EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
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Report of a Pest Risk Analysis for *Acidovorax citrulli*

This summary is based on a PRA prepared in the framework of the Prima Phacie project (MacLeod *et al*., 2012), according to EPPO Decision support scheme for quarantine pests (PM 5/3(4) revised by PRATIQUE) and subsequent discussions in the EPPO Panel on Phytosanitary Measures.

Pests: ***Acidovorax citrulli***
PRA area: EU
Assessors: Prima Phacie project:
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Date: December 2011 for the risk assessment part. January 2012 for the risk management part.

EPPO Core members (Jose Maria GUITIAN CASTRILLÓN, Salla HANNUNEN, Tami LEVI, Corinne LE FAY-SOULOY, Lucio MONTECCHIO, Nursen USTUN, Arild SLETTEN) reviewed the PRA between January and February 2013 and concluded that this PRA was relevant to make EPPO recommendations.
The PRA report was reviewed by the Panel on Phytosanitary Measures on 2013-10-29 and 2014-03-05

STAGE 1: INITIATION

Reason for doing PRA: Because *A. citrulli* can be a serious threat to cucurbit crops (in particular melon and watermelon), the EPPO Panel on Bacterial Diseases considered that it should be added to the EPPO Alert List in 2009.
This assessment was initiated as a case study pest to be examined within EFSA project CFP/EFSA/PLH/2009/01 (Prima phacie). *Acidovorax citrulli* had been selected as a case study pest because it satisfied a number of criteria needed to provide a range of contrasting pest examples for consideration in the project.

Taxonomic position of pest: Betaproteobacteria: Burkholderiales: Comamonadaceae
Genus: *Acidovorax*
Species: *citrulli* (Schaad *et al*., 1978) Schaad *et al*., 2008, comb. nov.
Synonym: *Acidovorax avenae* subsp. *citrulli* (Schaad *et al*., 1978) Willems *et al*., 1992

STAGE 2: PEST RISK ASSESSMENT

PROBABILITY OF INTRODUCTION

Entry

Geographical distribution:
(see PRA record for references)

EPPO region: Several incursions have been detected in Greece, Hungary, Israel and Italy.
The pest has been declared eradicated in Israel (Zioni pers. comm. 2013), Italy (for the outbreak in Emilia-Romagna, Finelli, pers. comm., 2013). No detection of the pest has occurred in Hungary since 2007 (Szalkai, pers. comm, 2013).
Present in Turkey.

North America: USA (Alabama, Arkansas, California, Delaware, Florida, Georgia, Illinois, Indiana, Iowa, Maryland, Mississippi, Missouri, North Carolina, Oklahoma, Oregon, South Carolina, Texas), Canada (Ontario)
South America: Brazil (Ceará, Minais Gerais, Pernambuco, Rio Grande do Norte, Rio Grande do Sul, Roraima).
Central America and Caribbean: Costa Rica, Nicaragua.
Africa: South Africa (unconfirmed). Sato (2009) lists bacterial fruit blotch in South Africa, no other details are provided. When contacted, Dr. Sato replied that the source of information is through personal communication with a colleague in S. Africa (Sato pers. comm., 2011) thus its presence in South Africa remains unconfirmed. The presence in Africa is not recorded in PQR not in the CABI map of Pant Diseases (CABI, 2011)
Asia: China, Indonesia, India, Iran, Israel, Japan, Korea Republic, Taiwan, Thailand, Turkey.
Oceania: Australia (Queensland), Guam, Northern Mariana Is.

Major host plants or habitats:
(see PRA record for references)

Acidovorax citrulli causes the disease bacterial fruit blotch on cucurbits, specifically on watermelon (*Citrullus lanatus*), and cantaloupe and honeydew melon (*Cucumis melo*) which are the most susceptible hosts. Other cultivated hosts include cucumber (*Cucumis sativus*), pumpkin (*Cucurbita moschata*) and squash (*Cucurbita pepo*). Citronmelon (*Citrullus lanatus* var. *citroides*) is a weed host. Recently betel vine (*Piper betle*: Piperaceae) was reported as a host in Taiwan.
Alternative hosts have been also suggested based on artificial inoculations: solanaceous plants/fruits and papaya fruits (Nascimento *et al.*, 2004).

