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

Detection and Delimitation Survey Methods for *Elsinöe australis* (Sweet Orange Scab of Citrus)

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United States Department of Agriculture (USDA)
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Center for Plant Health Science and Technology (CPHST)

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Introduction

This survey document proposes three different approaches for collecting information on disease incidence that can be considered collectively or separately in order to determine the extent of the infestation of *E. australis* in the U.S. Each state or region should choose the most appropriate method based upon the particular circumstances experienced at that time. Recent multiple detections of sweet orange scab (SOS) from a two state region support the likelihood that the pathogen may have established in the U.S. some time ago, since its conidial spore stage is known for limited spread and pathogen establishment. In fact, a researcher at Texas A&M University suggests that he has seen symptoms of SOS in that state for an extended period, but earlier attempts at isolating the pathogen from diseased plant tissues were unsuccessful. While his statements are interesting, they are observational, nonetheless, due to the fact that the pathogen was never isolated.

This pathogen produces short-lived, fragile conidia (asexual spores) that are known to spread by droplet splash, rather than by wind. Additionally, researchers postulate that due to their inherent fragility, spores may be most successfully spread amongst susceptible hosts if tissues (leaves, branches, and fruit) of a diseased plant directly rub against tissues of a healthy plant. This would result in transferring fungal spores from one host to the other via direct contact without the impact associated with droplet splash. With this theory in mind, it may be useful to examine citrus established in mature hedge rows or other overlapping, or shared canopy arrangements. It should be kept in mind however, that little is known of the sexual spore stage of this disease (ascospores) which occurs in the same host tissue.

To best promote our understanding of the extent of the SOS infestation, conduct ***'random' detection surveys***. This type of survey can be conducted in commercial citrus growing areas of states as well as high-risk residential areas where common citrus scab (caused by *E. fawcettii*) is already established. Surveying commercial, rather than residential citrus, allows APHIS PPQ to obtain disease incidence data rapidly and efficiently. However, it is acknowledged that residential citrus may have fewer inputs (fungicide applications) which will, consequently, support survey objectives better than a citrus production environment. This survey will provide needed information as to whether SOS is widely distributed and may allow us to understand if

SOS was previously overlooked or misdiagnosed as common citrus scab or other malady. To protect the integrity of our citrus industry, a ***targeted detection survey*** of citrus nursery stock propagation facilities should be implemented. This type of survey will help establish whether the pathogen is present, but undetected, in citrus nursery stock. Human-mediated, unintentional movement of SOS-diseased nursery stock is a distinct possibility since movement of diseased citrus plants is the only known means for this disease to achieve long-distance spread. If diseased nursery stock is identified, it is likely that SOS may already be widely spread. On the other hand, if nursery stock is healthy, SOS distribution may be more limited. Either way, surveillance and protection of citrus nursery stock should be an important objective of the Program. Conduct a ***delimitation survey*** in areas where citrus hosts have been confirmed to be diseased with SOS. This type of survey may be the greatest operational priority if detection occurs in an isolated fashion, as it may inform us on how widespread SOS is in the area. Completing this type of survey is important when a plant with SOS is identified, and the methods we propose mirror those of the surveys that have already been initiated.

'Random' Detection Survey

A random detection survey for SOS should be conducted to determine whether SOS has either been overlooked or previously misdiagnosed in citrus production areas where citrus scab (caused by *E. fawcettii*) is established. Survey implementation requires preplanning of routes, notification of growers, and obtaining permission to survey and sample groves for symptomatic tissues. Routes should be planned along primary and secondary roadways used most often for movement of citrus to processing facilities. This survey is considered random in nature only because no predetermined point of origin (confirmed positive plant, citrus nursery stock facility) exists.

