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Efficacy of a postharvest treatment aiming at eradication of all developmental stages of *Tecia solanivora* in ware potatoes

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Abstract

The European Commission requested the EFSA Panel on Plant Health to prepare and deliver a scientific opinion on the efficacy of a postharvest treatment aiming to eradicate all developmental stages of Guatemalan potato tuber moth *Tecia solanivora* (Lepidoptera: Gelechiidae) in ware potatoes. The Panel evaluated the scientific publication describing the elevated CO_2 treatment, which was defined as: 10-day exposure to 30% CO_2, 20% O_2 and 50% N_2 in controlled atmosphere at 17°C on the variety Negra Yema de Huevo (Papas Antiguas de Canarias, PDO potatoes, *Solanum chaucha*). In the scientific publication, the treatment was applied under semi-commercial and commercial conditions on artificially and field-infested tubers. The effect of the pest developmental stage on the treatment efficacy was investigated with artificial infestation of potato tubers with eggs, neonate and second instar larvae. Pupae and adults were placed in separate containers during the treatment. However, the third and fourth larval instars were not investigated. Further limitations were the sample size in the experiments, the mortality rate in the control group and the unknown level of infestation of the naturally infested potato tubers. It was not possible to evaluate the degree of pest freedom due to incomplete data on the conditions of production, i.e. the infestation level in the field. The Panel was able to conclude that although no surviving insects were observed in the performed experiments, the statistical evaluation of the presented results from the commercial trial indicate that it cannot be excluded that insects would survive the treatment. For example, based on the data provided the 95% confidence interval of the survival rate for eggs was: 0%–0.453%.

Keywords: potato, *Tecia solanivora*, CO_2 treatment, European Union, plant health, plant pest, quarantine

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1. Introduction

1.1. Background and Terms of Reference as provided by European Commission

1.1.1. Background

Regulation (EU) 2072/2019[1] provides for a prohibition of introduction of ware potato tubers originating in third countries, in point 17 of Annex VI. Point 17 (b) also provides two conditions to be fulfilled by a third country requesting a derogation to this prohibition.

The first condition provided in point 17(b)(i) is that the third country must be included in the list of eligible third countries. The second condition provided in point 17(b)(ii) is that either the third country is recognized as free from *Clavibacter sepedonicus* or that its legislation is recognized as equivalent to the EU rules concerning protection of against *C. sepedonicus*.

In addition, special requirements for introduction of ware potatoes are provided in Annex VII of the Regulation, as regards several quarantine pests listed in its Annex II. Concerning specifically the Guatemalan potato moth (*Tecia solanivora*), which is not established in the Union territory, point (15) of Annex VII provides for the requirement of an official statement that tubers originate in:

- a country where *T. solanivora* is not known to occur; or
- an area free from *T. solanivora*, as established by the national plant protection organization.

In 2022, Spain submitted justifying documents, in addition to an earlier request to the Commission for a derogation to the prohibition, for introduction of ware potatoes from the Canary Islands. The phytosanitary status of the area is considered as similar to the one of a third country under the Regulation 2016/2031 pursuant to Article 1(3). However, the Canary Islands fulfil the first condition provided in point 17(b)(i) of Annex VI to Regulation 2072/2019, i.e., they are included in the list of third countries eligible for a derogation to the prohibition. Nevertheless, *T. solanivora* is widespread in the Canary Islands. Therefore, Spain provided a specific postharvest treatment aiming at eradication of *T. solanivora* before imports into EU territory. The treatment is proposed as an alternative standalone measure to the current requirements against *T. solanivora* provided in point 15 of Annex VII to Regulation 2019/2072.

In support of the request, several background documents were submitted to the Commission, including a peer-reviewed publication submitted to the Commission by the Spanish National competent authority in February 2022.

1.1.2. Terms of Reference

In view of the above and in accordance with Article 29 of Regulation (EC) No. 178/2002,[2] the Commission asks EFSA to provide a scientific opinion.

Taking into account the technical information and the results of the treatment provided in peer-reviewed publication provided by Spain, EFSA is requested to assess the likelihood of pest freedom from *T. solanivora* in ware potatoes after the treatment.

