

SCIENTIFIC OPINION

Statement on the dossier for a derogation request of the US authorities concerning cold-treated strawberry plants intended for planting¹

EFSA Panel on Plant Health (PLH)^{2,3}

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ABSTRACT

Following a request from the European Commission, the EFSA Panel on Plant Health examined the report “Evaluation of Strawberry Nursery Plant Cold Treatments on Survival of the Whitefly, *Bemisia tabaci*”, submitted to the European Commission by the United States Department of Agriculture (USDA). The report serves as technical justification for a derogation from requirements listed in Annex IV, Part A, Section 1, point 46 of the Council Directive 2000/29/EC. The Panel concluded that due to shortcomings in the report itself and in the experimental design of the study, this report does not demonstrate the effectiveness of the cold treatment proposed for the elimination of *B. tabaci* from strawberry plant consignments prepared for shipment to the EU. In addition, the Panel conducted a preliminary review of the available literature on viruses of strawberry and concluded that no *B. tabaci*-transmitted viruses are currently known to infect strawberry and that no viruses of *Fragaria* listed in European Council Directive 2000/29/EC are known to be transmitted by *B. tabaci*. However, adult whiteflies of *B. tabaci* on strawberry consignments can carry plant viruses irrespective of whether strawberry is a host plant for these viruses and thus represent a pathway for the introduction of non-European viruses. There is also some uncertainty concerning the extent to which known or unknown *B. tabaci*-transmitted viruses could infect strawberry. Irrespective of the presence of *B. tabaci*, strawberry transplants from the US may represent a pathway for the introduction of any non-European virus (and other potentially harmful organisms) of *Fragaria*.

KEY WORDS

Bemisia tabaci, viruses of strawberry, United States, EU, cold treatment, *Fragaria*, pallidosis.

SUMMARY

Following a request from the European Commission, the Panel on Plant Health was asked to provide a scientific opinion on the report “Evaluation of Strawberry Nursery Plant Cold Treatments on Survival

1 On request the European Commission, Question No EFSA-Q-2009-00937, adopted on 08 December 2009.

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3 Acknowledgement: EFSA wishes to thank the members of the Working Group for the preparation of this opinion: Thierry Candresse, Olia Evtimova Karadjova, Johan Coert van Lenteren and Stephan Winter and EFSA’s staff member Sharon Cheek for the support provided to this EFSA scientific output.

of the Whitefly, *Bemisia tabaci*⁴ submitted to the European Commission by the United States Department of Agriculture (USDA) as technical justification for a derogation from requirements listed in Annex IV, Part A, Section 1, point 46 of the Council Directive 2000/29/EC⁵.

The Panel reviewed the report to indicate methodological issues of concern and evaluated the effectiveness of the treatment proposed for the elimination of *B. tabaci* whiteflies from consignments to be shipped to the EU. In addition, the Panel clarified which viruses listed in the Annexes of Council Directive 2000/29/EC can be transmitted by strawberry plants and by *B. tabaci*.

The Panel concludes that due to the poor quality of the report submitted and to serious shortcomings in the experimental design, the report does not demonstrate the effectiveness of the cold treatment proposed. Although there is potential for the development of a cold treatment protocol for the elimination of *B. tabaci* from strawberry plant consignments, reliable mortality estimates for the proposed cold treatment of 28 degrees Fahrenheit (-2.2 degrees Celsius) for 2 weeks cannot be derived from this study.

No *B. tabaci*-transmitted viruses are currently known to infect strawberry and no viruses of *Fragaria* spp. listed in Directive 2000/29/EC are known to be transmitted by *B. tabaci*. However, adult whiteflies of *B. tabaci* on strawberry consignments can carry plant viruses irrespective of whether strawberry is a host plant for these viruses and thus represent a pathway for the introduction of non-European viruses. There is also some uncertainty concerning the extent to which known or unknown *B. tabaci*-transmitted viruses could infect strawberry. Two whitefly-transmitted viruses have been recently confirmed as associated with strawberry pallidosis disease occurring in the US: *Beet pseudo-yellows virus* (BPYV) and *Strawberry pallidosis associated virus* (SPaV) but are only known to be transmitted by the greenhouse whitefly *Trialeurodes vaporariorum* and not by *B. tabaci*.

Strawberry plants for planting are vegetatively propagated and are thus subject to infection by viruses. Irrespective of the presence of *B. tabaci*, strawberry transplants from US may represent a pathway for the introduction of any non-European viruses (and other potentially harmful organisms) of *Fragaria*. This applies for example to SPaV, which is not reported as present in the EU and, if introduced, could be further spread by its vector, the glasshouse whitefly *T. vaporariorum*, which is widely established in the EU.