If citrus production grove data are known, this survey should be stratified to improve inspection efficiencies. Stratification introduces an effective mechanism to prioritize grove inspections which can be locally adapted. The proposed system consists of a total ranking scale from '3' (low priority) to '8' (high priority) and is designed such that the highest cumulative rank score [e.g., sweet orange = 3, <15 years old = 2, and poorly managed grove = 3], would result in that

crop and production practices having the greatest survey priority. Groves with the following characteristics should be ranked for survey priority as described.

- Citrus species
    3. Sweet orange, grapefruit, mandarin
    2. Lemon, lime
    1. Other
- Tree age
    - Younger trees are more susceptible to SOS and if the pathogen has established recently, a greater number of younger vs. older trees may be diseased.
        2. < 15 years
        1. > 15 years
- Production practices
    - Application of fungicide will limit symptom expression, so every effort should be made to avoid groves if fungicide has been applied.
        3. Poorly managed/abandoned
        2. Limited management (no fungicide)
        1. Highly managed

At a minimum, leaves, twigs, and fruit of trees in one grove from each section (640 acres) where a commercial operation is located should be examined for sweet orange scab, common citrus scab and scab-like symptoms. At a minimum, two rows of trees out of 20 tree rows should be examined at each site. If trees are asymptomatic and not tissues are collected, this information should also be recorded before moving on to the next site.

Fruit, leaves, and twigs of citrus trees should be examined for evidence of SOS or other similar scab-like symptoms (see Appendix 3). Surveyors should examine as much of the tree as possible for SOS symptoms, with emphasis on shaded areas of the canopy that may promote infection and disease development. Tissues with suspect scabs should be collected (Appendix 1) and sent in for determination.

Targeted Detection Survey

A targeted SOS detection survey should be conducted of all citrus nursery stock propagators in states where *E. australis* has been confirmed. This does not include plants sold by citrus dealers or big box stores. Survey efforts should be extended to citrus hosts growing immediately outside the facility that are located up to 50 meters from where citrus nursery stock is maintained. This survey should be conducted at the 100% inspection level, examining leaves, twigs, and fruit (if present), of citrus plants for scab and scab-like symptoms. Tissues with suspect scabs should be collected (Appendix 1).

If *E. australis* is confirmed from a sample(s) collected within or immediately adjacent to a nursery stock or propagation facility, the delimitation survey described above should be conducted to establish the area of infestation. In addition, trace-forwards and trace-backs (if applicable), should be conducted to further enable USDA APHIS PPQ to determine the scope of the infestation.
Surveyors should follow the sweet orange scab sampling protocol provided by USDA APHIS PPQ when surveying residential properties (Appendix 1).

Delimitation Survey

Surveyors should begin surveying in an outwardly direction from a known positive site, conducting SOS symptom inspections of all (100%) citrus hosts within a 0.5 mile radius from the confirmed plant. Fruit, leaves, and twigs of each citrus plant should be examined for evidence of SOS, common citrus scab or other similar scab-like symptoms (see Appendix 3). Surveyors should examine as much of the tree as possible for symptoms, with emphasis on shaded areas of the canopy that may promote disease development. Host plant tissues with suspect scabs should be collected (Appendix 1) and sent to the appropriate identifying authority (Appendix 2).

If *E. australis* is confirmed from a sample(s) collected within the initial 0.5 mile delimitation arc, an additional 0.5 mile arc should be drawn around the most distal positive location(s) from the initial positive. For example, nine positive sites are illustrated in Figure 1 within the original 0.5

mile arc (shown in gray). At each positive disease site located nearest the initial delimitation arc perimeter, additional 0.5 mile arcs are drawn. The survey should continue in an outwardly direction (shown in blue) until *E. australis* is no longer confirmed from diseased plant samples collected within any of the subsequently drawn arcs. The area encompassed by each new 0.5 mile arc should be surveyed at 100% coverage until the disease is not confirmed from the outermost arc.

Surveyors should review and follow the SOS sampling protocol provided by USDA APHIS PPQ when surveying residential properties (Appendix 1).