2. Assessment

2.1. Data and methodologies

2.1.1. Data provided by EC and authors of the scientific publication

The Panel considered all the data and information provided by EC and additional information provided by Dr Maria Gloria Lobo, author of the scientific publication submitted as Annex II on 18 and 27 October, after EFSA's request. The documents are managed by EFSA.

[1] Regulation (EC) number 2019/2072 of 28 November 2019 establishing uniform conditions for the implementation of Regulation (EU) 2016/2031 of the European Parliament and the Council, as regards protective measures against pests of plants, and repealing Commission Regulation (EC) No 690/2008 and amending Commission Implementing Regulation (EU) 2018/2019. OJ L 319, 10.12.2019, p. 1–279.

[2] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, pp. 1–24.

The overview of the information received are in Table 1.

Table 1: Overview of the submitted documents

No.	Overview of contents	Filename
1	Mandate	COM-ARES- 6277020-to EFSA_Mandate Tecia solanivora.pdf
2	Annex I Background and Terms of Reference	Annex I_BackgroundToR_treatment against Tecia solanivora.pdf
3	Annex II Scientific publication (Lobo et al., 2021)	Annex II Scientific publication.pdf
4	Additional information received on 18 October 2022	Agosto 2014.xlsx Diciembre 2014.xlsx Evaluación Ensayo 1 tratamiento cuarentenario postcosecha contra polilla guatemalteca.docx Evaluación Ensayo 2 tratamiento cuarentenario postcosecha contra polilla guatemalteca.docx Evaluación insecto Ensayo 1.xlsx Evaluación insecto Ensayo 2.xlsx Oral presentation EFSA 11 october 2022.pdf Protocolo exportación papa (enero 2016).docx Pictures and videos: Adults.JPG CO2 treatment in chambers.MP4 Larvae coming out potatoe.mp4 Photo treatment in chamber.JPG Potato low affected peeled.JPE Potato low affected.JPE Potatoes at the beginning of the treatment.JPG Potatoes at the end of the treatment.JPG Rearing camara.JPG
5	Additional information received on 27 October 2022	Answers to specific questions enclosed in the email. *Tecia Solanivora* reply.msg

The scientific publication and supporting data provided by Dr Gloria Maria Lobo formed the basis of this opinion.

2.1.2. Evaluation methodology used by EFSA

The Panel evaluated the scientific publication (Annex II) entitled 'Development of a Quarantine Postharvest Treatment against Guatemalan Potato Moth (*Tecia solanivora* Povolny)' submitted by the Spanish National competent authority in February 2022 to European Commission with reference to the following:

- Guidance on methodology for evaluation of the effectiveness of options for reducing the risk of introduction and spread of organisms harmful to plant health in the EU territory (EFSA, 2012).
- Pest categorization of *Tecia solanivora* (EFSA, 2018).
- ISPM No.44: Requirements for the use of modified atmosphere treatments as phytosanitary measures (IPPC and FAO, 2021).
- Additional scientific literature as relevant.

2.1.3. Methodology

When developing the opinion, the Panel followed the EFSA Guidance on methodology for evaluation of the effectiveness of options for reducing the risk of introduction and spread of organisms harmful to plant health in the EU territory (EFSA 2012).

According to EFSA guidance, the checklist of all requirements was created and used by the experts commissioned to analyse this request. It included two parts:

- assessment of the effectiveness of the presented option in reducing pest infestation in potatoes under laboratory conditions.

- assessment of the effectiveness of the presented option in reducing pest infestation in potatoes under operational (commercial) conditions.

The Panel evaluated then the following factors that may have an effect on the treatment's efficacy:

- Number, size and variety of potato.
- Age and the developmental stages of *T. solanivora* at the moment of treatment.
- Density of pest in potato tubers to be treated.
- Variation in response to the treatment among pest populations.
- Efficacy tests under practical conditions and natural infestation.