⁴ Available at <http://registerofquestions.efsa.europa.eu/roqFrontend/questionsListLoader?panel=ALL>

⁵ Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. OJ, L.169, 10.7.2000, p. 1-112.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION⁶

Annex IV, Part A, Section I: 46 of Council Directive 2000/29/EC provides that plants intended for planting, originating in countries where *Bemisia tabaci* Genn. (non-European populations) or other vectors of a number of specified viruses are known to occur, should be accompanied by an official statement that no symptoms of the relevant harmful organisms have been observed on the plants during an adequate period, and that (a) the plants originate in areas known to be free from *Bemisia tabaci* and other vectors of the relevant harmful organisms; or (b) the place of production has been found free from *Bemisia tabaci* and other vectors of the relevant harmful organisms on official inspections carried out at appropriate times; or (c) the plants have been subjected to an appropriate treatment aimed at eradicating *Bemisia tabaci*.

A derogation request for these requirements was submitted by the US phytosanitary authorities. The request pertained to acceptance of cold treatment at 28 degrees Fahrenheit (-2.2 degrees Celsius) for 2 weeks as an alternative to the requirements of Council Directive 2000/29/EC.

The derogation request originally was irrespective of plant species but was subsequently confined by the US phytosanitary authorities to strawberry plants intended for planting. The supporting technical justification submitted by the United States Department of Agriculture consisted of a short report of scientists of the University of California, Riverside, entitled 'Evaluation of Strawberry Nursery Plant Cold Treatments on Survival of the Whitefly, *Bemisia tabaci*'. The technical justification was discussed in the Standing Committee on Plant Health on 2-3 February 2009 and considered of insufficient quality to warrant submission to EFSA. On 6 October 2009, the dossier was discussed with the US authorities, who requested clarification of the requirements for a scientifically sound dossier, in particular as concerns controls, replications and statistical analysis. Clarification was also required on which viruses listed in the Annexes of Council Directive 2000/29/EC can be transmitted by strawberry plants and by *Bemisia tabaci*.

Since the study report pertains to the mitigation of risks posed to plant health by regulated harmful organisms (*Bemisia tabaci* and viruses vectored by this insect), the Commission is seeking the advice of the European Food Safety Authority in the form of a scientific opinion on the requested derogation concerning cold treatment of strawberry plants to eliminate *Bemisia tabaci* whiteflies from consignments to be shipped to the EU.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA is requested, pursuant to Article 22(5) and Article 29(1) of Regulation (EC) No 178/2002, to provide a scientific opinion on the dossier for a derogation request of the US concerning cold-treated strawberry plants intended for planting. In particular, EFSA is requested to include in its scientific opinion:

- (1) Evaluation of the methodological aspects of the dossier with particular attention to potential methodological concerns.
- (2) Evaluation of the proposed cold treatment of strawberry plants intended for planting in terms of its effectiveness in elimination of *Bemisia tabaci* whiteflies from consignments to be shipped to the EU.
- (3) Clarification which viruses referred to in Annex IV, Part A, Section I: 46 of Council Directive 2000/29/EC can be transmitted by *Bemisia tabaci* on strawberry plants.

⁶ Submitted by European Commission, ref. SANCO E1/RB/svi D(2009) 510563, available at <http://registerofquestions.efsa.europa.eu/roqFrontend/questionsListLoader?panel=ALL>

EVALUATION

1. Introduction

This document presents a statement of the Panel on Plant Health in response to a request for a scientific opinion on a derogation request and technical justification submitted by the US phytosanitary authorities to the European Commission. The technical justification comprises a report entitled “Evaluation of Strawberry Nursery Plant Cold Treatments on Survival of the Whitefly, *Bemisia tabaci*”⁷. Clarification was also requested on which viruses referred to in Annex IV, Part A, Section I, point 46 of Council Directive 2000/29/EC can be transmitted by *B. tabaci* on strawberry plants.

The scope of the request and of this opinion is restricted to plants of strawberry (*Fragaria L.*).

1.1. Protective measures against *B. tabaci*

B. tabaci (Gennadius) is listed as a harmful organism in Community plant health legislation and protective measures are taken to prevent its introduction and further spread within the European Community. It is highly polyphagous and a vector of a wide range of plant viruses, which include agents listed as harmful organisms within the Annexes of Council Directive 2000/29/EC. In addition, the introduction of *B. tabaci* represents a pathway for the introduction of non-European viruses, particularly begomoviruses, which are transmitted in a circulative manner with the consequence that adult whiteflies can remain viruliferous for their entire life span.