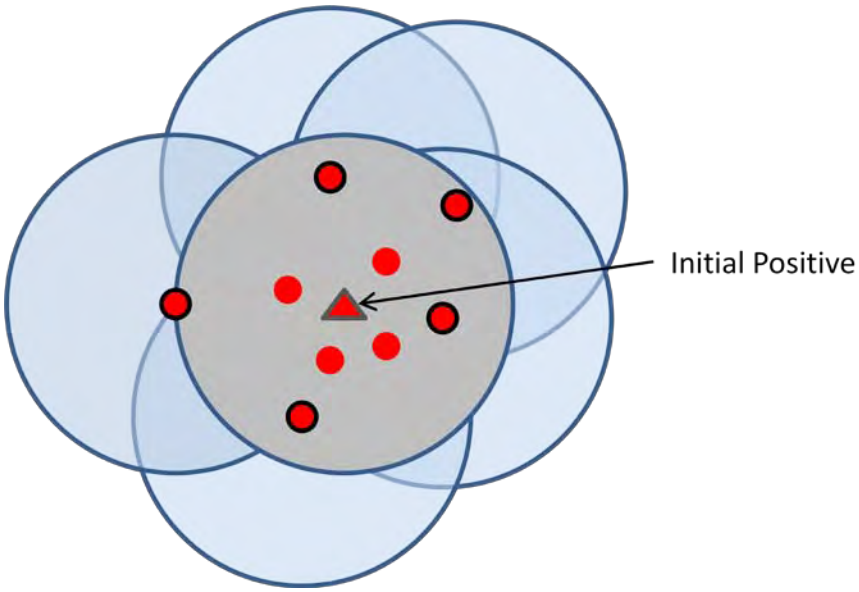


Figure 1. Survey arcs extending out 0.5 mile from distal disease positive locations.

Appendix 1. Sweet Orange Scab (SOS) Survey and Sampling Protocol

Sanitation is a very important part of the survey protocol. It is our responsibility as Regulatory Officials to take strict measures to prevent plant pathogens contamination between inspections. In addition for legal liability reasons, it is important to minimize public perception in viewing Regulatory Officials as "pathogen spreaders" we need to follow a sampling protocol. Utilize sanitation protocols prior to entering a facility and prior to returning to vehicles.

Upon arrival at residence:

1. Contact homeowners/residents, inquire about citrus on property and request permission to enter property.
2. Provide SOS pest information sheet with contact phone number.
3. If host material is present, take carrier with:
    - gloves
    - resealable bags w/labels
    - paper towels
    - garbage bags(for gloves and towels)
    - pruners
    - spray bottles with bleach solution, GX-1027 disinfectant solution, and water
4. Don shoe covers or disinfect shoes with GX-1027 solution prior to entering host area.
5. Wear gloves while handling material.
6. Disinfect pruners prior to cutting plant material.
7. Locate host tree(s).
8. Collect <u>symptomatic leaves and fruit only.</u>
9. Place sample material from each tree in separate resealable bags w/ labels and seal.
10. Place sample bag into second resealable bag.
11. Place flagging on sampled tree.
12. Disinfest pruners with bleach solution and remove gloves between sampling trees and after sampling in area is completed.
13. Disinfest hands and shoes with GX-1027 between samples per location and before leaving residence.
14. Store samples in cooler with ice packs until return to office.

15. Complete paper survey/sampling worksheet for sampled residence. MAKE SURE THAT SAMPLE LABEL CORRESPONDS WITH SAMPLE SHEET.
16. Take GPS reading and complete any record sheets used during survey.

Upon arrival to Incident Command Post:

1. Place double-bagged samples in walk-in cooler. (See segregated SOS sample area.)
2. DO NOT OPEN INNER BAG WITH SAMPLE.
3. Give sample sheets to proper data entry personnel for input into Pest ID. Print out will be placed between the two bags with the sample.

