

Validation and Safety of Unmanned Aerial Spraying Systems (UASS) in Agroforestry Environments





) MINISTERIO DE AGRICULTURA, PESC Y ALIMENTACIÓN



LEUROPEA uropeo Agricola sarrollo Rural te en las zonas rundes

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# Introduction

Implementing the European Commission's Farm to Fork Strategy requires a quick development of new plant protection product application technologies that provide effective crop protection while ensuring the sustainability and competitiveness of European agriculture. Precision agriculture, understood as the set of techniques and technologies that enable actions to be carried out at the right time, in the right place and in the right quantities, improving the efficient use of production tools and minimising the impact on the environment and human's safety, is part of the response to achieve the goals set. In recent years, the development of innovative plant protection treatment solutions for precision agriculture has intensified considerably.

Spain is committed to speeding up the arrival of new technologies that will make possible precision agriculture implementation in our production systems as soon as possible. A clear example is the support for the development of Unmanned Aerial Spraying Systems (UASS) by the Ministry of Agriculture, Fisheries and Food, through the Innovation Project Co-funded by Spanish Ministry of Agriculture & European Agricultural Fund for Rural Development (EAFRD) in 2021, through the Operational Group (OG) Phytodron Project, which was set up to analyse the validation and safety of Unmanned Aerial Spraying Systems (UASS) in the agroforestry environment. The aim of this OG was to gather experimental information to propose a revision of the current Directive 2009/128/EC on the Sustainable Use of Plant Protection Products, which considers UASS as part of the aerial treatments prohibited in the Directive. Depending on the results, the use of UASS could be viewed as different from treatments with manned aerial spraying systems (aeroplanes and helicopters), contributing to the development of precision agriculture.

The Phytodron OG has conducted experiments in vineyards, citrus, pine trees and olive groves to evaluate data relating to human and environment safety, application quality and efficacy. The OG has worked with leading experts in each subject studied regarding the development of the protocols and their execution.

The trials contrasted UASS treatment field data with conventional ground-based treatments in tests of efficacy, residues, drift and aerial deposition; and UASS treatment field data with existing models for aerial and ground-based treatments regarding drift, operator, worker, bystander and resident exposure.

Prior to conducting the tests, extensive work was carried out to configure the UASS in accordance with the following ISO standards:



- > Characterization of indoor equipment Cross-sectional distribution ISO 5682:2017
- > Characterization of outdoor equipment Cross-sectional distribution ISO NWI 23117-2

These trials were conducted in vineyards, citrus, pine trees and olive groves to compare human and environmental exposure in UASS vs. conventional terrestrial and aerial spraying.

This publication contains the following protocols designed within the Phytodron OG:

- 1. Humans' non-dietary exposure trials during treatment of grapevines to determine the exposure of operators in auxiliary activities, pilot in application, and resident/bystanders during treatment carried out by foliar spraying using UASS.
- 2. Efficacy and residue trials during treatment on citrus to determine treatment efficacy and active substance residues in crop after treatment with foliar spraying on citrus trees, comparing treatment using UASS and terrestrial application (quads or tractors).

By carrying out these trials, validated by these protocols, we have sought to differentiate the plant protection treatments carried out with UASS from conventional aerial treatments (with manned aircrafts) framing UASS in a new category: Precision Agriculture.

## OPEX test protocol Operator/pilot and resident/bystander exposure



#### Title of the protocol

Determination of Non-dietary human exposure during treatment by UASS on vines.

#### Purpose of the test

Comparison of OPerator EXposure (pilot and auxiliary activities<sup>1</sup>), bystander & resident drift exposure during UASS application vs. conventional application (EFSA OPEX Guide<sup>2</sup>) during a foliar spray treatment using UASS on vines.

<sup>1</sup> mix and loading (M&L), purge, battery changes and maintenance and cleaning activities <sup>2</sup> doi: 10.2903/j.efsa.2022.7032

# EXPERIMENTAL CONDITIONS

**Crop** Guyot training wine grapevines

#### Location

Commercial plot in Trebujena, Cádiz

#### Plots

Trials on commercial plots with the following specifications:

- > Total plot: At least 8 ha, simulating one day of treatment.
- > Same plot to be treated 4 different days.

#### $N^{\underline{o}}$ of trials

4

Nº of replicates

Nº of applications 1 treatment per trial



- > To measure exposure to the crop protection product during mixing, loading, application, purge, battery changes and maintenance and cleaning activities, treatment using UASS shall be carried out on a plot of at least 8 hectares on at least 4 different days on the same plot.
- > To measure the exposure of bystanders and residents to the crop protection product, a plot of at least 1 ha will be treated with a UASS on 4 different days.