Which pathway(s) is the pest likely to be introduced on:

- **Seed intended for planting of Cucurbitaceae.**

A. citrulli survives on and in cucurbit seed. Infested seed have been intercepted in USA and Israel.
An outbreak of bacterial fruit blotch in Nicaragua was linked to imported seed (EPPO, 2009a) as was an outbreak in Turkey (Demir, 1986; Nursen, 2008) and in Indiana in USA (Latin & Hopkins, 1995) so it is possible that there will be some association at origin.
Infected fruit may contain *A. citrulli* infected seeds regardless of whether the fruit shows symptoms or not.
Seed treatments are applied to reduce incidence of the disease but do not eliminate the pathogen.

- **Seedlings (transplants) of Cucurbitaceae.**

The pathogen has been found in association with seedlings of cucurbits in trade. Trade from infested area occurs. Seedlings may be asymptomatic.

Pathways considered less likely:

- **Fruit of Cucurbitaceae**

Some infested fruit may be symptomless. Cucurbit fruit are not regulated by the EC plant health directive and so no specific phytosanitary measures are in place.
However the probability of transfer to a host crop is very unlikely as cucurbit fruit will be consumed and waste disposed of.

- **Solanaceous seeds**

There is one report from Israel suggesting that *A. citrulli* may be transmitted by solanaceous seeds – the pathogen has been isolated from tomato and aubergine seeds imported to Israel (Assouline *et al*., 1997). However, this pathway was not examined due to these kinds of hosts not being reported again. Nevertheless with more time such a potential pathway would be worth assessing, particularly if *A. citrulli* expands the

range of hosts on which disease is expressed.

- **Cucurbit seed for food or feed (cucurbit grain)**

Processed (toasted) pumpkin seeds can be found in supermarkets and health food shops in the EU. If not already processed, such seeds should be toasted before consumption. However, the trade of cucurbit seeds for food or feed was excluded as a possible pathway, since it was assumed that only a small volume is involved and the intended use of the commodity prevents establishment of the disease.

- **Cucurbit, mainly watermelon, waste derived from industrial activity (fruit juice production).**

Lack of information on this pathway prevented its analysis.

Establishment

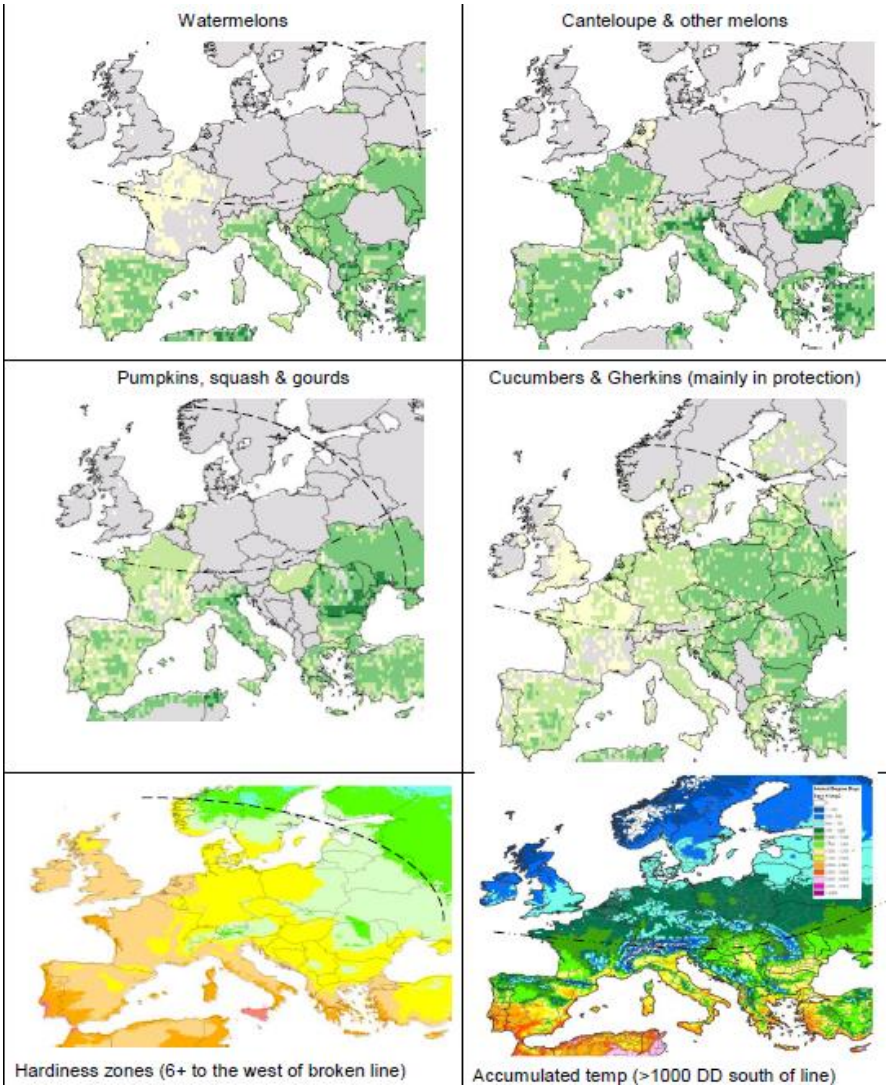
Plants at risk in the PRA area:

Cucurbits, specifically watermelon (*Citrullus lanatus*) and cantaloupe and honeydew melon (*Cucumis melo*) which are the most susceptible hosts. Other cultivated hosts include cucumber (*Cucumis sativus*), pumpkin (*Cucurbita moschata*) and squash (*Cucurbita pepo*).
Outside of protected cultivation, host crops occur in many EU countries and are most densely grown in southern and eastern Europe.

Climatic similarity of present distribution with PRA area (or parts thereof):

Acidovorax citrulli occurs on most continents (a suggestion that it occurs in South Africa has not been confirmed). Given that there have been outbreaks of bacterial fruit blotch of cucurbits in Greece, Italy and Hungary survival is known to be possible in these areas already.
Optimum conditions for disease expression are 55% relative humidity and temperatures between 24°C and 38°C. However the climatic conditions to sustain the pathogen have not been well studied and the pathogen is likely to survive and be able to establish in areas outside the conditions where disease expression is optimal.
The pathogen appears to occur in plant hardiness zones 5 or 6 up to plant hardiness zones 10 or 11. In the EU, crop hosts occur outdoors in plant hardiness zones 6 (e.g. Romania, Poland) to 10 (southern Portugal, Spain and Italy). Melons and cucumbers are grown under glass in northern Europe, where it is not appropriate to consider plant hardiness zones.
With regard to accumulated temperature *A. citrulli* generally does not appear to occur in areas with less than 1,000 day degrees, above a base of 10°C (southern and central Europe). However, there is insufficient detailed information for the distribution of *A. citrulli* to accurately determine the accumulated temperature required by *A. citrulli* to express disease symptoms. When outbreaks of bacterial fruit blotch are reported in the literature, there is often mention of hot and humid conditions. There is uncertainty as to whether environmental conditions in the EPPO region are optimal for disease expression.

Maps in figure 1 show the potential occurrence of *A. citrulli* in relation to hardiness zones, accumulated temperature and crop host production (extracted from Mc Leod *et al.,* 2011). Humidity is not taken into account.



Characteristics (other than climatic) of the PRA area that would favour establishment:

There is no information regarding whether abiotic factors such as soil type influences establishment. In the absence of evidence, it is assumed that such factors are not critical.

Which part of the PRA area is the area of potential establishment:

The area of potential establishment can be considered as all glasshouses where cucurbits are grown and areas outdoors from southern and central Europe. However, there is uncertainty as to whether environmental conditions in the EPPO region are optimal for disease expression.

POTENTIAL ECONOMIC CONSEQUENCES

How much economic impact does the pest have in its present distribution:

The most important impact of the disease is likely to be on watermelon fruits. Bacterial fruit blotch has become increasingly important in the watermelon and melon industry since 1987 when the disease destroyed entire fields of watermelon crops on Guam and Tinian in the Mariana Islands (Wall & Santos, 1988; Wall *et al*., 1990). The disease has now rapidly emerged as a serious pathogen of watermelon and melon crops and is a major threat to these industries around the world. Losses of up to 50% and even 100% of marketable fruit have been reported several countries in particular in fields of watermelons. The extent of losses is likely to depend on the initial inoculums level and environmental conditions. Loses range from 5 to 100%. Specific measures are needed to control the disease (use of clean planting material, crop rotation, elimination of debris and volunteers). However, surveys in the USA show that the disease can be sporadic with an incidence varying from 4% to 47% (Latin and Hopkins, 1995).

Outbreaks have occurred in Greece, Italy, Hungary and Turkey, but generally no important damage has been recorded. There is uncertainty as to whether environmental conditions in the EPPO region are optimal for disease expression. Studies conducted in the framework of the EU FP7 TESTA project show that initial infection and disease progression is closely linked to seed infestation level (Stefani, *pers. comm.* 2014). It is consequently important that seeds are free from this pathogen.