The Panel evaluated limitations of the experimental setup and the statistical methodology presented in the scientific publication (Lobo et al., 2021) and performed a re-evaluation of the data as follows:

Statistical methods for assessing the effectiveness of the treatment to reduce pest infestation were applied. A conclusion on the likelihood that *T. solanivora* will survive the treatment was re-evaluated using 95% confidence intervals on the results of the semi-commercial and commercial experiments. Clopper–Pearson approximations were used for the total sample size and corrected to the level of survival in the control groups. A simulation using @RISK 7.6 software was done to integrate the survival of the treatment over the random survival rate in the control groups.

2.2. Application of the controlled atmosphere as a phytosanitary treatment

Controlled atmospheres enriched with carbon dioxide (CO_2) have been developed to control insect pests that affect storage foods (grains, fruits, seeds or dry fruits). Elevated CO_2 levels cause insects to open their spiracles resulting in insect death from water loss. At CO_2 concentration above 10%, spiracles remain permanently open producing toxic effects on the nervous system (Nicolas and Sillans, 1989). Lepidopteran larvae in advanced stages are more tolerant to high CO_2 levels than young larvae (Mitcham et al., 2006). ISPM 44 provides guidance on modified atmosphere treatments and their application as phytosanitary measures. It identifies parameters to be considered when applying modified atmosphere treatments but it does not give guidance on efficacy levels to be reached.

2.3. Description of elevated CO_2 treatment

The submitted scientific publication (Lobo et al., 2021) proposes a postharvest treatment of potatoes to eliminate all developmental stages of the *T. solanivora* by the application of a controlled atmosphere enriched in carbon dioxide. In the publication four experiments with *T. solanivora*-infested potato tubers are described.

The first two experiments were performed *in vitro* in laboratory conditions and investigated the effect of a duration of exposure and different levels of CO_2 on mortality of the different developmental stages of the *T. solanivora*. Based on the results of these experiments, the proposed post-harvest treatment of ware potatoes of the variety Negra Yema de Huevo, (Papas Antiguas de Canarias, Protected Designation of Origin (PDO), *Solanum chaucha*) with a diameter of 28–45 mm was set at a 10-day treatment with 30% CO_2, 20% O_2 and 50% N_2 controlled atmosphere at 17°C (semi-commercials) (Figure 1).

In the second two experiments (commercial scale trials), the proposed post-harvest treatment schedule was tested under practical conditions with 12 European pallets of ware potato tubers in dedicated treatment chambers (Figure 2). According to Dr Lobo (pers comm on 18/10/2022 and 27/10/2022), only one commercial trial was using variety Negra Yema de Huevo potatoes while the second trial used another potato variety Druid (Irish potato *S. tuberosum*) with size of 45–65 mm. The proposed measure is therefore evaluated primarily for the variety Negra Yema de Huevo (PDO potatoes) size 28-45 mm. The semi commercial trials were conducted with one European pallet of 384 kg potato tubers (PDO) in a controlled atmosphere chamber of 11.75 m^3. The commercial trials were conducted with 12 European pallets in a controlled atmosphere chamber of 38.5 m^3. The first trial used 4,608 kg of PDO potatoes and the second one used 7,920 kg of potatoes of the variety Druid.

The experiments were performed in 17 $\pm$ 2°C as indicated in Material and Methods (Sections 2.5 and 2.6 of the scientific publication), while in the abstract and in the conclusion, it is reported that the temperature was set to 18 $\pm$ 2°C. The Panel concluded that the treatment is defined with 17°C.

After the controlled atmosphere treatment for 10 days, experimental tubers were not dissected but evaluated for 8 weeks and the insect survivors counted.

The Panel decided to evaluate the experimental setup (Sections 2.5 and 2.6 of Lobo et al., 2021) and results (presented in Tables 4 and 5 of Lobo et al., 2021) of the post-harvest treatment in the (semi)-commercial trials.

Figure 1: Semi-commercial trial (Lobo et al., 2021). © Lobo 2021

Figure 2: Commercial trial (Lobo et al., 2021). © Lobo 2021

2.4. Relevant biological parameters

Information on the biology and development under different conditions of *T. solanivora* can be found in Pest categorization of *T. solanivora* section 3.1.2 (EFSA, 2018).