#### Product to be applied

Spintor 480 SC at the authorised rate, adapting the volume of liquid when spraying so that the maximum dose per hectare is never exceeded, with a maximum of 0.15 L/ha. Four applications shall be made with at least 7 days interval between applications and respecting the 14-day preharvest interval for the last application.

#### Execution

The treatment will be carried out by the company FTS. Four trials will be carried out on vineyards, coordinated by the National Institute for Safety and Health at work (INSST) and with the participation of University of Seville (US). Care shall be taken to ensure that different operators apply the treatments in each of the 4 trials.

#### Design

Foliar application to the whole crop. The test scheme is shown below:



#### When to apply

The treatment shall be carried out **in compliance with the authorised conditions of the product** (BBCH  $\geq$ 85 summer). Four applications shall be made with 7-day intervals between treatments, and a preharvest interval of 14 days.

#### Dates

The spraying shall be adapted to the phenological conditions of the crop at the harvesting stage, bearing in mind that early varieties will be used, whose harvesting dates, depending on the weather, might be between the end of July and mid-August.



As the last treatment should be 14 days before harvest, the 4 treatments are planned for the following dates:

- > 1<sup>st</sup> Application: 14 July.
- > 2<sup>nd</sup> Application: 21 July.
- > 3<sup>rd</sup> Application: 28 July.
- > 4<sup>th</sup> Application: 4 August.

#### Acceptable weather conditions

Wind speed: < 3 m/s; no more than 10% of the data should be obtained below 1 m/s. If the wind speed exceeds 2 m/s, the wind direction should be +/-  $30^{\circ}$ . No more than 30% of the data should exceed  $45^{\circ}$ 

- > Temperature: 10- 25 ºC.
- > Relative humidity.
- > Record rainfall 7 days before and 7 days after.
- > Without thermal inversion (measured at two heights or 3 D anemometer).

#### **UASS specifications and operational conditions**

A Dron Hispania Y10 UASS (10 L) will be used, and 10 L of solution/hectare will be applied.

AESA (Spanish Aviation Safety and Security Agency) permits depend on the UASS and its weight. This is important for planning purposes (before the test).

SPECIFICATIONS OF AERIAL EQUIPMENT			
Manufacturer	DRON HISPANIA		
Model	Y10		
Height above the crop	1,50	2,00	
Swath width Nozzle type XR101001VS (*)	3,40	4,10	
Swath width Nozzle type TXVK8 (*)	2,75	2,90	
Swath width according to manufacturer	4,00 - 5,00 m with 4 nozzles / 1,5 - 3 m		

(\*) Estimated values based on the study conducted (flight width assumption + 50% of the distance to the edge).

- > When applying in vineyards, the flight to apply the treatment takes place 1.50-2 m above the crop line.
- > In principle, type XR110001VS nozzles could generate more drift due to their wider operating range.
- > In principle, the TX-VK8 type nozzles could provide better results on 3D crops as they concentrate the spray thanks to their lower exit angle, which results in a narrower spray swath.



#### UASS - equipment configuration:

- > Hydraulic/centrifugal spraying.
- > Nozzles (position, type).
- > In accordance with environmental requirements (CD ISO 23117-1, Jan 2022).

#### UASS - operational conditions:

- > Cross-sectional distribution (LAB: ISO 5682-1)(FIELD: CD ISO 23117-2, Jan 2022).
- > Overlapping working width.
- > Height (distance between nozzles and target): 1.5-2 m above the crop.
- > Travel speed (depends on UASS and nozzle; manoeuvrability): 4-6 km/h, but this will depend on the nozzle.
- > Pre-calibration on test plot (PHS).

## METHODOLOGY USED IN THE EXPERIMENTAL FIELD PHASE

### Equipment calibration (according to OECD Guide No. 105 series on pesticides)

#### Drone calibration (according to OECD Guide No. 105 series on pesticides)

- **1.** Determination of the output rate from the UASS in a given period under normal operating conditions.
  - > Measure the flow rate out of each nozzle on the ground.
  - > Measure the volume of the final product used in flight: measure the initial and final product.
- **2.** Determination of the swath width distribution (pattern) (determination of the swath distribution pattern by measurement of the applied material from suitable collectors).
- **3.** Determination of the maximum effective swath width with the corresponding uniformity of distribution for overlapped swaths.
  - > Optimum flight speed.
  - > Best spraying height (above ground and distance above the crop)
  - > Mean volumetric diameter (MVD) in microns (=optimal droplet size), depending on wind speed and relative humidity.
- 4. Application rate: according to authorization.

#### Operator exposure when mixing, loading and applying

Regarding operator exposure, measurements will be made during treatments involving UASS (drones), and comparisons with conventional application systems will be made using current exposure estimation models.



The INSST will coordinate the tests in collaboration with US.

