NPAG Report
***Phyllosticta citriasiana* Wulandari, Crous and Gruyter: Citrus tan spot**
Ascomycetes: Dothideales
NPAG Chair Approval Date: November 09, 2012

This report is an internal PPQ document, intended to be used as an aid in PPQ decision making. The technical recommendations listed at the end of this document do not necessarily represent PPQ policy.

Initiating Event and Pest Identification: Don Seaver (USDA APHIS PPQ CPHST) alerted NPAG on June 26, 2012 to a newly described fungal pathogen of Citrus in China, *Phyllosticta citriasiana* (Seaver, 2012; Wulandari et al., 2009). Since *P. citriasiana* is not present in the United States, infects mature fruit and leaves of *Citrus maxima*, moves in trade with fruit, and the extent of its host range is not clear (Wang et al., 2012; Wikee et al., 2011; Wulandari et al., 2009), NPAG was asked to evaluate the pathogen.

Synonym: None

Current PPQ Port Policy: The Pest ID database lists the genus *Phyllostica* as reportable/ actionable, but it does not list *Phyllostica citriasiana*. There are 28 pages of other *Phyllosticta* species listed in PestID, many non-reportable and many others reportable, among those that are reportable is the related citrus pathogen, *Phyllosticta citricarpa* (*Guignardia citricarpa* – sexual stage) (PestID, 2012).

Pest Situation Overview:

Exotic status: *Phyllosticta citriasiana* is not present in the United States, and infected fruit, leaves, or planting material of *Citrus maxima* from infested countries could provide a pathway for its introduction. The fungus causes necrotic spots only on mature fruit and leaves of *Citrus maxima* (Wang et al., 2012; Wikee et al., 2011; Wulandari et al., 2009), although the full extent of its host range is not yet known. It has been reported from Asia (China, Thailand, and Vietnam) (Wikee et al., 2011).

Known pest status: Fruit symptoms are similar to those caused by *Guignardia citricarpa*, the causal agent of Citrus Black Spot**.** *Phyllosticta citriasiana* causes necrotic spots on mature fruit and leaves of *Citrus maxima*, the spots on the fruit continue to expand, even after fruit are harvested and in storage, although it appears only the rind is affected and the interior of the fruit is not impacted (Wang et al., 2012; Wikee et al., 2011; Wulandari et al., 2009). It is not clear what other impacts the pathogen has on infected plants, nor has extensive host range testing of *Citrus* species been conducted.

Biology: The teleomorph, or sexual stage, of *P. citriasiana* is unknown, therefore the biology described relates to what is known about the asexual stage (Wulandari et al., 2009), with some supplemental information about the related citrus pathogen, *Phyllosticta citricarpa* and its teleomorph *Guignardia citricarpa* (Schubert et al., 2010). Related species of *Phyllosticta* and their corresponding teleomorphs in the genus *Guignardia* have been recorded as endophytes, saprophytes, and plant pathogens, some causing leaf blotch and black spots on fruit of various plants (Wulandari et al., 2009). Lesions caused by *P. citriasiana* vary in appearance and form primarily on fruit, but also on mature leaves. The lesions often contain black fruiting bodies, or pycnidia, which produce conidia (spores) that can be splash-dispersed to infect host plants (Wang et al., 2012; Wikee et al., 2011; Wulandari et al., 2009). As yet, it has not been studied, but if this pathogen has a life cycle similar to *P. citricarpa*, then it is possible that when infected mature leaves senesce, fall to the ground, and decay, the pathogen may form its sexual stage and produce air-borne inoculum in the form of ascospores (Schubert et al.,

David R. Prokrym, Chair
david.r.prokrym@aphis.usda.gov
(919) 855-7578

USDA/APHIS/PPQ/CPHST/PERAL
1730 Varsity Drive, Suite 300
Raleigh, NC 27606-5202

Christie Bertone, Executive Secretary
christie.a.bertone@aphis.usda.gov
(919) 855-7509

2010). It is unknown what conditions favor infection of fruit and mature leaves, and *P. citriasiana* infections (like *P. citricarpa*) may remain latent for months, becoming visible as fruit begin to ripen, and even more noticeable after harvest in storage or during shipment (Schubert et al., 2010; Wang et al., 2012; Wikee et al., 2011; Wulandari et al., 2009). Unlike *P. citricarpa*, which infects a broad host range of *Citrus* species (Schubert et al., 2010), *P. citriasiana* has only been reported on *C. maxima* (Wang et al., 2012; Wikee et al., 2011; Wulandari et al., 2009).

Prevalence and global distribution: This species has only been reported from **Asia** - China, Thailand, and Vietnam (Wang et al., 2012; Wikee et al., 2011; Wulandari et al., 2009).

Host range: Rutaceae- *Citrus maxima* (Burm.) Merr. (pomelo, pummelo, or shaddock) (Wang et al., 2012; Wulandari et al., 2009).

