**Pest risk assessment for the European Community: plant health: a comparative approach with case studies**

# Pest Risk Assessment: Revised Test Method 4b[1]
# With specific risk reduction options in place

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[1] Method 4b describes risk elements using an ordinal scale of 5 categories (very low, low, medium, high, very high)

**Preface**

Pest risk assessment provides the scientific basis for the overall management of pest risk. It involves identifying pests and characterizing the risks associated with those pests by estimating their probability of introduction (entry, transfer and establishment) as well as the severity of the consequences to crops and the wider environment as a result of their introduction.

Risk assessments are science-based evaluations. They are neither scientific research nor are they scientific manuscripts. The risk assessment forms a link between scientific data and decision makers and should expresses risk in terms appropriate for decision makers.

**Note**

Risk assessors will find it useful to have a copy of International Standards for Phytosanitary Measures No. 5, the Glossary of Phytosanitary Terms (IPPC, 2007)[1], ISPM No. 11, Pest risk analysis for quarantine pests, including analysis of environmental risks and living modified organisms (IPPC, 2004)[2] and the EFSA guidance document on a harmonized framework for pest risk assessment (EFSA, 2010)[3] to hand as they read this document and conduct a pest risk assessment.

[2] ISPMs Nos. 5 and 11 available at https://www.ippc.int/index.php?id=ispms&no_cache=1&L=0

[3] EFSA Journal 2010, **8**(2),1495-1561, Available at http://www.efsa.europa.eu/en/scdocs/doc/1495.pdf

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**Supporting documentation**

- Annex 1: Questionnaire template distributed to EFSA Plant Health Network

## Executive Summary


*Provide a technical summary reflecting the content of the assessment (the questions addressed, the information evaluated, and the key issues which resulted in the conclusion)*

The purpose of this pest risk assessment was to inform the evaluation of risk reduction options by assessing the plant health risk associated with *Acidovorax citrulli* in a scenario where risk reduction options are in place, within the framework of EFSA project CFP/EFSA/PLH/2009/01.

**Pest biology** (*see datasheet for details*)

- Identity of the pest

*Acidovorax citrulli* (Schaad *et al*., 1978) Schaad *et al*., 2008, comb. nov.

Betaproteobacteria: Burkholderiales: Comamonadaceae

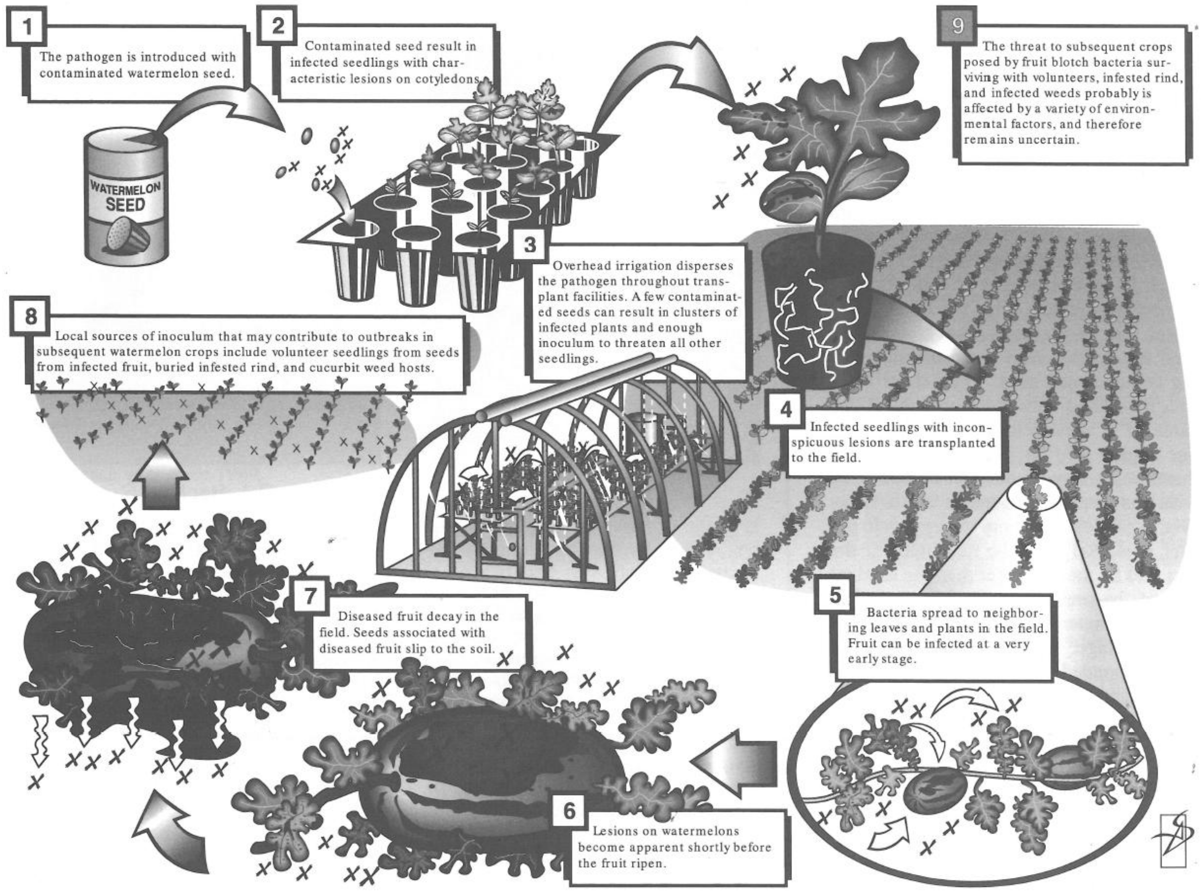
Synonym: *Acidovorax avenae* subsp. *citrulli* (Schaad *et al*., 1978) Willems *et al*., 1992