Appendix 2. Procedure for Packaging and Sending Suspect *Elsinöe australis* Samples to the PPQ Molecular Diagnostics Laboratory

Before sending samples to the PPQ Molecular Diagnostics Laboratory (MDL) for confirmation, survey personnel collecting plant samples should have training and familiarity in recognition of sweet orange scab symptoms. Consult the photos of typical symptoms contained in this document. Ideally, a plant pathologist familiar with the symptoms of *Elsinöe fawcetti* and *E. australis*, along with other citrus diseases and conditions should conduct the training and be available to examine all samples before selected for being sent for confirmation.

Suspect samples to be submitted for confirmation are double bagged and sealed in resealable zip bags. Ensure the bag is labeled with a local collection number, and include in the outer bag a hardcopy of the completed PPQ form 391 with the collection number written in block number 1. Attempt to identify the citrus species to include on the form. If being submitted by a PPQ identifier, the 391 information should be entered in the AQAS PestID system. In that case, print out the form from the database to include with the sample being sent.

All plant samples must be packaged with cool packs to help keep the plant samples fresh. Do not freeze the plant samples before sending and do not send with dry ice. Seal in a sturdy, crush proof box or ice chest. Send to the following address:

Dr. Mary E. Palm
USDA/APHIS/PPQ
PPQ Molecular Diagnostic Lab
B-580, BARC-East
9901 Powder Mill Rd.
Beltsville MD 20705
Phone: 301-504-7154 or 504-5700 ext 327

Send by overnight carrier. Only send samples Monday through Thursday. Samples collected on Friday or the weekend should be saved in a refrigerator and shipped on Monday.

Communications

Notify by e-mail the following persons that samples are being forwarded, including the types, amount, the overnight carrier tracking number: mary.palm@aphis.usda.gov, gloria.abad@aphis.usda.gov , laurene.e.levy@aphis.usda.gov , and joel.p.floyd@aphis.usda.gov , and be sure to copy the SPHD, SPRO, regional program manager, and charla.hollingsworth@aphis.usda.gov on the notification. If no Pest ID data entry had been made, send a PDF or other electronic version of the completed 391 in the e-mail notification to help facilitate data entry during the analysis.

Sample results will take several days because sequencing is required for positive confirmation. The MDL will send their results to the PPQ Domestic Diagnostics Coordinator who will forward them to the National Survey Coordinator, in Emergency and Domestic Programs, Riverdale, Maryland. He will e-mail the results to the SPHD, SPRO, and program managers.

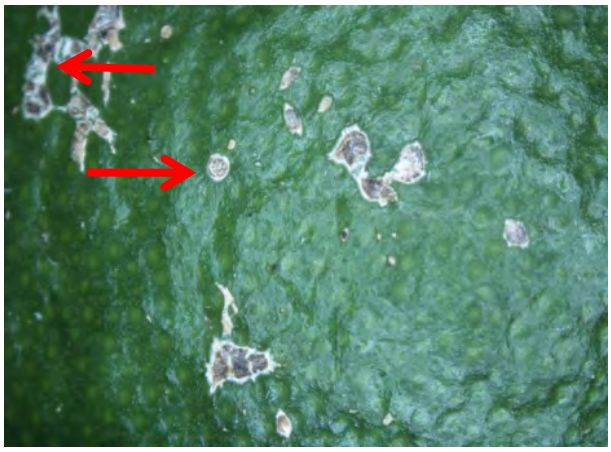
Appendix 3. Symptoms of Sweet Orange Scab (SOS, Elsinöe australis) and other similar symptoms.

Sweet Orange Scab (*Elsinöe australis)*

Symptoms on Fruit and Stems

The initial scab will form during the early stages of fruit development, and is slightly raised and pink to light brown in color. As the pustule continues to develop it will become warty and cracked and the coloration can change to a yellowish brown and eventually to a dark gray. Lesions on orange and grapefruit are sometimes flatter and less raised. Scabs are usually dispersed in the same pattern as water being splashed on the fruit. Symptoms that occur in a narrow straight line across the fruit are more likely wind damage. Lesions can also form on young stems. These symptoms resemble an area of dieback that has scabbed over.