Citrus maxima is the only host on which *P. citriasiana* has been confirmed. In extensive surveys in China, over 400 *Phyllosticta* strains were isolated from lesions on *Citrus reticulata*, *C. maxima*, *C. sinensis* and *C. limon* (Wang et al., 2012). Among these, 74 strains were selected for further characterization and phylogenetic analysis, and *P. citriasiana* was collected only from infected *C. maxima* and not from other species surveyed (Wang et al., 2012). No host range testing has been conducted, so we cannot definitively state that *P. citriasiana* would not infect other species of *Citrus*.

Potential pathways of introduction: The Rutaceae is a highly regulated plant family, and restrictions are already in place to limit introduction of Citrus pests. The movement of fruit in trade, as well as smuggling of plant material could provide a pathway for introduction of *P. citriasiana*. Viable pathogen moves with infected fruit of *C. maxima*. Wang et al. (2012) and Wulangari et al. (2009) readily isolated the pathogen from samples of infected *C. maxima* fruit and leaves to characterize *P. citriasiana* and other *Phyllosticta* pathogens of *Citrus*. *Phyllosticta citriasiana* was regularly intercepted on consignments of *Citrus maxima* originating in Asia entering the EU in the Netherlands (PPSN, 2009). Since September 2005, the Netherlands has intercepted and rejected a large number of consignments of *Citrus maxima* with necrotic spots similar to those caused by the EU regulated pathogen, *Guignardia citricarpa*, which originated in China, Thailand and Vietnam (PPSN, 2009). To date, molecular testing of intercepted consignments of *Citrus maxima* in the Netherlands confirms presence of *Phyllosticta citriasiana* on symptomatic fruit and not *G. citricarpa* (PPSN, 2009).

Phyllosticta citriasiana has not been intercepted in the United States; however *G. citricarpa* and *P. citricarpa*, which cause similar symptoms, have been intercepted on *C. maxima* entering the United States (PestID, 2012). The Plant Protection Service of the Netherlands (PPSN) reported consignments of *Citrus maxima* infected with *P. citriasiana* with necrotic spots similar to those caused by the regulated pathogen *Guignardia citricarpa* (PPSN, 2009), so *P. citriasiana* could have been mistaken for *G. citricarpa* on U.S. imports as well. Fruit of *C. maxima* have been intercepted from China (52 times), Hong Kong (8), Taiwan (25 - not sure if *P. citriasiana* is present in Taiwan), Thailand (47), and Vietnam (66) primarily in passenger baggage, with a few interceptions in mail and ship's stores (PestID, 2012). Among these interceptions on *C. maxima* from Asia, there were over 40 interceptions of *Guignardia citricarpa* or *Phyllosticta citricarpa* (PestID, 2012), which may instead be *P. citriasiana*.

Potential distribution in the United States and spread: If the host range is truly limited to *C. maxima*, which is grown as an ornamental or novelty plant and requires warm growing temperatures (US plant hardiness zones 9-11) (Christman, 2009), then the potential for distribution of *P. citriasiana* in the United States may also be quite limited. Little is known about climatic requirements for infection, survival and reproduction of *P. citriasiana*, making it difficult to predict where it might become established beyond where the host plant can grow.

Detection and control: Detection: Symptoms share a lot of similarities with those of *G. citricarpa* (*P. citricarpa*), as described in Wang et al., 2012. On fruit, symptoms of *P. citriasiana* infection appear

when pummelo fruit begin ripening. “Initially, spots are small, pin-point like, red-brown, and secrete amber gum. Spots eventually enlarge to 1–6 mm, usually 2–3 mm in diameter, become sunken, crater-like, red-brown to black-brown, then grey-brown to grey in the center with a red-brown rim. Tiny and slightly elevated black dots (pycnidia) develop in the center of the spots, and green halos often surround the spots. During storage, transportation or marketing, slightly sunken tan spots of various sizes and shapes further develop on the fruits. Finally, the whole rind becomes grey-brown and shriveled, but the infection is limited to rind. Foliar symptoms are only observed on mature leaves. Spots are initially tan, pin-point like, slightly sunken, then enlarge to 2– 3 mm in diameter, and fade to grey from the center. Black dots (pycnidia) also are commonly present in center of leaf spots (Wang et al., 2012).” *Phyllosticta citriasiana* has readily been isolated (single conidia or mycelium) from infected *C. maxima* lesions (Wulandari et al., 2009). Combined approaches have been used to positively identify *P. citriasiana*, employing morphological, cultural and biochemical characteristics, phylogenetic analyses (based on sequences of internal transcribed spacer region (ITS1, 5.8S nrDNA and ITS2), partial translation elongation factor 1-alpha (TEF1) and partial actin gene (ACT)), and specific primers for detection (Wang et al., 2012).