The current requirements for plants intended for planting are set out in Annex IV Part A, Section I, point 46 of Council Directive 2000/29/EC. The relevant harmful organisms transmitted by *B. tabaci* are listed as *Bean golden mosaic virus*, *Cowpea mild mottle virus*, *Lettuce infectious yellows virus*, *Pepper mild tigré virus* and *Squash leaf curl virus* and includes “other viruses transmitted by *B. tabaci*”. In addition, point 46(b) includes “other vectors of the relevant harmful organisms”.

1.2. Evaluation procedure

The Panel examined the US report entitled “Evaluation of Strawberry Nursery Plant Cold Treatments on Survival of the Whitefly, *Bemisia tabaci*” and provides a statement based on:

- Shortcomings identified in the report and in the experimental methodology relevant to the evaluation of the effectiveness of the proposed cold treatment of strawberry plants for the elimination of *B. tabaci*,
- A preliminary review of the literature on the viruses relevant to strawberry (*Fragaria* spp.) listed in the Annexes of Council Directive 2000/29/EC, to determine the viruses infecting strawberry and those viruses of strawberry which can be transmitted by *B. tabaci*.

2. Analysis of the submitted document

2.1. General comments

A cold treatment for strawberry transplants at 28 degrees Fahrenheit (-2.2 degrees Celsius) for 2 weeks is proposed as an alternative to the requirements of Council Directive 2000/29/EC. The Panel

⁷ Available at <http://registerofquestions.efsa.europa.eu/roqFrontend/questionsListLoader?panel=ALL>

reviewed the report provided and considers that the effectiveness of the proposed cold treatment for the eradication of *B. tabaci* is not demonstrated in this study due to the following shortcomings:

- The experiments are not clearly described,
- The effect of the cold treatment cannot be derived from the reported experiments as the experimental design lacks proper controls,
- The results are not clearly presented, and relate to an incomplete experiment so that conclusions cannot be drawn from the data,
- No reference is made to the extensive literature available on *B. tabaci* which may be relevant to the study.

2.2. Specific comments on the experimental methodology

- High mortality of *B. tabaci* on strawberry at a temperature of 30 °C does not support the conclusion that strawberry is not a suitable host plant for *B. tabaci*, and does not represent a viable control;
 - Mortality arising from the direct transfer of a *B. tabaci* population from a favoured host plant (cucumber and cotton) to strawberry cannot be excluded, as populations of whiteflies have been shown to adapt to new host plants when exposed to them for several generations but can show high mortality immediately following transfer to a new host,
 - A population reared on strawberries for several generations should be used for experiments to test (i) survival of adults and (ii) egg laying and mortality on strawberry.
- For a reliable test of the proposed treatment, use of a favoured host plant of *B. tabaci* would enable host plant mortality effects to be evaluated independently of the mortality effect from the cold treatment alone,
- The number of test plants and insects used are insufficient to derive reliable mortality estimates to determine the effectiveness of the cold treatment,
- The effectiveness of the treatment and reliability of the results is not supported by appropriate statistical analysis,
- The treatment protocol is not clearly described and does not indicate how the data relates to operational or simulated operational conditions,
- The effectiveness and reliability of the treatment proposed is not supported by time/temperature experiments confirming mortality effects over time,
- Survival of insects at low temperatures is affected by the temperatures at which they were previously exposed, the rate of cooling, the duration of cold conditions and other environmental parameters which should be clearly described.

The shortcomings identified by the Panel concerning both the experimental design and the poor quality of the evidence presented in the report, do not enable conclusions to be drawn on the effectiveness of the proposed cold treatment. Studies on low temperature exposure of *B. tabaci* (e.g. Bosco and Caciagli, 1998; Lin *et al.*, 2007) indicate, however, the potential for development of a cold treatment for the eradication of *B. tabaci*.

3. Clarification of viruses transmitted by *B. tabaci* and reported on strawberry

3.1. Viruses vectored by *B. tabaci* and listed in 2000/29/EC

In Annex IV, Part A, Section I, point 46, *B. tabaci* Genn (non-European populations) is listed as a vector of viruses such as *Bean golden mosaic virus*, *Cowpea mild mottle virus*, *Lettuce infectious yellows virus*, *Pepper mild tigré virus* and *Squash leaf curl virus* and “other viruses transmitted by *B. tabaci*”. In addition to those specifically named above, *Euphorbia mosaic virus* and *Florida tomato virus* are further listed in Annex IAI (7) as viruses vectored by *B. tabaci*.