Schaad *et al.* (2008) proposed that the taxonomic position of the pathogen be elevated from subspecies to species level (*i.e. A. citrulli* instead of *A. avenae* subsp. *citrulli*). The new name was then validly published in the International Journal of Systematic and Evolutionary Microbiology in 2009 (Schaad *et al.,* 2009). However, this new nomenclature has not yet been adopted by the scientific community (Bahar & Burdman, 2010). In this assessment, the most recent name, *Acidovorax citrulli* is used.

- Life history / Disease cycle and epidemiology: (*briefly*)

*Acidovorax citrulli* survives **on** and **in** cucurbit seed from where it can spread to infect the cotyledons as they emerge from the seed coat. Seedlings germinating directly in the field or in glasshouses can be infested. Seedlings grown in warm and humid glasshouses are more susceptible to disease establishment and the rate of local disease spread can be high. Infected seedlings may or may not show symptoms depending on the level of inoculum, virulence level of the strains, environmental conditions and host plant. Symptomless and yet infected seedlings can be transplanted into the field. As plants grow in the field, the pathogen is disseminated by wind-driven rain, irrigation water, thundershowers or by mechanical means via farm workers and cultivation equipment. Cucurbit fruit can then become infected via natural openings or wounds. Fruits may also be infected as a result of infection of female blossoms. Fruit can eventually rot in the field and their seeds, infested by contact with contaminated tissues, slip to the soil. The bacterium can overwinter in volunteer seedlings from seeds from infected fruits, infested rind or other infested crop residues as well as cucurbit weed hosts (especially wild citron, *Citrus medica*). Such plant material then acts as a local source of inoculum that may contribute to outbreaks in subsequent cucurbit crops. The bacterial fruit blotch of cucurbits disease cycle, from the introduction of the pathogen in transplant facilities with contaminated seed to the survival of the pathogen in the field, is illustrated in Latin & Hopkins (1995) and Walcott (2005). (Further details and references are provided in the datasheet).

**Figure ES1:** The bacterial fruit blotch of cucurbits disease cycle. Steps 1 to 9 illustrate the cycle from the introduction of the pathogen with contaminated seed to the survival of the pathogen in the field.




Source: Figure reproduced from Latin & Hopkins (1995) with permission from R.X. Latin

- Host range

*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).

- Means of dispersal / spread

Long distance dispersal of the pathogen is via contaminated seed or transplant seedlings moved in trade. Once *Acidovorax citrulli* has been introduced to cucurbit production, local / natural spread occurs via wind-driven rain splash and mechanical means. Spread is most rapid on hot summer days with heavy afternoon thundershowers that includes blowing rains. In glasshouse systems overhead irrigation can spread the disease.

**Time period considered by this assessment**

*State the time horizon that you considered during this assessment. If climate change is taken into account, note the climate change scenarios considered.*

Much new information has been published on this pathogen since 2009. More scientific papers are expected in the next few years. This assessment looks ahead up to five years and does not take climate change into account. It is suggested that the assessment be updated in the next few years to take into account any new information published in scientific literature.