The relevant aspects to the evaluation of the proposed treatment are summarised below:

The mean fecundity is around 200 eggs per female. According to Torres (1989) and EFSA (2018), females oviposit eggs individually or in small clusters on the soil surface or when in storage onto the skin of exposed potato tubers (EPPO, 2021). After egg hatching, larvae penetrate through the potato skin and bore irregular galleries that run into the tubers. Development of the pest usually occurs inside a potato tuber and goes through four larval stages (Hilje, 1994). The last larval instar usually exits the potato tuber to pupate, although pupation has been observed to occur inside potato tuber.

The pest's lifespan as well as eggs and larval development depends on the temperature. According to Torres et al. (1997) and EFSA (2018), the mean duration of developmental stages at 15.5°C is: 15, 29, 5 and 26 days for eggs, larvae, prepupae and pupae, respectively. Adult males live for 16 days, while adult females live for about 20 days.

It has also been recorded that development time at 20.4°C was 8.9 days for eggs, 24.4 days for the larval stages and 14–16 days for pupae (data used to develop the phenology model published by Schaub et al., 2016, as provided by J. Kroschel).

Under the experimental conditions reported in the scientific paper, the development time of the eggs to hatch was 10 days (pers comm with Dr Lobo and additional information provided).

The development of the larval stages under the experimental conditions was not investigated (Lobo et al., 2021) and is thus unknown but estimated by the Panel to be about 30 days to complete the four larval stages.

3. Evaluation

3.1. General comment

The design of the controlled atmosphere treatment with the elevated CO_2 in the submitted scientific publication and additional information provided were in agreement with ISPM No. 44. However, the Panel did not evaluate the monitoring of the practical implementation and level of official supervision of the treatment. It only focused on the evaluation of the presented experimental protocols, statistical significance and biological relevance of the presented results.

3.2. Assessment of the factors that may have an effect on the treatment efficacy

3.2.1. Number, size and variety of potato

The effect of size of the potato tubers on the efficacy of the treatment was not systematically investigated. The proposed measure is evaluated primarily for Negra Yema de Huevo of diameter 28–45 mm. For the semi-commercial trial, 100 potato tubers were artificially infested with eggs and 180 tubers for the commercial trial. Similar number of potato tubers were used in these trials to study effectiveness of the treatment for larvae. The number of naturally infested tubers was not determined in the semi-commercial trials, for the commercial trials 25 and 15 tubers per box per pallet were used for the first and second trial respectively.

3.2.2. Age and developmental stages evaluated in experiments

The developmental stage of the pest at the moment of the treatment was investigated using artificial infestation of potatoes with eggs or neonate and second instar larvae. Potatoes infested with larvae were obtained by infesting them with eggs and incubating them for 10 days. Pupae and adults were placed in the pallets in separate containers.

According to Lobo et al. (2021) and additional information provided, at the start of the experiment eggs were 1–2 days old. Before placing them on the potato tubers, they were examined, and only viable eggs were picked. Eggs hatched 10 days after infestation. Therefore, the panel assumes that the larval age was 1–2 days (neonate larvae) at the start of the treatment. The developmental stage of the larvae at the end of the pre-treatment incubation period was not verified. However, based on the age of the larvae at the start of the incubation (1–2 days) and an incubation of 10 days and the low developmental temperature of 17°C, the Panel estimates that only the first larval instars (first and second) were present on the treated potato tubers. Therefore, the effect of treatment on older stage larvae (third and fourth) inside the potato tubers was not investigated. According to the authors, the advanced stage larvae were probably present in the experiments with field collected potatoes classified by farmers as 'infested potatoes'; nevertheless no tests (including dissection) were performed to estimate the infestation rate and stage of the larvae inside these tubers.

3.2.3. Density of the pest in potatoes to be treated

Two pest densities were tested. The number was 5 eggs per potato in the semi-commercial trials and the number was 15 eggs per potato tuber in commercial trials.

