern for those two regions.

The objectives were therefore different between, on the one hand, regions which must protect one or two key moments of the growth cycle and, on the other hand, regions where the disease pressure remains high and constant throughout the season and are specifically increased by episodes of intense rainfall.

Meteorological events such as frost or hail also showed their importance. In addition to their dramatic impact on yield, these events greatly weaken the grapevine. They lengthen the growth cycle, especially when happening at the flowering stage, thus promoting plant vulnerability and the development of downy mildew (Gardea 1987, Evans 2000, AWRI 2021), among other impacts.

At Château Rioublanc, under Organic Production (BIO) mode, the new treatment protocol allowed for an important reduction of copper use until bunch closure, maintaining a better protection performance compared to the usual treatment protocol (UTP). However, the new protocol of protection (NTP) was not enough to shield grapes from the downy mildew outbreak resulting from a hail event, leading to near total production loss in this modality, whereas UTP managed some level of protection avoiding total loss.

At Château L'Hospitalet , under Organic Production (BIO), due to low downy mildew pressure, NTP did not perform any better than UTP in protection of yield.

At Quinta do Sairrão, under Integrated Protection mode (PRODI), due to low downy mildew pressure, it was possible to totally avoid the use of copper in UTP keeping total protection of yields. In this situation, NTP, that included the use of copper, did not perform any better protection.

Regarding Cu levels assessed for the different selected matrices (soil, grapes and leaves), distinctive conclusions can be withdrawn. For total and available Cu in soils, applied treatments on vineyards didn't result in significantly different inputs of this metal for this compartment when comparing the two different protocols (UTP vs. NTP). At Quinta do Sairrão, the conducted experiment with trays of artificial soils also corroborated these results. Although this result was not expected, this may be explained due to low downy mildew pressure resulting in a low number of applications during the trial year, and, consequently, in a low input of Cu to the soil at the end of the phytosanitary protection campaign. Nevertheless, the experiment with trays allowed us to estimate the total input of copper to the soils under NTP, which corresponded to 0.324 kg Cu/ha, knowing that the total copper metal dose applied was 0.599 kg Cu/ha. Furthermore, and despite the total Cu levels in soils, it was possible to realise that the available levels were low, corresponding in average to approximately 4% of the total levels.

In vine leaves analyses, results differ between locations. At Château Rioublanc, UTP appeared to have resulted in a significantly higher Cu accumulation in leaves than NTP, for both sampling periods, which is in accordance with Cu doses applied for each protocol. At Château l'Hospitalet, whilst the same was observed for the first collected samples, Cu levels were similar across both treatments at the end of applications. This could be predicted from equal Cu application doses for both UTP and NTP at this site. Finally, results for the first sampling campaign at Quinta do Sairrão can be a reflection of the treatment approach undertaken at this site under Integrated Production (PRODI) mode: more Cu was found in leaves grown under NTP, than under UTP. However, at the end of the trial, no differences could be identified between Cu levels in leaves.

The same conclusions also seem to be drawn from Cu levels in grapes, with no significant differences between UTP and NTP. At both Château Rioublanc and Château l'Hospitalet despite differences recorded on leaves, no significant differences were observed on grapes at these two trial sites, as well.

In summary, although for reasons that could not be controlled due to unfavourable meteorological conditions for downy mildew infections in two out of the three trial sites, in a general way it was not possible to infer significant conclusions in terms of a better environmental performance of NTP, regarding the reduction of Cu inputs. However, Château Rioublanc results suggest that, in fact, NTP may bring some context-dependent advantages in providing an adequate level of protection against low to medium downy mildew disease risk, while significantly reducing copper input to the environment. If yield is to be protected, it is important to underscore that, when using NTP, a contingency plan for quick action is essential to respond to an event suddenly increasing disease risk (heavy rain downpours over several days, hail, etc.). Even with such a plan, in such situations, results at Chateau Rioublanc demonstrated that, under NTP, severe losses of yield may be unavoidable.

