United States Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection and Quarantine

Technical Working Group (TWG) Report

Sweet Orange Scab (*Elsinöe australis)*

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United States Department of Agriculture (USDA)
Animal Plant Health Inspection Service (APHIS)
Plant Protection and Quarantine (PPQ)
Center for Plant Health Science and Technology (CPHST)

Table of Contents

Introduction

Trees showing symptoms highly suspect for Sweet Orange Scab (SOS), caused by the fungal pathogen *Elsinöe australis*, were identified on 15 June 2010, in Harris County Texas by USDA APHIS PPQ Houston Plant Inspection Station (PIS) personnel. The Agency was alerted to the potential detection through the work of researchers at the Texas A&M University-Kingsville Citrus Center in Weslaco. The leaf and fruit samples, from a lemon tree located on a residential property, were forwarded to the USDA APHIS PPQ Molecular Diagnostics Laboratory (MDL) in Beltsville, MD for diagnostic testing.

USDA APHIS PPQ MDL performed polymerase chain reaction (PCR) testing along with DNA sequencing and confirmed the presence of *E. australis* from the submitted samples. Samples were then submitted to USDA APHIS PPQ CPHST National Plant Germplasm Biotechnology Lab (NPGBL) in Beltsville, MD for confirmation of the identity of the pathogen.

On 13 August 2010, a TWG meeting was held via teleconference. During the meeting, participants and observers were introduced and the charge to the TWG was outlined (See Appendix 1 for a list of participants). Specifically, the TWG met for approximately one hour and was asked to provide specific scientific input on eight questions developed in support of APHIS program objectives concerning SOS detection and mitigation. This document reports information shared during this meeting as well as supporting scientific literature discussed and outlined during the meeting. Information reported here targets the most successful approaches to delimit the disease, contain the pathogen, and treat known infested areas. Questions asked of TWG participants and responses are listed in Appendix 2.

This document does not represent, or summarize, an extensive literature search for SOS. Additional reading materials are listed at the conclusion of this report if readers would like to gain a more in-depth understanding of the pathogen or the disease it causes. Because the distribution of *E. australis* is limited, much of the information presented in this report is based on scientific expertise obtained from research targeting *E. fawcettii*, the cause of citrus scab (also known as common scab or sour orange scab).

Summary Outcomes

- Eradication Potential
    - It is theoretically possible if the disease is not established over a wide area. While there are no reported instances of successful eradication, there have also been no known attempts at eradication.

- Spread Potential
    - Natural spread is probably limited, although biology of the pathogen is not well described, e.g. sexual stage. Information on epidemiology is also unknown from U.S. environments where the pathogen has been detected. Whiteside (1975),

utilizing another citrus pathogen in the same genus as *E. australis*, determined that *E. fawcettii* conidial infections can occur at a distance of 30 meters from a severely diseased lemon tree while trap plants placed 400 meters away from any known inoculum source experienced no infection. *E. fawcettii* produces spindle-shaped conidia that can be air-borne for short distances, whereas *E. australis* produces only hyaline conidia. Thus, the ability of *E. australis* to spread should be even less (Timmer, 2010 *personal communication*).

- Human-mediated spread poses the greatest risk and is the most likely means of long distance transport.

- Detection
  - Infection usually occurs on young plant tissues and subsequent lesions (scabs) remain identifiable until tissues senesce.
  - Diseased plant tissues may include leaf, stem and fruit.
  - Symptoms generally develop one week after infection depending on environmental conditions that promote disease development, e.g. warm temperatures and moist plant tissues.
  - If the pathogen is present, the best time to detect disease is during early spring when new tissues and fruit are expressed.
  - Misdiagnosis of this disease is also likely. Scabby lesions appear similar to citrus scab caused by *E. fawcettii*. Sweet orange scab symptoms may be routinely overlooked as a result.

- Delimitation Survey

  - One mile^2 from the initial infestation is adequate for delimiting natural spread from an initial detection site, but cannot account for human-mediated spread or long-term, undetected establishment across a much larger area.
  - Focus efforts on fruit symptoms and foliar tissues which are expressed within a one week period after infection
  - Carefully examine shaded plant tissues and specifically those that remain moist for 2.5-3.5 hours (CABI, 2006; EPPO/CABI, 1997). Warm air temperatures (24 to 27° C) and prolonged periods of plant tissue wetness (2 to 24 hrs.) promote infection and symptom development.