To obtain experimental potatoes infested with young larva, individual potatoes were inoculated with 5 (semi commercial trail) or 15 eggs (commercial trial) for 10 days (Section 3.2.3).

The actual number of neonate and second instar larvae per potato at the moment of treatment was not verified.

The density of larvae present in field-collected potatoes was unknown and only qualitatively based on classification criteria by the farmers ('healthy' and 'infested').

3.2.4. Variation among populations

Preliminary experiments were conducted with laboratory-reared insects, and the semi-commercial scale experiments were conducted with laboratory-reared insects and field infested potatoes.

3.2.5. Efficacy tests under commercial and natural infestation conditions

The experiments evaluated were conducted on a commercial scale with controlled atmosphere treatment chambers with a maximum volume of 38.5 m^3. In addition to the artificially infested potatoes, naturally infested potatoes were placed in boxes used for export and tested.

3.2.6. Limitations of experimental setup and statistical methodology

The authors published only the summary of final outcomes, no descriptive statistics on the replications such as standard errors (SEs) were shown for the semi-commercial and commercial trials in the scientific publication. On EFSA request, raw data were provided.

3.2.6.1. Limitations of the experimental setup

The Panel concluded that the experimental condition in the semi commercial and commercial trials were not optimal for the insects. There was an unusual mortality in the control group (no CO_2 treatment) shown by large variation in survival rate of eggs (20–89%), larvae (45–98%) and pupae (33–96%).

During the semi-commercial trials, the mortality in the control group was:

- in the first control experiment 224 eggs out of 500 have survived,
- in the second trial only 98 eggs out of 500 have survived,

whereas in the commercial trials:

- in the first experiment 261 eggs out of 450 have survived in the control,
- in the second trial with the variety Druid 399 eggs out of 450 have survived.

The survival of larvae in the control group during the experiments for two repetitions of semi commercial was as follows

- in the first control experiment 227 larvae out of 500 have survived,
- in the second trial only 235 larvae out of 500 have survived,

whereas in the commercial trails

- in the first experiment 279 larvae out of 450 have survived,
- in the second trial with the variety Druid 441 larvae out of 450 have survived.

The argument used by the authors for the reason why the experiments mainly focused on young larvae (scientific publication and pers comm) is that only 'healthy' potatoes with no symptoms of larval damage are presented for export certification. These potatoes for export are assumed to have only eggs or neonate larvae of *T. solanivora*, if present. There was no further evidence shown that these are the only stages that would be present inside the potatoes. The preselected potato tuber classified by farmers as healthy were infested (as reported in Lobo et al., 2021); therefore, the preselection sorting may not provide certainty that all infested tubers will be detected prior to the proposed treatment.

Elevated CO_2 treatment effect has been studied on pupae but only outside tubers (small vase, placed in the box with tubers) although pupation has been observed to also occur inside potato tubers, where pupae are more protected from the CO_2 treatment.

A potato tuber was infested with a number of eggs or larvae, and these infested potato tubers were placed in one box. Analyses were done considering eggs or larvae as statistical units but could also have been done using the tuber or box of potatoes as a statistical unit. This would result in reduction of independent repetition units to a very low number for the treatment.

3.2.6.2. Assessment of the level of the pest survival after elevated CO_2 treatment

The efficacy of the treatment was demonstrated with zero surviving insects in the experiments. Therefore, the evaluation of the certainty is based mainly on the sample size (i.e. number of insect individuals treated). Calculations were made for the:

a) Number of *T. solanivora* placed in the experiment.
b) Number of *T. solanivora* that would have survived the experiment without treatment (estimated from the control group).
c) The simulated number of possible survivors in the treated group (combined results).

Using the Clopper–Pearson approximation of the 95% confidence intervals gives following results: Combined results for the semi-commercial trials, are:

- Survival rate of *T. solanivora* as eggs: 0%–0.928%.
- Survival rate of *T. solanivora* as larvae: 0%–0.647%.
- Survival rate of *T. solanivora* as pupae: 0%–0.927%.
- Survival rate of *T. solanivora* as adults: 0%–3.242%.