Describe damage to potential hosts in PRA area:

Infected watermelon fruits develop dark olive green blotches on their upper side. Early in development, blotches appear usually as small (less than 1 mm in diameter), irregularly shaped, either water-soaked, dull gray green or dark green lesions. The fruits may ultimately rot ('watery rot' symptom) and collapse due to secondary decay organisms invading the blotches.

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How much economic impact would the pest have in the PRA area:

Watermelons grown outdoors in warm and humid environments are most susceptible. As stated above, there is uncertainty as to whether environmental conditions in the EPPO region are optimal for disease expression. Outbreaks have occurred in Greece, Italy, Hungary and Turkey, but generally no important damage has been recorded.. However, it cannot be excluded that in specific situations (e.g. humid summer) damage could occur and potentially be "high" (although uncertainty means the range of impacts spans "low" to "very high"). See also previous comment on seed infestation level.

CONCLUSIONS OF PEST RISK ASSESSMENT

Summarize the major factors that influence the acceptability of the risk from this pest:

Acidovorax citrulli is a pathogen of cucurbits, especially damaging to watermelons, which has spread its geographic distribution in recent years and when environmental conditions are suitable, major crop loss can result.

Estimate the probability of entry:

The probability of entry is considered **moderately likely to very likely with a medium uncertainty**.

There is trade in cucurbit seed, seedlings and fruit from countries where *A. citrulli* occurs but there is a limited number of interceptions of infested consignments around the world, together with the few incidents of outbreaks in the EU. However it should be noted that seeds have been identified as a source of outbreaks in recent years.

Estimate the probability of establishment:

The probability of establishment is **moderately likely (with a medium uncertainty)** in all of the watermelon growing area of the EU. In general, conditions must be warm and wet for *A. citrulli* to cause disease (bacterial fruit blotch of cucurbits). Optimal conditions for disease development suggests 55% relative humidity and temperatures between 24°C and 38°C are optimal, although the wider range of temperature and humidity over which the disease can develop is unknown. This is the greatest uncertainty

around identifying exactly where the pathogen could establish, and how likely it is to establish. However, based on where the disease has been reported outside of Europe, almost all of the watermelon growing area of the EU is likely to provide suitable conditions within which the pathogen could establish.

Estimate the probability of spread:

The rate of spread of the pests is likely to be **very high (with a low uncertainty)** as they pest can move with seed and plants for planting. The rate of natural spread (via overhead irrigation, or wind-driven rain splash) is considered as moderate (1-10 km in a year) with medium uncertainty. However, there have been outbreaks in the EU without extensive further spread in the following years.

Estimate the potential economic impact:

Based on the information for areas where the pest occurs, the potential economic impact is considered as **major with high uncertainty**. As stated above, there is uncertainty as to whether environmental conditions in the EPPO region are optimal for disease expression. However, it cannot be excluded that in specific situations (e.g. humid summer and use of infested seed lots) damage could occur and potentially be "high" (although uncertainty means the range of impacts spans "low" to "very high").
In the EU most watermelon is grown in southern regions, which is where losses would be greatest.
No environmental impacts are anticipated.

Degree of uncertainty

The main uncertainties are :
- likelihood of the pathogen being associated with the commodity at the start of the pathway,
- how likely infected seedlings would be detected and removed from the pathway during routine quality checks
- detailed environmental and cultural conditions required by the pathogen to cause damage.

OVERALL CONCLUSIONS

Acidovorax citrulli is a pathogen of cucurbits, especially damaging to watermelons, which has spread its geographic distribution in recent years and when environmental conditions are suitable, major crop loss can result. Introduction of the pest in the PRA area is rated as moderately likely. Interceptions have already occurred. Transfer and spread to other plants will occur via overhead irrigation, or wind-driven rain splash. As stated above there is uncertainty as to whether environmental conditions in the EPPO region are optimal for disease expression However, it cannot be excluded that in specific situations (e.g. humid summer and use of infested seed lots) damage could occur and potentially be "high" (although uncertainty means the range of impacts spans "low" to "very high").
Conditions in glasshouses that use overhead irrigation would provide suitable conditions for the pathogen.