Exposure from mixing/loading, application, maintenance and cleaning of equipment and any other operation that may involve exposure, e.g. battery change, shall be analysed.

#### Workers

Plant protection treatments using UASS (drones) are generally carried out by two people to optimise tasks, minimise the risks involved and avoid dosage errors, among other aspects. Therefore, one operator will be responsible for mixing and loading the equipment, changing batteries and cleaning and maintaining the UASS in the field, coming into frequent contact with the equipment, while the pilot applicator will be responsible for operating the UASS without coming into contact with the equipment. Therefore, 2 people will be sampled in each test, requiring 8 people to carry out the four tests. Efforts shall be made to select different operators for each test.

#### **Operator PPE (mixing/loading and pilot)**

It is common for operators to wear protective clothing type 4, a full-face mask and double nitrile gloves, avoiding exposure in case of breakage of the outer gloves and cross-contamination. Double nitrile gloves are worn by the operator and the pilot in case the outer glove is torn.

#### Working hours

The 8-hour working day starts with the journey to the farm and ends once the worker has returned. Therefore, they are not applying the treatment for 8 hours. Although there is no specific limit to how long the pilot can operate the drone, breaks must be taken to avoid pilot fatigue, which can also lead to errors in applying the treatment. After the 2nd hour of applying the treatment, a break is taken.

The amount of work done shall be typical of a normal working day and shall be documented in the exposure study. Sampling time shall coincide with the standard time required to carry out the operation. A record should be kept of the time spent on each task.

If, during the working day, personnel need to remove their gloves or coveralls for any reason (food, service, etc.) or if they break, they shall be provided with new gloves or coveralls to continue working. At the end of the working day, the two or more gloves or coveralls worn by the same person will be considered a single sample for analysis.

#### Photographs and recordings

The treatments will be recorded, either using a tripod-mounted camera or a camera on another UASS, and photographs will be taken of all the activities carried out by the workers and the people applying the product.

#### **Typical mixing-loading conditions**

Make the mixture each time directly in a test tube - deposit each time and pour it into the UASS tank.



#### **Application Protocols**

Standard application protocols are based on a checklist for the various tasks and phases of the working day

#### Dosimeters to be analysed

Dosimeters shall be used to compare the potential exposure of the body and the actual exposure of the hands. The results shall be compared with those obtained when applying the EFSA OPEX Guidance for terrestrial applications.

The operator performing mixing/loading, cleaning and maintenance activities shall wear cotton outer coveralls and cotton underwear (long-sleeved T-shirt and long trousers). Both garments shall be analysed to obtain potential exposure. The pilot/applicator shall only use the outer coveralls, as it is considered that the actual exposure due to the spray mist will be negligible. Cotton gloves shall be worn under nitrile chemical protective gloves during mixing/loading, cleaning and maintenance of equipment. The pilot/applicator shall wear cotton gloves. The operator and the pilot shall wear a cotton hood (balaclava).

The dosimeters for the body (coveralls and underwear in mixing/loading and outer coveralls in application tasks, and cotton balaclavas) and the cotton gloves (used under nitrile gloves) used during mixing/loading, and the cotton gloves used by the pilot shall be analysed. The overalls will be divided into 3 sections for analysis: arms, legs, and torso.

Whenever the sampling units are deemed likely to reach their saturation point, they should be replaced.

#### Control samples for fortified testing

To meet the quality requirements and regarding the possible impact of the sampling conditions on the recovery efficiency of the extraction method used, controlled contamination or spiking of the samples shall be carried out with known quantities of the solution applied, which shall be prepared specifically in the field and the same solution shall be used on all the fortified samples. This will be done each day of sampling and at each location.

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The control samples for skin exposure consist of a pair of cotton hand gloves and portions of outer coveralls and inner coveralls used for mixing/loading that have not been exposed to the treatment. At least 3 blank samples shall be taken from each matrix(gloves, balaclava, cotton coveralls) and 3 fortified samples from each matrix (gloves, balaclava, outer and inner cotton coveralls) to which 2 known levels of a solution containing the active substance of the plant protection product used in the study shall be added. The 2 fortification levels shall be established prior to sampling. Only 3 of the 4 samples taken shall be analysed, as 1 additional reserve sample is always taken to be able to replace a sample that might be damaged if necessary.



		Per Operator		Total per Activity 1 operator/applier x 4 days		Samples analyse
		Mix/loading	Application	Mix/loading	Aplication	(TOTALS)
Inner Dosin	neters					
Gloves		1	1	4	4	8
Coveralls	Arms	1		4		4
	Legs	1		4		4
	Torso	1		4		4
Outer Dosir	neters					
Gloves			1		4	4
Coveralls	Arms	1	1	4	4	8
	Legs	1	1	4	4	8
	Torso	1	1	4	4	8
Total samples (mixing/ loading and applying)		7	5	28	20	48

#### Table 1. Number of operator samples

#### Exposure of residents and bystanders

Regarding bystander exposure, measurements will be made during treatments involving UASS (drones), and comparisons with conventional application systems will be made using current exposure estimation models.