- Treatment Recommendations

  - Tree Removal
    - Is the most effective treatment for limiting disease spread.
    - Remove all trees with confirmed *E. australis* infections and destroy all vegetative material by burying or burning.
    - Replant using healthy citrus nursery stock.
  - Tree Pruning
    - In theory, aggressively pruning trees should remove *E. australis* infected tissues. Trim tree branches until limbs appear like deer antlers (buck-horning) to remove all diseased foliage, fruit and stems. This plant

denuding treatment should promote healthy re-growth. Destroy all vegetative material by burying or burning. This procedure will need to be done throughout the grove to ensure that incipient disease in adjacent host plants is not missed.
        - The treatment is labor intensive, but may preserve grove integrity in the long term.
    - Fungicide Application
        - Is estimated to be the least effective treatment at limiting spread of this disease and will not achieve eradication.
        - Fungicides can be applied to trees when fruit are developing according to label use regulations to control disease.
        - All fungicides that are currently labeled for use on common scab should effectively control SOS. Read and follow all label instructions.
    - Integrated Control
        - Tree pruning (buck-horning) and fungicide application can be used as an integrated approach to reduce the risk of re-infection of healthy plant tissues. This approach has not been researched and there are no data to support it. Its effectiveness at controlling disease spread is therefore unknown.

- Protection of Citrus Nursery Stock

    - Leaf tissues should be kept dry to prevent spore germination, and consequently, plant infection.
        - Drip irrigation or other means of irrigation that does not wet leaf, fruit, and stem tissues should be employed.
        - A solid roof material or covering should be used to keep plants dry during rain events.
        - Apply prophylactic fungicide treatment at flush when new leaf tissues have emerged and immediately following fruit set. Read and follow all label instructions.

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Appendix 1. TWG co-chairs, participants, and observers.

Invitees	Organization	Affiliation
Russ Bulluck	APHIS PPQ CPHST	Co-Chair
Charla Hollingsworth	APHIS PPQ CPHST	Co-Chair
Jim Adaskaveg	U. California, Riverside	Participant
Ignacio Baez	APHIS PPQ CPHST	Participant
Megan Dewdney	U. Florida	Participant
Bob Griffin	APHIS PPQ CPHST	Participant
Megan Henderson	APHIS PPQ CPHST	Participant
Madurababu Kunta	Texas A&M-Kingsville Citrus Center	Participant
Laurene Levy	APHIS PPQ CPHST	Participant
Mary Palm	APHIS PPQ MDL	Participant
Maria Perez	APHIS PPQ	Participant
Natalia Perez	U. Florida	Participant
Don Seaver	APHIS PPQ CPHST	Participant
Mani Skaria	Texas A&M-Kingsville Citrus Center	Participant
Pete Timmer	U. Florida, Professor Emeritus	Participant
Valerie DeFeo	APHIS PPQ EDP	Observer
Pat Gomes	APHIS PPQ EDP	Observer
Phil Mason	APHIS PPQ	Observer
George Nash	APHIS PPQ	Observer
Shashank Nilakhe	Texas Department of Ag	Observer
Ray Prewett	Texas Citrus Mutual	Observer
Justin Wall	APHIS PPQ	Observer
Sergio Garran	INTA Argentina	Invited, but not attending
Juan Pedro Agostini	INTA Argentina	Invited, but not attending
Michael Priest	DPI, New South Wales, Australia	Invited, but not attending
Daniel Ploper	Estación Experiment Station, Argentina	Invited, but not attending
Renato Reis	UNESP–Departamento de Fitossanidade, Brazil	Invited, but not attending
Jae Wook Hyun	National Institute of Subtropical Agriculture, Korea	Invited, but not attending

Appendix 2. Questions asked of TWG participants and responses recorded.

1. *Can this disease be eradicated?*

   In theory yes, but little is known about: (*i*) the sexual stage, (*ii*) how the pathogen spreads, (*iii*) inoculum sources other than fruit, (*iv*) and other pathogen lifecycle and host infection information. In short, there are too many unknown factors to provide a definitive answer.

   a. *Have there been any instances where this disease has been successfully eradicated?* There are no reported instances where it has been eradicated or where eradication has been attempted.
   b. *Can one eradicate this disease by physically removing all developing fruit from the tree?* No. Removing fruit does not seem to prevent recurrence of disease, especially in this case where disease has been detected on leaves.
   c. *Can an application of hormone to prevent fruit set be used to eradicate this disease?* This approach is unlikely to be successful and should not be attempted.