Flatter lesions

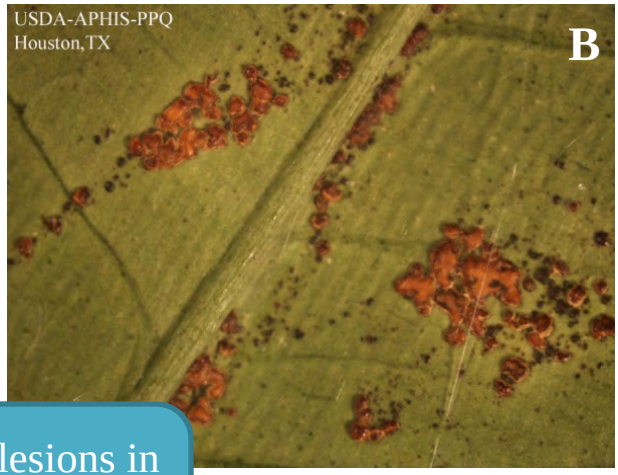


Appendix 3. Symptoms of Sweet Orange Scab (SOS, Elsinöe australis) and other similar symptoms.

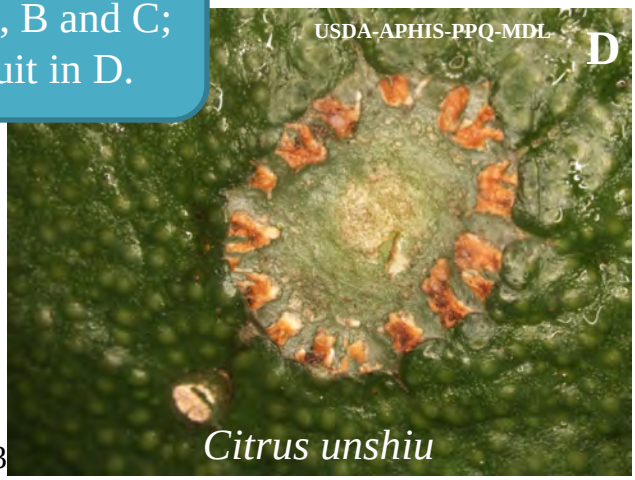
Symptoms on Leaves

Lesions begin on leaves as water soaked spots which evolve into pustules that are typically found on the underside of the leaf near the mid-vein or the border. Foliar lesions that form on young tissue are typically raised, while lesions formed on more mature tissues are typically flatter. Infected leaves can become irregularly shaped, stunted or puckered.