- > The INSST will coordinate the tests in collaboration with the US.
- ▶ 6 mannequins/test: 3 adults + 3 children (4 trials = 12 adults + 12 children in total).
- > Positioning of the mannequins: in 1 place, downwind, 5 m from the crop, spaced 10 m apart.

Adult 5 m	Child 5 m	Adult 5 m	Child 5 m	Adult 5 m	Child 5 m
↑↑↑↑↑↑↑↑↑ (wind direction) <b>Plot</b>					



Dosimeters for persons not involved in the treatment: 1 internal dosimeter (*long-sleeved T-shirt* and *long cotton trousers*) and *balaclava* (*head and neck*), and 1 external dosimeter (*short-sleeved T-shirt* and *short cotton trousers*) to compare the potential exposure of applications by UASS with the data obtained using the EFSA model for land applications.

The sample matrices are made of cotton: from the balaclava and from the outer and inner cotton garments.

The inner dosimeters shall be divided into 4 samples:

- > Uncovered arms due to short sleeves.
- > Uncovered legs due to wearing shorts.
- > Torso and upper arms covered by short sleeves.
- > Waist and legs covered by shorts.

There shall be a total of 4 body dosimeters for each mannequin:

- > Outer dosimeter, shorts and short-sleeved T-shirt.
- > Inner dosimeter, arms and legs not covered.
- > Inner dosimeter, torso and upper arms covered by short sleeves and waist and legs covered by shorts.
- > Balaclava

#### Table 2. Number of skin exposure samples Bystanders/Residents

	Per Mannequin	Total Samples 24 Mannequins: (3 adults + 3 children) x 4 days	Samples to analyse
Inner Dosimeters			
Inner dosimeter, arms not covered by short sleeve	1	24	24
Inner dosimeter, legs not covered by shorts	1	24	24
Inner dosimeter, torso and upper arms covered by short sleeve	1	24	24
Inner dosimeter, waist and legs covered by shorts	1	24	24
Balaclava	1	24	24
Outer Dosimeters			
Outer dosimeter, arms, short sleeve, and torso	1	24	24
Outer dosimeter shorts	1	24	24
Total inner + outer	7	168	168



#### Table 3. Number of samples fortified in the field

	Total Samples	Total to Analyse
Fortified samples		
Gloves	6	6
Outer coveralls	6	6
Cotton garments (outer and inner dosimeter on mannequins and inner operator dosimeter)**	6	6
Inner+outer coveralls	6	6
Balaclava	6	6
Total Fortified Samples	30	30

\* As the same matrix is used for operators and residents/bystanders, the same fortifications shall be used when testing together.

#### Table 4. Number of blank samples

	Total Samples	Total to Analyse
Blank samples		
Gloves	3	3
Outer dosimeter	3	3
Cotton garments (outer and inner dosimeter on mannequins and inner operator dosimeter)*	3	3
Balaclava	3	3
Total Blank Samples	12	12

#### Other requirements

The sampling time shall coincide with the standard time required to carry out the operation. The actual sampling time shall be recorded. Whenever the sampling units are deemed likely to reach their saturation point, they should be replaced.

To comply with the quality requirements and concerning the possible impact of the sampling conditions on the recovery efficiency of the extraction method used, controlled contamination or fortification of the sampling media will be carried out using known quantities of the solution applied, which shall be prepared specifically in the field and the same solution shall be used for all fortifications. This will be done for each day of sampling and at each location. The fortified samples for the operator shall be valid for persons not involved in the treatment.



Skin exposure control samples consist of a portion of the balaclava for the head and neck and portions of the outer and inner cotton overalls for the body. At least three samples shall be taken as blank samples (one each from the balaclava, the outer coveralls and the inner coveralls) and two replicates as fortified samples to which two known levels of a solution containing the active substance of the plant protection product used in the study shall be added, both for the balaclava and the inner and outer coveralls. As they coincide with the tests for operator exposure and use the same matrices, it would only be necessary to take a blank sample and one to be fortified from the outer short-sleeve dosimeter at two levels.

After collecting the samples, the control samples shall be fortified on the same day in an area of the same field where the treatments are being applied.

#### Photographs and recordings

The treatments will be recorded, either using a tripod-mounted camera or a camera on another UASS, and photographs will be taken of all the activities carried out.

# METHODOLOGY GOVERNING THE ANALYTICAL PHASE

Validate the analytical methods in accordance with current European guidelines.