2. *How far out from detections should a delimitation survey be conducted?*

   One mile delimitation is adequate for natural spread, but a survey of this distance is unlikely to detect human movement of host material (e.g., nursery stock trade or other).

   a. *How far can Elsinöe australis spread naturally following establishment?* If spread was solely natural, only a very limited survey would be necessary since natural spread is meters at most. Conidial spread is probably insignificant. Natural spread of scab is limited and most spread will be facilitated from humans moving plant materials. Researchers attempting to establish *E. fawcettii* in healthy trees through inoculation, failed to get it to establish or spread. Conidia are fragile and need water splash transport to new plant tissues. Diseased plant material is the primary way to spread this disease unless the fungus produces ascospores, but that information is unknown to scientists at this time.

3. *Should diseased trees be removed when identified?*

   This is always desirable and the most effective way to control spread. It will however, require molecular testing of each tree to distinguish sweet orange scab from citrus scab. Buck-horning trees may get rid of the disease without destroying the trees, but the labor demands associated with it may be too high for industry to adopt.

4. *Can this disease be spread in nursery stock? If yes, how can spread be mitigated?*

   Spread through nursery stock is the most likely source of new infestations. Experiments which involved infecting a tree and then tracking natural spread of the disease on adjacent trees indicated that the disease does not readily move to nearby trees. Physical contact between a diseased tree and a healthy tree is probably required to spread the disease as conidia are short-lived and splash movement is required.

5. *What is the best growth stage of the host to visually detect symptoms of SOS? How long does it take for symptoms to develop after host tissue has been infected?*

   Infection usually occurs on young tissue and will remain visible for the life of that tissue. Symptom development takes a few days and is dependent on environmental conditions that promote disease development, e.g. warm temperatures and moist plant tissues. If the pathogen is present, the best time to detect disease is during early spring when new tissues and fruit are forming. Symptoms will remain until tissues senesce. Symptoms generally develop one week after tissue infection.

6. *What part of the tree canopy would one expect to find SOS especially early in the infection cycle? Are there effective methods for early detection?*

   In Texas the disease is not more prevalent in any particular area of the grove. Symptoms are usually spread throughout diseased trees. However, symptoms can be localized in the tree canopy due to droplet splash dispersal. Shaded areas may be more conducive to infection due to the moist microclimate associated with these areas. Fruit is only susceptible for approximately six weeks after petal fall.

7. *How long are spores viable?*

   Conidia are fragile and ephemeral, maintaining viability for approximately 1.5 hours. Lesions can produce spores for an extended period of time, but the number of spores released declines as lesions age. It is unknown whether detached stems and leaves are a source of spores. If environmental conditions are not conducive at the time of leaf growth or fruit set, disease symptoms do not develop. From year-to-year, sweet orange scab incidence will vary.

   a. *If infection does not occur, how long can spores serve as an inoculum source?* Conidia are very short-lived (e.g., 1.5 hrs), germinate quickly, and infect quickly. If infection does not occur, spores lose viability rapidly. Lesions can produce viable spores for an extended period of time although the number of successful infections decline as fruit mature and leaves age. The best time for disease spread is during the spring as spores are produced both in older and younger lesions.

   Researchers in Florida ran an experiment with *E. fawcettii* and were able to quantify conidial production (approx. 10^5-10^6 conidia/ml for a lesion area of approx. 0.5 cm^2; Megan Dewdney, 2010, *personal communication*) from lesions for 45 days (duration of the experiment). A similar outcome is assumed of *E. australis*.

8. *Are young trees more susceptible to infection than older, mature trees?*

   Yes, but only as a result of the proportion of young tissue associated with younger trees.

Appendix 3. Questions posed to CPHST by USDA Emergency and Domestic Programs.

1. *Given the prevailing conditions in Spring, TX, how far out from the epicenter/foci should a delimitation survey be conducted to have a high degree of confidence that the extent of infestation has been determined?*

   See Appendix 2, Question 2

2. *How far does SOS spread each season?*

   See Appendix 2, Question 2.