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# Feasibility Report (E4.4.1)

**<u>Result from activity 4.4. Technical feasibility analysis of the itineraries proposed in different regions of the SUDOE space and in different production modes</u>**
**<u>E4.4.1. Feasibility Report</u>**

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Involved partners: PTV, ADVID, IFV, CVAN, UPC, SVBNA, EURECAT, JEAN LEON (TORRES), GREENUPORTO, SCEA (GERARD BERTAND)

## 1. Introduction

Copper remains a major phytosanitary agent in protecting grapevines from attacks of downy mildew (*Plasmopara viticola*). Its continued usage, however, leads to accumulation in soil with damaging effects for soil- and water-borne biodiversity. In reducing copper usage across different wine regions, a comprehensive evaluation of environmental and socioeconomic contexts (soil type and vulnerability, climate, disease pressure, usual farming practices, mode of production) is required to improve the environmental performance while retaining economic viability. This latter is translated by the protection of yield and value of grapes and by keeping production costs competitive. In Action 4.4, the partners involved in each of the three trials conducted an evaluation of the feasibility of the new treatment protocol as compared to usual practices.

## 2. Methodology

Each of the partners involved in the three trial sites described in E4.3.1 produced a context and feasibility analysis from the trial experience, projecting obtained results into a potential adoption of NTP as a protection protocol.

## 3. Results and Discussion

### a. Case studies results at three different SUDOE wine regions

#### Château Rioublanc (Bordeaux, certified organic production)

Copper accumulation in leaves, especially in UTP was significant for a year when the pressure was no more than the average recorded for previous years. We consider it to be largely due to treatments executed after the June hailstorm which weakened plants and required a special curative strategy. However, this did not translate in a significant way, neither for soil total or available copper contents nor for berry copper values.

The hailstorm clearly weakened the plot, facilitating the spread of mildew. The Glucosei modality (NTP) did not provide sufficient protection to obtain the same yield as the usual winegrower modality (UTP). Unfortunately, these unexpected events are forecasted to increase, compromising the use of treatments with lower levels of copper.

#### Chateau l'Hospitalet (Narbonne, certified organic production)

During this trial, some rows were left for the first time without any treatments: no spot of any disease was observed even on non-treated (control) rows. This seems to hint that in some years, under very dry climate, no copper is required to ensure no contamination of downy mildew on the vines.

However copper application remains essential as it is very hard to know from modelization if the disease is close to infecting the vines or not. From experience on this site, it is known that when disease pressure is high, losses can be serious. Nevertheless, during this phase of the year when treatments are made preventively, a small dose of copper can be used. In years like 2021 and 2022, without any pressure, it is possible to finish the season with an extremely low total of copper applied. And when confronted with a season with high pressure, it is possible to use more copper to resist the disease.

### Quinta do Sairrão, (Douro, certified integrated production)

Douro schists, low pH anthrosols present higher vulnerability to copper contamination with potential impacts in soil and water organisms, which may reduce local biodiversity and important ecosystem functions such as nutrient recycling, competition with pathogens or pest antagonism.

The experience obtained during the trial (as reported in E4.3.1) allowed us to understand that in integrated production (PRODI) mode, under low downy mildew pressure, the use of copper can be totally avoided. This may not be the case, however, in years with higher infection potential. In any case, close monitoring of weather conditions, crop growth and disease risk by local observation is paramount to avoid unnecessary copper applications with both environmental and economic advantages, while securing yields and quality of grapes.

## b. Technical viability evaluation

### Château Rioublanc (Bordeaux, certified organic production)

This trial confirmed the benefit of using a form of copper with a different formulation to improve its effectiveness at a lower dose. However, the alternative formulation used (Glucosei) still had copper and therefore could only be used to reduce applied doses and not for replacing copper. It seemed, in any case, to allow for a significant dose reduction still providing adequate protection under a normal downy mildew pressure.

A limiting point for feasibility of adopting this new protocol is the classification of Glucosei as a foliar fertiliser, meaning it cannot be used for phytosanitary purposes, which limits its application, particularly under organic farming, for which it is necessary to justify every foliar fertiliser input.