Leaves can become disfigured



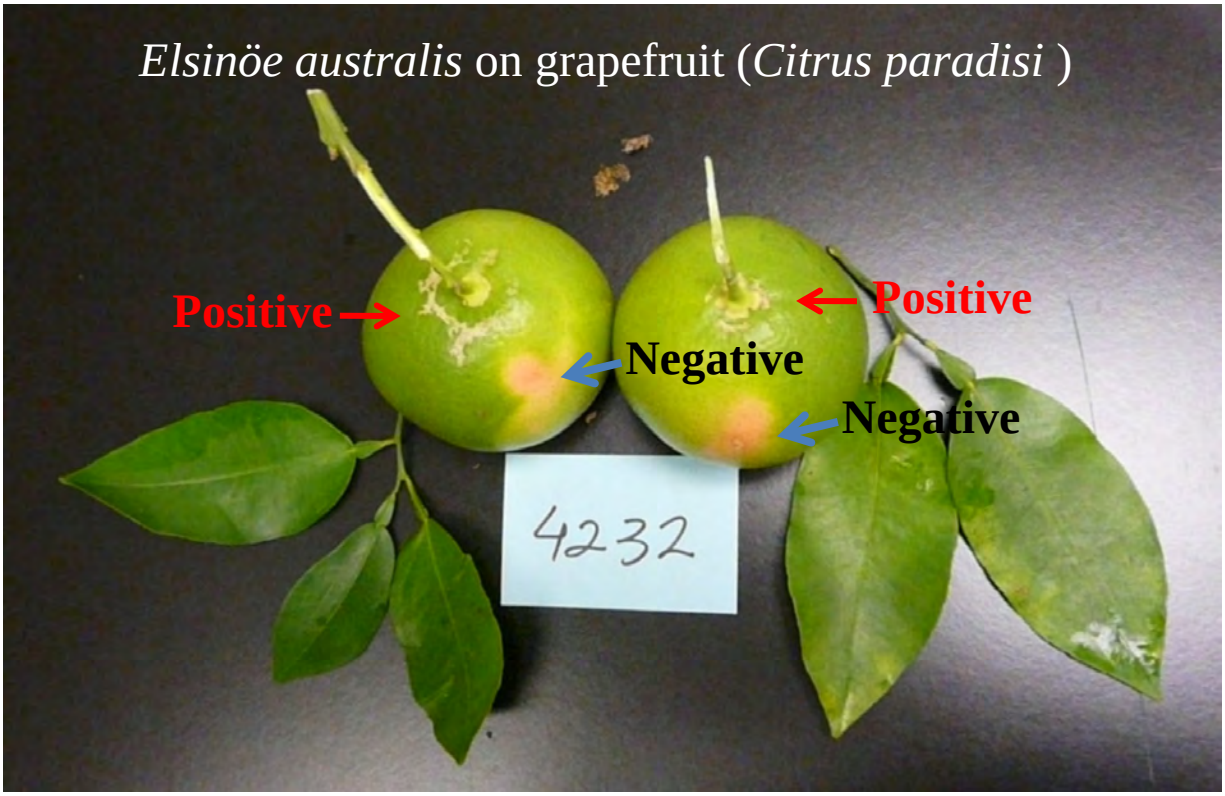
Close up of lesions in leaves in A, B and C; and in fruit in D.



Appendix 3. Symptoms of Sweet Orange Scab (SOS, Elsinöe australis) and other similar symptoms.

Multiple types of symptoms can be present on plant tissues

Plant tissues may have a number of symptoms present. This figure illustrates fruit discoloration that may result from other causes. In this case, sweet orange scab is also present, and appears to be minor in comparison.



Citrus canker can sometimes be confused with citrus scab

On young fruit, citrus canker lesions will be surrounded by a yellow halo. As the lesions age, the halo disappears and the lesions turn darker brown in color (as seen in the photograph).

Appendix 3. Symptoms of Sweet Orange Scab (SOS, Elsinöe australis) and other similar symptoms.

Injuries and other irregularities can be easily confused with Sweet orange scab symptoms, but should nonetheless be examined closely

Spots appearing similar to scabs can be caused by other pathogens, insects, or environmental injuries. Samples submitted to the USDA APHIS that were identified as free of *E. australis* are illustrated below. If symptoms in the field are ever in question, they should be submitted as official samples.

Insect damage

Environmental damage

Stem canker lesion (also seen in above image)

Appendix 3. Symptoms of Sweet Orange Scab (SOS, Elsinöe australis) and other similar symptoms.

Common Citrus Scab (*Elsinoe fawcettii*)

Symptoms on leaves and fruit

E. fawcettii symptoms are very similar to *E. australis*, with the exception of the lesions which are typically rounder and more raised. The lesions may be grouped singularly or in multiples which may overlap. Any plant tissue with citrus scab symptoms should be submitted for testing.

Front and back view of the same leaves

Appendix 3. Symptoms of Sweet Orange Scab (SOS, Elsinöe australis) and other similar symptoms.

Common Citrus Scab (*Elsinöe fawcettii*)