All samples taken during the field phase shall be analysed using the previously validated methods.

The matrices shall be made of cotton: gloves, balaclava, trousers and short-sleeved outer T-shirt for mannequins, inner T-shirt and long trousers for mannequins, also used as an inner dosimeter for the operator and outer coveralls for the operator.

The number of samples to be analysed has already been specified in the protocols for operators and for bystanders and residents, including the fortified samples.

The UASS loading samples (Table 5) must be in glass jars properly identified by labels. All textile samples must be cut into pieces, wrapped in aluminium foil, and placed in polyethylene freezer bags. The sample identification indicates the sample type and the previously established identification number.

The stability study previously carried out in the laboratory will determine the ideal conditions for transporting and storing the samples.

#### **Additional Information**

Apply for an aerial spraying permit to use UASS for the trial.



#### **Experimental product label**

Spintor 480

#### **Applicator safety**

People applying the product must wear gloves during mixing/loading and application operations and suitable protective clothing for their torso and legs.

Outdoors: When mixing/loading, chemical protective gloves must be used, and when applying, cleaning and maintaining equipment, chemical protective gloves, clothing (type 3 or 4 according to UNE-EN 14605:2005 + A1:2009), and appropriate footwear must be used. When cleaning the application equipment, the same protective equipment shall be used as during the application of the product.

If a tractor with a closed cab and air filtering device is used, protective equipment is not required if the windows are kept closed.

#### **Worker Safety**

Do not use this product in work involving mechanical activities, which may result in the deterioration of the worker's chemical protective gloves when the worker has to re-enter the farm.

Do not enter the area of the crop until the product is dry.

Re-entry period: is the minimum time in days that must elapse after application and before tasks involving prolonged contact with the crop, longer than 2 hours, can be carried out. For wine grapes, 5 days if the crop is sprayed twice, and 14 days if it is sprayed 3 times. For table grapes and grapevines, 2 days if the crop has been sprayed once and 10 days if it has been sprayed twice.

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#### Environmental risk mitigation

SPe3: To protect aquatic organisms, respect an unsprayed buffer zone to water bodies of 25 m in the case of vines, or 5 m when using 90% drift reduction nozzles.

The product is hazardous to bees and other pollinating insects. Treatments are prohibited during the flowering period and during exudate production (e.g. production of honeydew after aphid attacks). When plants are in flower or in exudate production periods in the field (in the case of mulched strips), their parts above ground must be destroyed or made unattractive to bees before the treatment (by cutting, uprooting or through selective removal of weeds).

SPe8: Dangerous to bees. To protect bees and other pollinating insects do not apply to crop plants when flowering. Do not use where bees are actively foraging.



In case of crops where insects and/or predatory mites (Amblyseius spp., Orius spp., Coccinella, Typhlodromus) or parasites of the Hymenoptera family (Encarsia formosa, Trichogramma brassicae, Aphidius) are to be released for biological control, treat with Spintor 480 SC preferably before the releases to start the crop with the lowest possible pest level. After releasing, apply the product once the populations are well established, and the weather conditions are still suitable for their recovery (spring, summer and early autumn).

#### Method of application

SPINTOR<sup>®</sup> 480 SC acts by ingestion, contact and has translaminar action. Apply at the beginning of the infestation, using standard spraying by tractor or by hand in the open air. Adapt the volume of liquid when spraying so that the maximum dose per hectare is never exceeded.

#### Wine grapevine

To control altica, pyralid, grape moths, thrips and white-spotted beetles, apply 20-25 cc/hL; 3 applications can be made at intervals of at least 7 days, with a maximum of 0.1-0.15 L/ha and with a maximum of 200-500 L/ha in terrestrial treatments.

#### Preharvest Interval

14 days.

#### **Crop Destruction**

Not applicable as the product is authorised for crops.

#### **Expected deadlines**

- > Beginning of the trial: 14 July 2022
- > End of trial: 4 August 2022
- > Final report: 28 February 2023

#### Protocol prepared, reviewed and approved by:

Prepared by INSST (Isaac Abril, Francisco Díaz and Isabel Lara) Review and approval by other members of the Phytodron WG project: AEPLA: M<sup>a</sup> del Carmen Márquez and Silvia de Luna Corteva: Jorge Martínez, M<sup>a</sup> del Carmen Sánchez, Inés Bernaldo and Esther García INIA: José Luis Alonso-Prados, Pilar Sandín España and Carmen López-Goti University of Seville: Manuel Pérez Ruiz and Luis Sánchez Fernández External collaborations with the Phytodron Operational Group project: Crop Life Europe: Edgars Felkers, Kathrin Bürling, Sarah Adham FTS Agroconsulting: Manolo Vargas

## Protocol Efficacy and Residue Trials on Citrus in Andalusia

October 2022



#### Title of the protocol

Establishment of the efficacy of the treatment and the residues of the active substance spinosad after treatment with Spintor Bait (Spintor-Cebo) to control *Ceratitis capitata* by foliar spraying applied by UASS on citrus trees.