There are no reports of the *B. tabaci*-transmitted viruses specifically listed in 2000/29/EC on strawberry (Martin and Tzanetakis, 2006). The Panel considers it unlikely that these viruses may infect strawberry as these viruses have, where known, a restricted host range. However, there is some uncertainty as no specific studies have been carried out to exclude the possibility that these viruses could infect strawberry.

3.2. Whitefly-transmitted viruses of strawberry

Members of the genus *Crinivirus* of the family *Closteroviridae* comprise one of the predominant groups of whitefly-transmitted viruses that have emerged following recent advances in molecular methods of virus detection and characterisation. In a review of strawberry viruses by Martin and Tzanetakis (2006) and summarised in Table 1 below, two closely related criniviruses are described, associated with the pallidosis disease of strawberry in the US. These recently identified viruses are *Strawberry pallidosis-associated virus* (SPaV) described by Tzanetakis *et al.*, (2004, 2005) and *Beet pseudo-yellows virus* (BPYV), a well known virus recently identified in strawberry by Tzanetakis *et al.*, (2003). SPaV is considered to be the main agent associated with pallidosis disease in the US. This disease causes root and runners reduction (Converse and Volk, 1990; Martin and Tzanetakis, 2006) and the pallidosis disease agent(s) shows synergistic detrimental effects when present in mixed infection with other strawberry viruses (Martin and Tzanetakis, 2006).

Criniviruses are transmitted in a semi-persistent manner by the whiteflies *Trialeurodes vaporariorum* (Westwood), *T. abutilonea* (Haldeman) and *B. tabaci*, but both SPaV and BPYV are only transmitted by the glasshouse whitefly *T. vaporariorum* and not by *B. tabaci* (Martin and Tzanetakis, 2006; Tzanetakis *et al.*, 2006). BPYV has the widest host range of all criniviruses, which normally have a restricted host range, and new hosts continue to be identified, such as blackberry (Tzanetakis and Martin, 2004) and pumpkin (Wintermantel, 2004) in the US.

For plants of *Fragaria* L. intended for planting, the relevant harmful organisms listed in European Community legislation are: *Arabis mosaic virus*, *Raspberry ringspot virus*, *Strawberry crinkle virus*, *Strawberry latent ringspot virus*, *Strawberry mild yellow edge virus*, *Tomato black ring virus* (Annex IVAI 19.2.); *Strawberry latent ‘C’ virus*, *Strawberry vein banding virus* (Annex IAI) and *Raspberry leaf curl virus* (Annex IIAI).

Table 1: Strawberry viruses: names, acronyms, natural modes of transmission and genera (adapted from Martin and Tzanetakis, 2006).

Virus name	Acronym	Mode of transmission	Genus
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<i>Apple mosaic</i>	ApMV	Pollen, seed	<i>Ilarvirus</i>
<i>Arabis mosaic</i> *	ArMV	Nematode, seed	<i>Nepovirus</i>
<i>Beet pseudo-yellows</i>	BPYV	<i>T. vaporariorum</i>	<i>Crinivirus</i>
<i>Fragaria chiloensis cryptic</i>	FCICV	Unknown	Unknown
<i>Fragaria chiloensis latent</i>	FCILV	Pollen, seed	<i>Ilarvirus</i>
<i>Raspberry ringspot</i> *	RpRSV	Nematode, seed	<i>Nepovirus</i>
<i>Strawberry chlorotic fleck</i>	StCFV	Aphid	<i>Closterovirus</i>
<i>Strawberry crinkle</i> *	SCV	Aphid	<i>Cytorhabdovirus</i>
<i>Strawberry feather leaf</i>	NA ^(a)	Unknown	Unknown
<i>Strawberry latent</i>	StLV	Unknown	<i>Cripavirus</i>
<i>Strawberry latent C</i> **	SLCV	Aphid	<i>Nucleorhabdovirus</i>
<i>Strawberry latent ringspot</i> *	SLRSV	Nematode, seed	<i>Sadwavirus</i>
<i>Strawberry mild yellow edge</i> *	SMYEV	Aphid	<i>Potexvirus</i>
<i>Strawberry mottle</i>	SMoV	Aphid	<i>Sadwavirus</i>
<i>Strawberry necrotic shock</i>	SNSV	Thrips, pollen, seed	<i>Ilarvirus</i>
<i>Strawberry pallidosis associated</i>	SPaV	<i>T. vaporariorum</i>	<i>Crinivirus</i>
<i>Strawberry pseudo mild yellow edge</i>	SPMYEV	Aphid	<i>Carlavirus</i>
<i>Strawberry vein banding</i> **	SVBV	Aphid	<i>Caulimovirus</i>
<i>Tobacco necrosis</i>	TNV	Chytridiomycetes	<i>Necrovirus</i>
<i>Tomato black ring</i> *	TBRV	Nematode, seed	<i>Nepovirus</i>
<i>Tomato ringspot</i>	ToRSV	Nematode, seed	<i>Nepovirus</i>