3. *Does SOS infect leaves and twigs?*

   It is not typical for SOS to infect leaves and twigs, but the pathogen can infect these tissues when environmental conditions are conducive. Scientific literature states that scabs can form on leaves, twigs and fruit (EEPO/CABI, 1997).

4. *What portion of a tree should be sampled?*

   Fruit provides the best samples for *E. australis*. See Appendix 2, Question 2.

5. *What is the best time of year to visually detect symptoms of SOS?*

   See Appendix 2, Question 5.

6. *What part of the tree canopy would one expect to find SOS, especially early in the infection cycle?*

   See Appendix 2, Question 5.

7. *What fungicides can be used to control SOS?*

   Current fungicides in use for citrus scab (*E. fawcettii*) should be effective. Products such as Topsin® (thiophanate methyl), Abound® (azoxystrobin), Gem® (trifloxystrobin), Headline® (pyraclostrobin), ferbam, and copper fungicides are currently labeled for use in Florida for citrus scab.

8. *What disinfectants can be used to devitalize/kill SOS?*

   Quaternary ammonium products, bleach solution and other topical disinfectants labeled for use on citrus canker in Florida should be effective to decontaminate hard surfaces. However, the disinfectants will not effectively reduce inoculum present inside of citrus fruit lesions.

9. Should infected trees be removed when found?

This action would remove inoculum, but a case-by-case determination (as well as delimitation of the current infestations) will need to be considered. See Appendix 2, Question 3.

10. Are young trees more susceptible to infection than older mature trees?

Young tissue is susceptible, regardless of tree age. Scab pustules produced on spring flush serve as inoculum for infection of fruit (Bushong and Timmer, 2000).

11. Can this disease be eradicated? Have there been any instances where this disease has been successfully eradicated?

See Appendix 2, Question 1.

12. What distance from an infected tree should be quarantined to prevent further spread of the disease?

This question should be determined by the regulatory program.

13. Should movement of nursery stock be prohibited?

Final determination will need to be made by the regulatory program. See Appendix 2, Question No. 4.

14. Are there effective treatments that can be applied to nursery stock to prevent infection?

Prophylactic foliar fungicides can help (see Question No. 7 above).

15. Can you defoliate nursery stock to mitigate risk of infection or spread of the disease?

Not exclusively. Infection can occur on stems and fruit also. See "Tree Pruning" in the "Treatment Recommendations" section.

16. Can this disease be cultured?

Yes (Hyun et al, 2001). Recent detections have resulted in a U.S. isolate of *E. australis* which is being maintained by the USDA.

17. Do we have a validated diagnostic for this disease?

The NPGBL in Beltsville is currently working to validate a molecular diagnostic test.

18. Are there effective methods for early detection?

See Appendix 2, Question No. 6.

19. *What is the latency of symptom expression once a tree has been exposed or infected?*

Typically short, but depends greatly on environmental conditions. See Appendix 2, Question No. 5.

20. *Can spores of this disease be dispersed by wind? If so, how far?*

Spores of *E. australis* may be dispersed over short distances by air (Chung and Timmer, 2005). See question No. 2 above.

21. *How long are spores viable?*

See Appendix 2, Question No. 7.

22. *Can spore traps be used to enhance delimitation/early detection?*

It is much easier to scout for this disease than to trap spores because symptoms are expressed soon after infection. Spore traps are good research tools, but not effective in delimitation because they sample a very small area, detected spores cannot be traced to a particular tree, and are labor intensive.

23. *Should leaf litter/debris be sampled?*

There is no need since disease symptoms are expressed on diseased leaves, twigs, and fruit on trees.

24. *Can you control or eradicate this disease by removing fruit from the tree?*

E. australis overwinters in the tree canopy on fruit and other plant organs that were infected during the previous season (CABI, 2006).

25. *Can you spray the tree with a hormone to prevent fruiting and disrupt the infection cycle of the disease?*

This would not be beneficial since the pathogen can also survive on leaves and twigs. See Appendix 2, Question No. 1.

26. *What parts of the tree are susceptible to infection?*

Inocula consist of conidia, and presumably ascospores, from scabs formed on leaves, twigs and fruit (EEPO/CABI, 1997). Younger host plant tissues are more susceptible to infection than aged tissues.