#### Purpose of the test

Compare treatment efficacy and residues of the active substance spinosad on fruit after treatment with Spintor-Cebo to control *Ceratitis capitata* by foliar spraying applied with UASS and quads on mandarin trees. Define the distribution of the solution on the vegetation.

# EXPERIMENTAL CONDITIONS

#### Location

A commercial plot of mandarin trees in Benacazón (Seville) affected by the pest.

The trial should preferably be conducted in a field with at least 30% in full production and in areas with a history of severe infestation (*Ceratitis capitata*). All the trees must be of the same variety.

Cultivation conditions (e.g., exposure, slope, soil type, fertilisation, pruning, irrigation) should be uniform across all trial/replication plots and should conform to local agricultural practices.

#### **Plots**

Trials on commercial plots that should be uniform, as square as possible. 4% of the plot shall be selected in the central area where 3 traps with specific commercial attractants (females) shall be placed diagonally. Applications will be made with the intervention threshold according to the Ministry of Agriculture's Integrated Pest Management guidelines: treat when the number of flies caught per trap is 2 flies/trap and per day before veraison or 0.5 after veraison. On citrus, the treatment is applied after the colour changes, with a 7-10 day interval between applications.



#### **Crop and variety**

Clemenville mandarin, with activity of Ceratitis capitata

#### $N^{\underline{o}}$ of trials

1

#### Design

3 treatments of 4 replicates of 1 hectare each in 1 mandarin trial. Each block divided into 3: control (1 ha if possible), conventional terrestrial treatment using a quad (1 ha) and UASS treatment (1 ha).

\* The ideal size of the control sample is 1 ha; if this is not possible, it should be at least 200 m<sup>2</sup>, and the reasons why it was not necessary to reduce the size of the control sample should be justified (compensation, pest pressure, etc.)

This block will be repeated 4 times with a random distribution of the 3 treatments. In the blocks, the distribution of treatments shall be randomised. If samples are to be taken from the centre of the plot, the rest of the plot (60%) will act as a buffer.

The repetitions do not have to be on the same farm if it is the same variety, but in the same period to avoid climatological variability. If the plots are close together, they can be completed in less time.

#### Acceptable weather conditions

The conditions should be as advised by good agricultural practice for any plant protection treatment, i.e. do not treat if:

- > Wind speed is >3m/s(10.8 km/h)
- > Temperatures > 25 ºC
- > Relative humidity < 50%

#### Efficacy

#### **Control sample management**

The plot itself acts as a barrier; the central areas are sampled. The central zones (net plots) should ideally be 100 m apart (although it can be less, between net plots, there should be at least 20 m distance), 5 buffer lines between plot and plot so as not to interfere. Monitor the control plot.

#### **Applications**

Localised application on the crop. In citrus, the treatment is applied after the colour changes, with a 7-10 day interval between applications. Start applications following the farmer's usual methods until harvest. Apply 1 L/ha of Spintor-Cebo, but distributed over the entire surface area (Treatment 1) or in 1 row out of 4 (treatment 2). In aerial applications,





apply to 40% of the surface. The quantity of Spintor-cebo is 0.4 l/ha. Once treatment 1 or 2 has been decided, the same treatment shall be applied to all plots so that they can be considered replicates. Likewise, once the dosage and the solution have been chosen, they shall be the same in all the plots so that they are considered replicates.

#### **Spintor Cebo Authorisation**

Between BBCH75 to BBCH87, apply at least 10 days apart. Preharvest interval: 1 day. Dose in terrestrial application (patch) 1-1.5 L/ha. Spray using between 4 and 10 L of liquid/ha and direct the application to the highest part of the tree and oriented towards the middle, and avoid wetting the fruit. Strips (aerial application) 1-1.25 L/ha. Spray using 6 to 8 litres of liquid/ha, treating only 40% of the area to be protected in strips.

**Method of application**: The spraying solution is applied only to the top of the trees (so only a tiny part of the fruit is treated (<1%). In the case of a quad, instead of spraying, a fine jet is applied. In the case of the UASS, use nozzles at an angle so that the treatment width is not too broad. This is only applied to one side of the trees. It should be documented whether the same row is always treated or whether different rows are treated in the 4 treatments. If the same row is always treated, we would be in the most disadvantaged case from a residue point of view. As the treatment is applied from the top of the tree, the residue samples shall be taken from there (treated area).