**Geographic Distribution**

| | |
|---|---|
| North America: | Present, USA in several, mostly southern States, Canada. |
| South America: | Present, Brazil. |
| Central America: | Present, Costa Rica (eradicated in Nicaragua) |
| Europe: | Outbreaks under eradication in Greece, Hungary and Italy. |
| Africa: | Present, 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. |
| Asia: | Present, China, Indonesia*, India, Iran*, Israel, Japan, South Korea, Taiwan, Thailand, Turkey. |
| Oceania: | Present, Australia, Guam, Northern Mariana Is. |

***** According to EPPO PQR v 5.0.1500 database (EPPO, 2011) the pest record in this country is not reliable.

**Pathways**

Three pathways are considered. The principle pathway which is most likely to facilitate spread is through use of contaminated cucurbit seed intended for planting; the other pathway likely to provide a route for entry into the EU is the use of contaminated seedlings, grown from contaminated seed lots, then transplanted and intended for cucurbit production; a third pathway considered, though much less likely to provide a route for establishment, is through import of contaminated cucurbit fruit such as watermelons, other melons, pumpkins or cucumbers. However, the likelihood of bacteria transferring from contaminated fruit to growing plants is extremely small, unless fruit is disposed of carelessly, very close to a site of production.

## Summary of risk elements

- Likelihood of entry and transfer (*summarise 2.08*)

*Acidovorax citrulli* is most likely to enter the EU via contaminated watermelon seed, or contaminated watermelon seedlings that have been grown from a batch of infected seed. Transfer and spread to other plants is almost certain via overhead irrigation, rain splash, or mechanical means. Combining risk elements that contribute to the likelihood of pest entry and transfer using a BBN model (described elsewhere) suggests that with risk reduction options in place the likelihood of entry and transfer over the next five years shifts from mostly medium-high to mostly medium. However, there is considerable uncertainty around this and there is some chance that the likelihood of entry could actually be high or very low. Further study of how risk elements and pathways are combined in the BBN model is justified.

- Potential area occupied at time horizon (*summarise extent of spread*, *3.02*)

In general, conditions must be "warm and wet" for *Acidovorax citrulli* to cause disease (bacterial fruit blotch of cucurbits). Conditions used to drive disease development in seed testing suggests 55% relative humidity and temperatures between 24°C and 38°C are optimal, although the specific range of temperature and humidity over which the disease can develop is unknown. Based on the locations of where the disease has been reported outside the PRA area, almost all of the watermelon growing area of the EU is likely to provide suitable conditions within which the disease could develop. Conditions in glasshouses that use overhead irrigation would also provide suitable conditions for the pathogen to cause disease.

With swift action taken to destroy infested crops and strict hygiene measures implemented to prevent local spread, effectively eradicating the pathogen at a local scale, spread would be much reduced. Outbreaks in the USA and elsewhere suggest that occurrence of the disease is very much based on the use of contaminated seed or seedlings. There is not much spread from sites of initial introduction. In a scenario where action is taken to inhibit spread, the likely area occupied after 5 years is very likely to be less than 10% of the potential host area where establishment is possible whereas without action being taken, it is possible that the pathogen could spread to up to 1/3 of the area suitable for establishment.

- Consequences (*summarise the combination of 3.03 and 3.04*)

Bacterial fruit blotch was first recognised as a significant pest in 1987 when the disease destroyed entire fields of watermelon crops on Guam and Tinian in the Mariana Islands. Since then the disease has rapidly emerged as a serious pathogen of watermelon and melon crops in the USA and is a major threat to these industries around the world (Bahar & Burdman, 2010). In warm and wet conditions the consequences to the most susceptible crops such as watermelons and melons can be severe. For example, of 500ha affected in south western Indiana nearly 100 ha incurred losses approaching 90%. If similar losses occurred in the EU, the impact could be regarded as high or very high.

No serious environmental impacts are expected to be caused by the pathogen.