Another important issue in the progress towards copper reduction was highlighted by this trial: the impossibility of large-scale testing of new products of interest that have not yet obtained approval. None of the innovative products of GT1 could be tested in large plots due to the difficulties of obtaining derogation in France and other countries in Europe (unless destroying the entire harvest). To this is added in organic production mode the automatic downgrading of the harvest in the case of the use of a non-referenced input.

Finally, its difficulty of supply in France, undoubtedly linked to the elements previously mentioned, further limits its feasibility to employ in organic farming. A joint work with the manufacturer, as well as possible changes in regulation could be a way of solving this obstacle by obtaining a registration of this and other possible alternative products under development in the phytosanitary category.

### Chateau l'Hospitalet (Narbonne, certified organic production)

Once the supplier is identified, it is very easy to obtain the Glucosei, named Labicuper Evo in France and provided by VitiVista. As it is a fertiliser, it should not be possible to use it alone in a phytosanitary program, but it can be integrated to stay at very low levels in copper at the beginning of the season and to use a more efficient product in case of increasing pressure.

In the case of high pressure for downy mildew, more treatments and more than 3kg/ha of copper are required. That's why it is necessary to compensate during years without pressure, this product obviously helping to reach this objective. Indeed, the biodynamic certification referential limits the use of copper at 15 kg/ha/5 years, it's the same logic for the European limitation which is 28 kg/ha/7 years but lower levels. The possibility to use less copper when there is less pressure is a huge advantage. And it should be possible to increase doses in years with higher pressure.

No technical point likely to avoid integrating this product in phytosanitary programs at this location were identified. Essential orange oil was already in use in phytosanitary programs for some time here. In addition, no investments regarding machinery and/or equipment were needed to implement this itinerary. Also, workers do not need any specific training to perform the new treatment which is an advantage of this itinerary.

To increase technical viability, it is very important to disseminate results from credible trials conducted with adequate experimental design and a sound use of statistical analysis. It is hoped that as many people as possible will become aware about trials and results. It would be useful for every winemaker around the world to find a way to share these kinds of results. Currently, most of the trial results to make choices of phyto-protection programs are made by retailers and they don't publish the results of their trials. Making cooperative field trials together with independent organisations and wine estates seems very useful to advance in the issue of reducing copper use.

## Douro trial site (certified integrated production)

Copper presented a very good compromise between protection efficiency and cost. Its use in certified integrated production could be alternated with other active substances against downy mildew, a practice that accounted for lower copper inputs while ensuring adequate protection and overall sustainable production.

Considering these soils were more susceptible to copper impacts depending on their properties, data obtained in this study (GT2.2) suggested that lower pH values were within the soil characteristics that accounted for a great environmental risk of copper in vineyard soils. All opportunities to reduce copper use are valuable and PRODI offers a reliable and sustainable way of balancing plant and environmental protection at a reasonable cost for a region having some of the highest costs of production in Europe. The development and validation of the economic feasibility of new protocols and products to reduce copper should also take into account non-tangible goods as soil quality and the services provided, at least by prioritising their implementation in most vulnerable soils.

The adaptation of copper dosage to vegetation volume, by using remote sensing monitoring technology, to address some canopy variables, the use of spray recovery panels in tractors and the choice of the right moment to spray, can all contribute to an important reduction in the levels of copper necessary to achieve crop protection. However, recent climate evolution has shown that weather is becoming more variable and extreme, requiring flexibility and readiness to act quickly when severe events may trigger what, under favourable spread conditions may become an explosive outbreak with serious consequences for both yields and value of the crop. Caution and contingency plans are of the essence for any strategy to reduce copper usage under these conditions.

Low commercial availability of alternatives such as Glucosei may therefore become a limiting factor in reducing copper amounts. In the Douro, the scarce and dwindling labour availability is also an obstacle as the use of these products offering lower safety against sudden outbreaks require the capacity to react quickly in terms of product application and canopy management. If temporary labour is not available at the right time, these itineraries may become an undesired liability.

### c. Replicability and SWOT analysis of proposed itineraries

The replicability of these results is informed by the application of the new COPPEREPLACE protocol across different regions (Bordeaux, Narbonne and Douro) and different production strategies (organic and integrated productions).