NA^(a) = Not available, indicates the virus disease has been described in the literature but that the authors are unaware of a known isolate of the virus currently maintained in a collection.

* = Viruses listed in Annex IVAI (19.2) of 2000/29/EC

** = Viruses listed in IAI of 2000/29/EC

The Panel concludes that no virus reported on strawberry plants is known to be transmitted by *B. tabaci*. However, adult whiteflies of *B. tabaci* on strawberry consignments can carry plant viruses irrespective of whether strawberry is a host plant for these viruses and thus represent a pathway for the introduction of non-European viruses. In particular, *B. tabaci* transmits begomoviruses, including those listed in Council Directive 2000/29/EC, in a circulative manner and adults can remain viruliferous for their entire life span.

3.3. Introduction of viruses in strawberry planting material from the US

Commercial strawberry (*Fragaria x ananassa* Duchesne) is propagated vegetatively and is thus subject to infection by viruses during plant propagation. "Non-European viruses" of *Fragaria* L. are listed in Annex IAI of Council Directive 2000/29/EC in addition to those agents specified. SPaV and BPYV are both associated with pallidosis disease of strawberry, which causes root and runners reduction (Converse and Volk, 1990; Martin and Tzanetakis, 2006). In addition, the pallidosis disease agent(s) shows synergistic detrimental effects when present in mixed infection with other strawberry viruses (Martin and Tzanetakis, 2006). BPYV is known to be present in parts of the European Union, e.g. Greece, Italy. SPaV is not known to occur in the EU.

The Panel concludes that irrespective of the presence of *B. tabaci*, consignments of strawberry planting material pose a risk of introducing non-European viruses such as SPaV in the EU.

CONCLUSIONS

The Panel considers that due to the poor quality of the report submitted and to serious shortcomings in the experimental design, the report does not demonstrate the effectiveness of the cold treatment proposed. Although there is potential for the development of a cold treatment protocol for the elimination of *B. tabaci* from strawberry plant consignments, reliable mortality estimates for the proposed cold treatment of 28 degrees Fahrenheit (-2.2 degrees Celsius) for 2 weeks cannot be derived from this study.

No *B. tabaci*-transmitted viruses are currently known to infect strawberry and no viruses of *Fragaria* spp. listed in Directive 2000/29/EC are known to be transmitted by *B. tabaci*. However, adult whiteflies of *B. tabaci* on strawberry consignments can carry plant viruses irrespective of whether strawberry is a host plant for these viruses and thus represent a pathway for the introduction of non-European viruses. There is also some uncertainty concerning the extent to which known or unknown *B. tabaci*-transmitted viruses could infect strawberry. Two whitefly-transmitted viruses have been recently confirmed as associated with strawberry pallidosis disease occurring in the US: *Beet pseudo-yellows virus* (BPYV) and *Strawberry pallidosis associated virus* (SPaV) but are only known to be transmitted by the greenhouse whitefly *Trialeurodes vaporariorum* and not by *B. tabaci*.

Strawberry plants for planting are vegetatively propagated and are thus subject to infection by viruses. Irrespective of the presence of *B. tabaci*, strawberry transplants from US may represent a pathway for the introduction of any non-European viruses (and other potentially harmful organisms) of *Fragaria*. This applies for example to SPaV, which is not reported as present in the EU and, if introduced, could be further spread by its vector, the glasshouse whitefly *T. vaporariorum*, which is widely established in the EU.

DOCUMENTATION PROVIDED TO EFSA

Request letter from DG SANCO to EFSA Executive Director sent on 18/11/2009⁸

ref: SANCO.E1/RB/svi D(2009) 510563 including:

Annex 1: Background and Terms of Reference of the request

Annex 2: Letter of September 17, 2009 from USDA containing the report "Evaluation of Strawberry Nursery Plant Cold Treatments on Survival of the Whitefly, *Bemisia tabaci*".

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