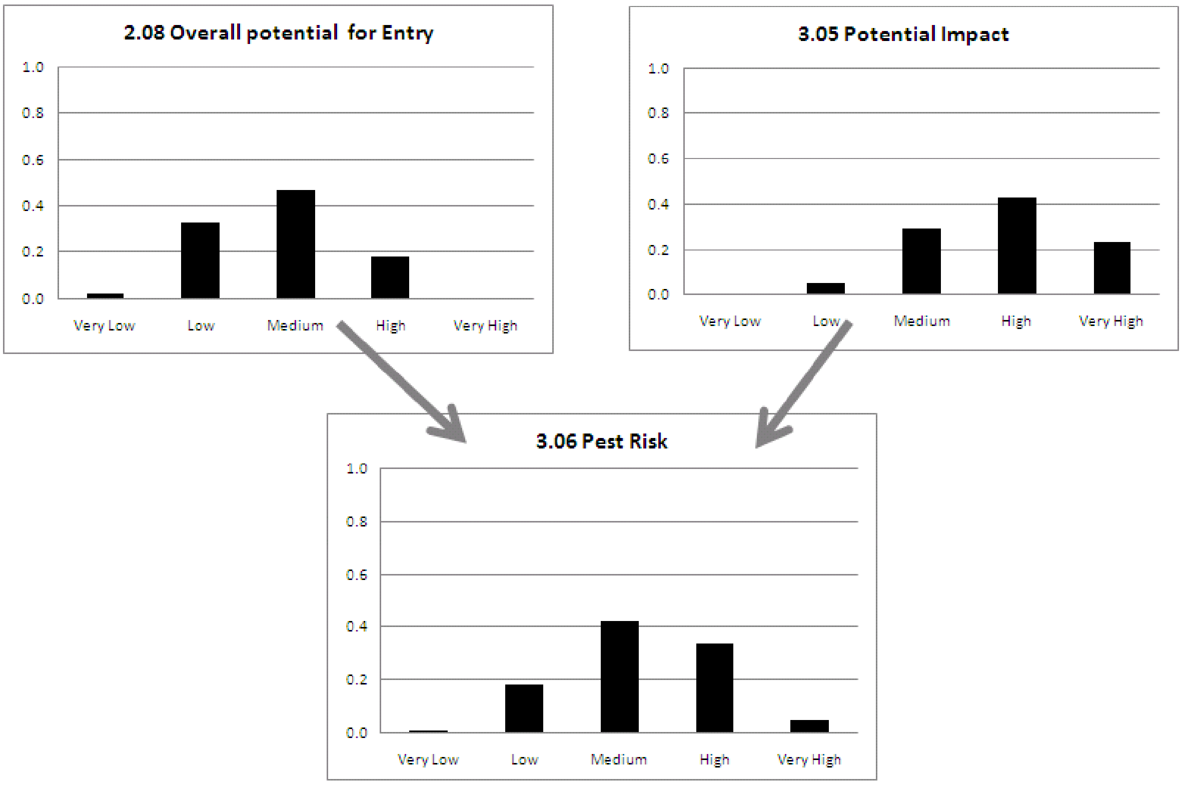
Whilst no risk reduction options are available to ensure recovery of a crop after infection (therapeutic measures), the disease is likely to have a moderate to severe impact on affected crops. Nevertheless, risk reduction options that inhibit introduction and spread of the pathogen (see above) would act to reduce total potential impact by reducing the area over which impacts are likely to occur.

- Pest risk (*summarise 3.06, the combination of likelihood of entry & transfer 2.0, with potential impact, 3.05)*.

*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.

In an experimental risk assessment method a BBN model (described elsewhere) was used to systematically combine elements contributing to risk. Model outputs are presented in a series of bar charts. Figure ES1 shows the final model output which combines scores for likelihood of entry and transfer with potential impact to give an overall pest risk profile. The profile shows much uncertainty with some likelihood of risk being minimal (*i.e.* very low) up to major (very high). Most likely the pest risk is actually medium. Taking into account the major impacts reported where outbreaks have occurred, but which are normally only reported in a small area, the overall rating of medium pest risk presented to the EU by *Acidovorax citrulli* seems a reasonable output for the model and is in line with assessors opinion. Risk reduction options appear to shift the risk profile slightly to the left (see "Evaluation of risk reduction options" elsewhere) and do not seem to have a large influence on risk. This could be due to the many uncertainties associated with the assessment and/or related to how the BBN model operates.

**Figure ES 1:** Graphical representation of Pest Risk (3.06), combining overall potential for pest entry and transfer (2.08) with potential impact (3.05).