**Time of application**: To facilitate obtaining the application permit, as the trials would involve aerial treatments, we should apply treatments under the authorised conditions of the product in October, depending on the weather and the crop's phenology. Under these conditions, there will be fruit and flies.

The number of applications and the date of each application shall be recorded. If available, locally established pest thresholds should be recorded.

For Spintor cebo, the lower the volume of the liquid, the higher the efficacy. Using the UASS, it can be applied at 4 L by calibrating the equipment and with the correct nozzle and speed of treatment.

Type, timing and frequency of evaluations: The growth stage (BBCH) of the crop must be recorded with each application and evaluation. Monitor the pest population using traps as an indicator of efficacy.

#### **Efficacy counts**

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These counts shall be conducted in the central area of each plot before each application.

Fruit assessment: count the % of damaged fruit at harvest on 500 fruit samples per plot, and conduct a midtrial assessment of the fruit on the trees.

Collect the samples and store them for evaluation and ensure the visibility of the attack. If at ambient temperature,



store for at least 5 days to detect recent bites. If in a cooler, the time can be reduced to 3 or 4 days.

Trapping in adult traps: count every 3-4 days one station per thesis, each station with 3 or 4 traps, e.g., they can be:

- 1. 2 Nadel traps with trimedlure, for males and 2 Tri-pack food traps, for females.
- 2. 1 Nadel trap for males and 3 for females.
- 3. 1 pheromone trap + 2 food traps (biammonium phosphate).
- 4. 2 Glass food traps (biammonium phosphate) +1 tripack food trap for females.

Irrespective of size, 500 fruits per treatment must be collected to evaluate efficacy. Source: EPPO standard PP 1/301(1) *Ceratitis capitata* - bait application. The area of assessment and placement must be equal to monitor the pest correctly. If possible, 10 metres between each trap. In each zone of each replicate and treatment, 25 pieces of fruit will be sampled randomly from each tree. Random sample collection. Sampling shall be done on every 2nd tree of the 40 trees in the 10% of 1 ha, on 3 or 4 levels.

**Phytotoxicity**: The crop shall be examined for the presence of phytotoxic effects caused by the product applied. In addition, any positive effects should be considered. The type and duration of such effects on the crop must be recorded, and whether or not there are any effects must also be recorded.

**Data on other plant protection products**: It is advisable to obtain a record of the products applied to the test plots. Potential interference with these should be avoided.

**Weather data**: On the days before and after spraying (e.g. 7 days before and 7 days after), record any weather data that may affect the development of the crop and/or pest and the performance of the plant protection product. This usually includes rainfall, relative humidity, and temperature data.

All data should be recorded at the test site preferably but may be obtained from a nearby weather station. Its location and distance from the test plot site should be recorded.

Any deviation from the design given in the EPPO standards shall be justified.

#### **Residue considerations**

In citrus, the preharvest interval for Spintor Cebo is 1 day. Therefore, the last treatment should be 1 day before harvest.

#### Residues

The study will aim to compare the residue levels found between the UASS and terrestrial applications. The trials shall follow European guidelines (OECD Guide n. 509, Crop Field Trials). To rule out cross-contamination, samples shall be taken from a control plot. According to the experimental design, there is an untreated control and two types of application. Therefore, untreated control samples; terrestrial treatment samples and UASS treatment samples should be submitted.



Following the preharvest interval after the last application (1 day), 1 kg of the sample (fruits) shall be collected from different sites within the plots, avoiding the edges, from a minimum of 4 different trees among the **treated trees**. Whole mandarins must be harvested from the high areas, as this is where the product will be applied. Ensure that the manual sample collection process has access to this height, and to the inner and outer areas of the trees.

The representative sample of each treatment shall be frozen as soon as possible (within a maximum of 24 h) and kept refrigerated until frozen. Shipment to the laboratory shall be carried out while maintaining the cold chain. The maximum stability periods of the active substance when frozen in matrices with high acid content should be taken into account (18 months at -20°C for Spinosyn A and D)(EFSA Journal 2018; 16(4):5252).

Substances included in the definition of residue for monitoring purposes shall be analysed in the samples (*monitoring* or *enforcement*): *Spinosad*, *sum* of *spinosyn* A and *spinosyn* D (Reg. 2015/603).

If 8 independent tests are unavailable, the individual values of the different types of treatments shall be compared.

#### Specifications of Reference Equipment and Operational Conditions (Conventional Equipment)

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#### Terrestrial Sprayer Specifications: Quad with motor pump and sprayer

- > Air support, geometry
- > Operational nozzles (number, type, pressure)
- > Process (boom height, pressure, airflow, speed)
- > Prior calibration on each test plot (PHS)

Speed of application: 6 Km/h

#### UASS specifications and operational conditions

A Dron Hispania Y10 UASS (10 L) will be used. A full cone spray nozzle with an orifice between 0.8 and 1 mm, low-drift fan nozzle AI9504EVS, will be used, the results of which give adequate results. To limit the swath width, they shall be positioned at an angle to the direction of travel.