To replicate the approaches and results obtained in this work, it is essential to consider the context where it will be applied. Soil vulnerability, climate and usual farming practices will need to be considered to manage the risk of disease adequately. The following SWOT analysis (Table 1) is meant to provide guidance in such replication. Because of the absence of downy mildew pressure in both Château l'Hospitalet and Quinta do Sairrão, we were unable to provide specific feasibility evaluations for place / mode of production binomes, as planned. In this way, the SWOT analysis tried to draw conclusions for the experiences in all three sites for application to the SUDOE space.

*Table 1 - SWOT analysis of COPPEREPLACE new treatment protocol*

| | |
|---|---|
| **STRENGTHS**<br>Possible reduction of copper dose.<br>Ability to restrain downy mildew when pressure is low.<br>No new equipment/investment needed | **WEAKNESSES**<br>GLUCOSEI classification as fertiliser.<br>Not adapted for high-pressure of downy mildew. |
| **OPPORTUNITIES**<br>Coupling with other technologies may increase efficiency.<br>Development of effective copper formulations with lower copper content.<br>Forces farmers to think in integrated way, instead of quick-fixes | **THREATS**<br>Use of this approach may improperly speed legal copper restrictions, as it still does not eliminate copper use.<br>GLUCOSEI with different availability across different areas in the SUDOE space. |

## 4. Conclusions and recommendations

COPPEREPLACE has reaffirmed the importance of product formulation in finding better ways to reduce the environmental impact of plant protection in viticulture. GT4 trials have shown how a better formulated cupric product may provide acceptable protection at a reduced copper dosage. Regarding price, GLUCOSEI cost per treatment is slightly higher (specially in Bordeaux) than the conventional treatment, but this may vary with disease pressure and number of applications. However, considering environmental impact/cost (reduction of copper accumulation) such difference may still be considered acceptable.

Unfortunately, and despite the careful planning, the three trials demonstrated great limitations associated with field testing of new treatment protocols and new products to replace copper. Such limitations were related with difficulties in performing experimental designs truly replicated not only because of the great variability in several environmental aspects (e.g., soil types, topography, sun exposure, regional and local climatic conditions), but also due to operational limitations, as it was not possible for partners to compromise the crop of large areas for experimentation. Furthermore, the timeframe of this research project was particularly limited for such purpose, as to overcome uncertainty caused by annual climatic variability, the protocols and the products need to be repeated at least for three years.

It is not currently feasible to provide adequate plant protection in either organic or integrated productions by totally avoiding copper use. Inversely, reducing copper use by employing better lower-impact copper formulations does seem a possible and feasible way of lowering copper dispersion to the environment. This requires, however, better knowledge of context conditions for all situations, namely, soil vulnerability to copper contamination and climate drivers of disease risk, spray dispersal and copper leaching in soils, as this project clearly demonstrated that any product or treatment protocol cannot be implemented without field validation, given the great impact of environmental conditions on their effectiveness

Under integrated production mode, the possibility to alternate cupric formulations with other protection products can significantly lower copper use and, for places and years with very low downy mildew pressure, totally replace its use by PRODI-approved active substances, reducing the values of copper found in soils, leaves and berries.

More precise spray application knowledge and technologies such as hydrosensitive paper testing, spray recovery panels, variable-rate application, automatic leaf area detection and remote sensing are important requirements. Particularly, this trial has demonstrated the interest of implementing periodic sprayer calibrations, adjusting sprayer efficiency and using protective panels to decrease spray dispersal. The adequation of copper dose to vegetation size (leaf area or canopy volume) was also employed to keep copper values low during the spray season.

These practices, while preserving crop value, also reduce the loss of sprayed products and present a good business case for cost management in viticulture.

The dissemination of these results through exchanges between research actors and professionals remains an essential part for wide implementation of these methods and technologies in the SUDOE space. The best tool for this will be the continuation and strengthening of the stakeholder network setup by COPPEREPLACE (GT6), perhaps even by converting it into a community of practice. This should also become the place for discussion with public authorities and policy-makers so that the latter have a clear vision of the progress achieved, the existing limitations and obstacles to feasibility of any proposed solutions, to best adapt regulatory developments and foster the increasing adoption of copper-smart viticulture practices.