**Uncertainties**

*Summarise major uncertainties and report on what happens if those risk elements that are most uncertain are changed (e.g. become less uncertain) – how does the overall risk profile of expected risk change? Describe changes in the bar chart showing expected risk if uncertainty is changed.*

Three pathways were considered (cucurbit seed for planting, cucurbit seedlings for planting and cucurbit fruit for consumption). The assessment focussed on watermelon seed, seedlings and fruit from countries where the disease has been reported. A critical uncertainty in all of the pathways was estimating the likelihood of the pathogen being associated with the commodity at the start of the pathway. The pathway of seedlings had uncertainty around how likely infected seedlings would be detected and removed from the pathway during routine quality checks. Further research on the detailed environmental conditions required by the pathogen to develop symptoms would better inform the likelihood of establishment in the risk assessment area and also reduce the uncertainty around identifying the endangered area.

**Conclusion**
The bacterial plant pathogen *Acidovorax citrulli* has the characteristics of a quarantine pest for the EU.

**Keywords:** bacterial fruit blotch of cucurbits, *Acidovorax citrulli*, pest risk assessment

## Stage 1 – Initiation

### 1.1 Background and Initiation

*Provide the background and terms of references as provided by the originator of the risk assessment request (European Commission, European Parliament, Member States, or EFSA)*

The purpose of this assessment is to evaluate the plant health risk of *Acidovorax citrulli* within the framework of EFSA project CFP/EFSA/PLH/2009/01 (Prima phacie).

The terms of reference are described in EFSA call CFP/EFSA/PLH/2009/01, Pest risk assessment for the European Community plant health: A comparative approach with case studies (EFSA, 2009). The text in Section 1.4 of the call, “Structure and essential requirements of the proposal”, pages 7-9, provide the terms of reference *e.g.* that a systematic review of risk assessment methodologies, with emphasis on quantitative and semi-quantitative approaches, used in pest risk assessment to analyse and predict the likelihood of entry, establishment and spread, the potential negative consequences, the overall risk characterisation and the associated level of uncertainties be assessed, together with a systematic review of the methods used to assess the effectiveness of management options in reducing the risk of introduction and/or spread. The quantification of economic losses in monetary values and the assessment of potential effects on export markets, employment and tourism were not to be included.

**Initiation Point**

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.

### 1.2 Identification of the risk assessment area

The risk assessment area is the 27 Member States of the EU with the focus on the continental European area, specifically excluding the ultra-peripheral regions, *i.e.* the French overseas departments, Spanish Canary Isles and Portuguese Azores and Madeira.

### 1.3 Available pertinent regulatory information

#### (i) Previous risk assessment or pest risk analysis?

A pest risk analysis was performed by Üstün Nursen from the Plant Protection Research Institute in Turkey (Nursen, 2008) following the finding of an emerging disease referred to as “watermelon fruit blotch” detected in Turkey in 1995. The analysis considered Turkey as the PRA area.

#### (ii) Available Pest Fact Sheets/ Pest Alerts etc.

A pest datasheet for *Acidovorax citrulli* was composed and submitted as Annex 1d to the first Prima phacie interim report (July, 2010). The datasheet has subsequently been updated with additional information, for example regarding its name (*A. citrulli* rather than *A. avenae* ssp. c*itrulli*), and its geographic distribution.

The CABI Crop Protection Compendium also has a datasheet for this organism, using the name *Acidovorax avenae* subsp. *citrulli* (last updated by CABI 08 April 2011 (CABI, 2011)). There is a short datasheet by EPPO and IMI on the pest (EPPO, 2009a; Saddler 1994)
Several extention units of US Universities (*e.g.* University of Missouri-Columbia, University of Illinois, Texas A & M University), have published a concise description of this disease (symtomatology, disease cycle, disease management), while a more detailed one is written by Walcott (2005) (open access at the American Phytopathological Society webpage).

**(iii) Current regulatory status**
What is the pest's status in the Plant Health Directive (Council Directive 2000/29/EC[4])?

*Acidovorax citrulli* (or its synonyms) is not specifically mentioned in the EC Plant Health Directive.

*Acidovorax citrulli* is not specifically mentioned in the EC Marketing Directive 92/33/EEC of 28th April 1992, although there is a requirement that vegetable propagating and planting material (other than seed) of *Cucumis melo* (melon), *Cucumis sativus* (gerkin), *Cucurbita pepo* (courgette), *Cucurbita maxima* (gourd) be substantially pest free.