Control: Recent papers describing this pathogen do not provide details about management of *P. citriasiana*; however recommendations for *G. citricarpa* are likely applicable, since these organisms are closely related species and likely share similar biology and epidemiology. The University of Florida released some guidance for growers to manage *G. citricarpa* and these recommendations include the following: use clean, certified nursery stock for planting; increase air flow in grove to reduce leaf wetness which may enhance spore germination and infection; reduce the accumulation of leaf litter on grove floor to decrease inoculum production; use available registered fungicides (Copper products, Strobilurins), applied to give maximum coverage of fruit and leaves (Dewdney et al., 2012).

Potential economic impacts: *Phyllosticta citriasiana* causes infections on ripening fruit, which can continue to develop after harvest, during storage and shipment (Wang et al., 2012), decreasing the quality and marketability of infected pummelo fruit. There is some small scale production of *C. maxima* in the United States (CA, FL) that could be impacted, although we do not know how many growers or how much acreage is involved to accurately estimate potential impact (USDA, 2011). If *P. citriasiana* only infects *C. maxima*, then we would expect very minimal impact in the United States and the pathogen could be managed. It is unknown if *P. citriasiana* can infect other species of *Citrus*, although it was not recovered from *C. limon*, *C. reticulata*, or *C. sinensis* in surveys in China (Wang et al., 2012). If it can infect other citrus species, such as grapefruit or tangelo, the impact could be broader. Grove management practices for *P. citriasiana* would be expected to be similar to those outlined for management of *G. citricarpa*, so could increase the cost of production of affected crops (Schubert et al., 2010).

Trade implications: The related Citrus pathogen, *Guignardia citricarpa*, is currently regulated in the United States and in the European Union (A1 quarantine pest) (EPPO/CABI, 1997), but *P. citriasiana* is not currently regulated by the EU (PPSN, 2009), nor in the United States. It is only present in Asia, reported in China, Thailand, and Vietnam (Wulandari et al., 2009), so it may become a concern for other Citrus producing countries worldwide, where the disease has not yet been reported.

Potential environmental impacts: The only reported host of *P. citriasiana* to date is *C. maxima* (Wang et al., 2012), which is a cultivated species, not naturalized in the United States (USDA - NRCS, 2012). Should additional rutaceous hosts prove susceptible, there are no endangered or threatened species of *Citrus*, but there are at least 16 Federally endangered species in the Rutaceae, in the genera *Melicope* (most in Hawaii) and *Zanthoxylum* in the United States, with a few additional state listed threatened species (USDA - NRCS, 2012), however to date there is no evidence that other hosts are infected. If the pathogen is introduced, there are few pummelo production areas which would be impacted, and additional management measures, including fungicides could have some environmental impacts; however we would expect the impacts to be small.

NPAG teleconferences: None held.

Current regulatory response and activities: No changes made to already relatively stringent import policies for rutaceous plant materials.

Need for new technology or knowledge:

- Conduct host range testing on other *Citrus* species, to determine if *P. citriasiana* is host-specific to *C. maxima*.
- Determine environmental conditions that are optimal for pathogen survival and disease development.
- Is there evidence of a sexual stage of *P. citriasiana* and what is its role in the life cycle of the pathogen?
- Assess *C. maxima* fruit from Asia, previously intercepted at U.S. ports with infections reported as *G. citricarpa.* Are there any specimens available to verify *P. citriasiana* vs. *G. citricarpa*?

National Plant Board consultation: None

The following technical recommendations are based on the best available science at the time of the report completion and are intended to be used as an aid in PPQ decision-making.

NPAG Recommended PPQ Port Policy: NPAG considers *Phyllosticta citriasiana* to be a threat and recommends that PPQ establish a reportable/actionable port policy.

Recommendations:

1. NPAG considers *Phyllosticta citriasiana* to be a threat and recommends that PPQ establish a reportable/actionable port policy because this pathogen is not known to be present in the United States, it infects ripening fruit and mature leaves of *Citrus maxima*, infected pummelo may provide a pathway for the pathogen to become established, and the extent of the pathogen's host range has not been determined. **Action Leader: Joe Cavey (PPQ-PHP-NIS**)

Direct referral: Joe Cavey (PPQ-PHP-NIS).

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Author: Lisa Ferguson

Chair's Approval: *David R Prokrym*

Signature Date: November 9, 2012

David R Prokrym

Digitally signed by David R Prokrym
DN: cn=David R Prokrym, o=APHIS PPQ PERAL, ou=USDA, email=David.R.Prokrym@aphis.usda.gov, c=US
Date: 2012.11.09 14:49:37 -05'00'

David R. Prokrym, Chair
david.r.prokrym@aphis.usda.gov
(919) 855-7578

USDA/APHIS/PPQ/CPHST/PERAL
1730 Varsity Drive, Suite 300
Raleigh, NC 27606-5202

Christie Bertone, Executive Secretary
christie.a.bertone@aphis.usda.gov
(919) 855-7509