AESA (Spanish Aviation Safety and Security Agency) permits depend on the UASS and its weight. This is important for planning purposes (before the test).





SPECIFICATIONS OF AERIAL EQUIPMENT			
Manufacturer	DRON HISPANIA		
Model	Y10		
Height above the crop	1,50	2,00	
Swath width according to manufacturer	4,00 - 5,00 m with 4 nozzles / 1,5 - 3 m		

Only one nozzle, the central nozzle, shall be used. Due to the application conditions of the product Spintor-cebo, it is to be applied in a jet.

In the case of the quad, to avoid fruit staining, it is better to spray thinly over the trees so that it does not touch and stain the fruit. The upper part of the tree has few fruits.

#### UASS - equipment configuration:

- > Hydraulic spraying.
- > Nozzles (position, type).
- > In accordance with environmental requirements (CD ISO 23117-1, Jan 2022).

#### UASS - operational conditions:

- > Cross-sectional distribution (LAB: ISO 5682-1)(FIELD: CD ISO 23117-2, Jan 2022).
- > Overlapping working width.
- > Height (distance between nozzles and target): 1.5-2 m above the crop.
- > Travel speed (depends on UASS and nozzle; manoeuvrability): 4-6 km/h but depending on the nozzle, up to 25 km/h (with UASS).
- > Pre-calibration on test plot (PHS).



#### METHODOLOGY USED IN THE EXPERIMENTAL FIELD PHASE

### Equipment calibration: (according to OECD guide No. 105 series on pesticides)

#### Test 1 (calibrated reference equipment)

A baseline characterisation of the reference equipment (hydro-pneumatic sprayer) shall be carried out by means of the following:

- 1. Vertical positioning test: adjustment of nozzles to the height of the crop.
- **2.** Transverse drift test.



#### Test 2 (UASS calibration) (according to OECD Guide No. 105 series on pesticides)

- **1.** Determination of the output rate from the UASS in a given period under normal operating conditions.
  - > Measure the flow rate out of each nozzle on the ground.
  - > Measure the volume of the final product used in flight: measure initial and final product.
- **2.** Determination of the swath width distribution pattern by measurement of the applied material from suitable collectors.
- **3.** Determination of the maximum effective swath width with the corresponding uniformity of distribution for overlapped swaths.
  - > Optimum flight speed.
  - > Best spraying height (above ground and distance above the crop)
- 4. Application dose: according to authorization.

### METHODOLOGY GOVERNING THE ANALYTICAL PHASE

A multi-residue method (QuEChERS) shall be developed to extract from mandarin samples the constituents included in the residue for monitoring purposes, as laid down in Reg. 2015/603 (monitoring or enforcement: Spinosad, sum of spinosyn A and spinosyn D) and subsequent detection and quantification by LC-MS/MS. The validation of the method shall be performed according to the current European guidelines (SANTE/12682/2019 and SANTE/2020/12830, Rev. 1). The matrix effect shall be checked by performing calibration lines in a solvent and matrix blank. The linearity shall be determined, in a solvent or matrix blank according to the procedure described below in a range of concentrations appropriate to the expected levels (30% LOQ to 20% above the highest expected value). If the range of concentrations is extremely wide, 2 calibration lines can be made. In case of very high concentrations, the samples can be diluted to be quantified. The method's accuracy shall be determined by the recoveries of the compounds included in the residue definition in matrix blank samples spiked at two concentration levels (LOQ and 10xLOQ); 5 replicates at each concentration level and 2 matrix blank replicates shall be performed. The precision of the method shall be determined based on the %RSD values of these replicates. The specificity and selectivity of the method shall be determined by monitoring two characteristic ions resulting from fragmentation in the mass spectrometer (MS/MS).

Field samples shall be partially thawed, cut and crushed prior to analysis. Once the sample has been homogenised, a portion of the sample is taken for analysis according to the following **pre-viously validated method. Each sample shall be analysed in duplicate.** 

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**Crop Destruction** Not applicable as the product is authorised for crops



#### Additional Information

Apply for an aerial spraying permit to use UASS for the trial.

Dilutions of SPINTOR-CEBO should be applied within 12 hours of preparation.

It is important not to store SPINTOR-CEBO spray dilutions in sealed containers.

#### Preharvest intervals

Citrus fruit: 1 day.

#### **Dates**

- > Beginning of the trial: 14 October 2022
- > End of the trial: 25 November 2022 (residue sample collection)
- > Final Report: 28 February 2023

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