STAGE 3: PEST RISK MANAGEMENT

IDENTIFICATION OF THE PATHWAYS

Pathways studied in the pest risk management

- **Seed of *Citrullus lanatus, Cucumis melo, Cucumis sativus, Cucurbita moschata* and *Cucurbita pepo* (*Citrullus lanatus* seed in particular)**
- **Seedlings (plants for planting) of *Citrullus lanatus, Cucumis melo, Cucumis sativus, Cucurbita moschata* and *Cucurbita pepo*, (*Citrullus lanatus* seedlings in particular)**

IDENTIFICATION OF POSSIBLE MEASURES

Possible measures for pathways

Measures related to the crop or to places of production:

- Pest free area

The seeds or seedlings should originate from a pest free area established on the basis of surveillance. The exporting country should provide surveillance data to demonstrate that the pest is absent from all or part of its territory and information on how pest freedom is maintained.
For a country where the pest is present in part of the country, measures should be in place to prevent that infested seeds are moved to the pest free area. Delimiting surveys should be conducted to determine the exact pest distribution.

- Pest free place of production or pest free production site

Place of production freedom reduces the risk to an acceptable level and should consist of a combination of the following individual measures:

- Pest should have been absent from the place of production at least since the two previous growing period
- Sanitation measures should be in place (e.g., prevention of infection with tools, equipment, etc.)
- The seeds/or seedlings should be produced from seeds free from the pest
- The place or site of production should be protected from wind-driven rains or a buffer zone of 1 km to 5 km depending on local climatic conditions (e.g. areas prone to storm) should be established. There is uncertainty on the minimum distance of such buffer zone.
- Testing during the growing period.

Measures related to consignments:

- Testing of seeds
    - Testing of seed by growing seedlings

Seed testing would be effective but require a high number of seed to be tested. Currently, it is recommended that the US seed industry test 10000–50000 seedlings per seedlot. Seedling inspection begins once the cotyledons start expanding and evaluations continue on a daily basis. The final inspection is conducted after 18 days or when the cotyledons are fully expanded and the first true leaves are expanding.
An alternative method is the 'sweat box assay'

    - Testing of seed by PCR

Several tests are available. A diagnostic protocol is in preparation for this pest in the framework of the EU TESTA project (tests include molecular tests such as Taqman PCR).

The following measures related to consignments are not considered efficient.

- Seed treatments

Thermotherapy: there are few experiments but no approved schedule as a phytosanitary treatment. Since the PRA was conducted, 2 articles were published (Feng et al. 2009 and Kubota *et al.* 2012) and concluded that the level of temperature needed to kill the pathogen will adversely affect the seed germination rate.

Chemical treatments: decontamination of infested seed by chemical treatments is not 100% effective. The inability for the compounds to penetrate the seed coat, the location of bacteria on/in the seed and the phytotoxic effects hinder their efficacy. In addition, it is not possible to guarantee pathogen-free seed, as seed testing actually refers to a representative sample of the seedlot.

- Testing of seedling (e.g. ELISA, PCR-based assays)

This is not considered appropriate as it is time-, space- and labour-consuming, and seedlings moderately infected or symptomless could be overlooked (*i.e.* the method is not sensitive).

- Inspection of seed and seedlings is not considered appropriate as they may be symptomless.

EVALUATION OF THE MEASURES IDENTIFIED IN RELATION TO THE RISKS PRESENTED BY THE PATHWAYS

The pest would be difficult to eradicate or contain if introduced therefore measures should be taken to prevent its further entry and spread in the PRA area.

Degree of uncertainty Uncertainties in the management part are:

- Possibility to maintain a PFA/PFPP
- Detection of asymptomatic plants
- Effectiveness of tests

IDENTIFICATION OF POSSIBLE MEASURES

PC= Phytosanitary certificate, RC=Phytosanitary certificate of re-export

Pathway	**Measures**
Seeds of *Citrullus lanatus*, *Cucumis melo*, *Cucumis sativus*, *Cucurbita moschata* and *Cucurbita pepo* (*Citrullus lanatus* seed in particular)	PC and, if appropriate, RC • Pest-free area or • Pest-free place of production or • Pest-free site under protection • Testing of seed by growing seedlings or by an appropriate method
Seedlings of *Citrullus lanatus*, *Cucumis melo*, *Cucumis sativus*, *Cucurbita moschata* and *Cucurbita pepo* (*Citrullus lanatus* seed in particular)	PC and, if appropriate, RC • Pest-free area or • Pest-free place of production or • Pest-free site under protection

References

Macleod A *et al.* 2012. Pest risk assessment for the European Community plant health: A comparative approach with case studies. Full report [1053 pp.] is available at http://www.efsa.europa.eu/fr/supporting/doc/319e.pdf
The PRAs for *Acidovorax citrulli* are available in Phase 4 Annexes 1 http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/319eax8.zip