Combined results for the commercial trials, are:

- Survival rate of *T. solanivora* as eggs: 0%–0.453%.
- Survival rate of *T. solanivora* as larvae: 0%–0.415%.
- Survival rate of *T. solanivora* as pupae: 0%–4.389%.
- Survival rate of *T. solanivora* as adults: 0%–3.272%.

The effective number of pupae (in commercial trials) and adults subjected to CO_2 treatment was small and subsequently gave a wide confidence interval.

For the judgement on the likelihood of pest freedom, the additional boxes of healthy/infested potatoes provided by the farmer in the control groups give some indications. From 2 to 47 *T. solanivora* (as provided in the raw data by pers comm), insects (the developmental stage was not specified in the data) were observed per untreated box. The level of infestation of the field collected tubers was not determined before the treatment. Tubers were not dissected, and it is not known at what stage the insect died.

The assessment of the final risk of importing potatoes was not further evaluated.

4. Conclusions

The Panel concludes that:

The effect of size and variety of potato tubers was not systematically investigated.

The developmental stage of *T. solanivora* at the moment of treatment was investigated with artificial infestation of potatoes with eggs, neonate and second instar larvae. The effect of the treatment was also tested on pupae and adults, but not on the third and fourth larval instars.

Only two pest densities were tested (in the semi-commercial trials 5 eggs per potato tuber and 15 eggs per potato tuber in the commercial trials).

The Panel observed a high mortality rate in the control group (no CO_2 treatment), which indicates that experimental conditions were not optimal for the insect. Furthermore, this leads to an overestimation of the efficacy of the treatment.

The effect of the treatment was not systematically investigated with field collected potato tubers and with unknown pest pressure.

The actual number of insects affected by the treatment in the experiments with artificial infestation was limited and the level of infestation on the production site and before export was unknown. Therefore, it was not possible to estimate the risk of infested consignments after the treatment.

Although no surviving insects were observed in the performed experiments, the statistical evaluation of the presented results from the commercial trial indicate that it cannot be excluded that insects would survive the treatment. For example, based on the data provided the 95% confidence interval of the survival rate for eggs was 0%–0.453%.

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Abbreviations

EPPO	European and Mediterranean Plant Protection Organization
FAO	Food and Agriculture Organization
IPPC	International Plant Protection Convention
ISPM	International Standards for Phytosanitary Measures
PDO	Protected Designation of Origin
PLH	Plant Health

Glossary

Control (of a pest)	Suppression, containment or eradication of a pest population (FAO, 1995, 2017)
Entry (of a pest)	Movement of a pest into an area where it is not yet present, or present but not widely distributed and being officially controlled (FAO, 2017)
Establishment (of a pest)	Perpetuation, for the foreseeable future, of a pest within an area after entry (FAO, 2017)
Impact (of a pest)	The impact of the pest on the crop output and quality and on the environment in the occupied spatial units

Introduction (of a pest)	The entry of a pest resulting in its establishment (FAO, 2017)
Measures	Control (of a pest) is defined in ISPM 5 (FAO 2017) as "Suppression, containment or eradication of a pest population" (FAO, 1995). Control measures are measures that have a direct effect on pest abundance. Supporting measures are organisational measures or procedures supporting the choice of appropriate risk mitigation measures that do not directly affect pest abundance.
Pathway	Any means that allows the entry or spread of a pest (FAO, 2017)
Phytosanitary measures	Any legislation, regulation or official procedure having the purpose to prevent the introduction or spread of quarantine pests, or to limit the economic impact of regulated non-quarantine pests (FAO, 2017)
Protected zone	A Protected zone is an area recognised at EU level to be free from a harmful organism, which is established in one or more other parts of the Union.
Quarantine pest	A pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled (FAO, 2017)
Regulated non-quarantine pest	A non-quarantine pest whose presence in plants for planting affects the intended use of those plants with an economically unacceptable impact and which is therefore regulated within the territory of the importing contracting party (FAO, 2017)
Spread (of a pest)	Expansion of the geographical distribution of a pest within an area (FAO, 2017)