**(iv) What is the pest's status in the European and Mediterranean Plant Protection Organisation (EPPO)?** (*put tick (✓) in box if relevant*) (www.eppo.org)

| EPPO List: | A1 regulated pest list | | A2 regulated pest list | | Action list | | Alert list | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | ✓ |

*Acidovorax citrulli* was added to the EPPO Alert List in July 2009 after the EPPO Panel on bacterial diseases considered that it was a possible seed-transmitted threat to cucurbit crops in particular melon and watermelon, following reports of its spread in other parts of the world (EPPO, 2009b).

**1.4 Strategy of data searching** (*identity of databases, data banks and information systems, key search terms and strategies applied, and the time period covered should be provided*)

Information searches were performed consulting several sources such as:

- Abstracting databases: *e.g.* AGRICOLA, CAB Abstracts, ISI Web of Knowledge
- Internet search machines: Google Scholar
- EPPO information systems: *e.g.* EPPO reporting service, EPPO PQR v 5.0.1500 (EPPO, 2011)

[4] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32000L0029:EN:NOT

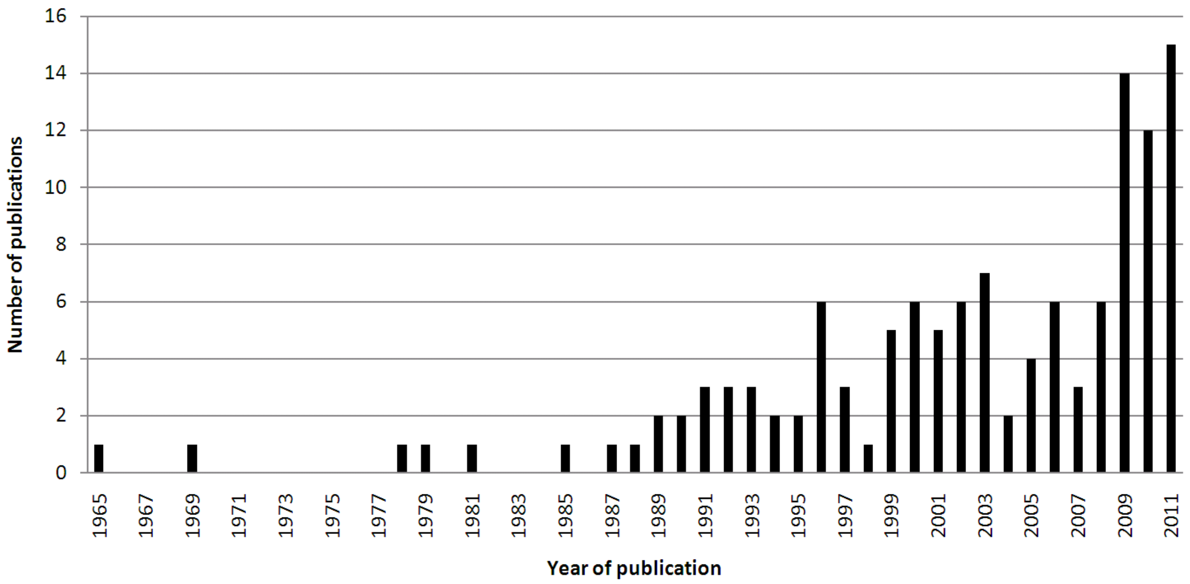
- Information from Member States on issues related to host distribution at a national level was acquired via a questionnaire prepared in the framework of the Prima Phacie project and distributed by EFSA to all NPPOs.

### 1.5 Time period considered in this assessment

(*Likelihoods of future events are only meaningful when a time period is specified. Choices that might be considered include: 3 months (short term, e.g. following discovery of an outbreak or other new potential threat), 5 years (medium term), or 30 years (long term). Choose a time scale and briefly explain why such a horizon was selected. – note whether climate change was taken into account. If climate change is taken into account, note the climate change scenarios considered.*

This assessment takes a medium term view, looking up to five years into the future. The chart below draws on the list of references cited in the Prima phacie datasheet for *Acidovorax citrulli*. The chart shows the number of publications that are cited in the datasheet against the year the citation was published, each year since 1965. Approximately one third of all references cited have been published in the last 4 years (*i.e.* since 2008). The trend shows that the rate of publications relating to this pest has increased in recent years. Anticipating that such a trend will continue for the next few years at least (as there is much uncertainty regarding epidemiological characteristics of the disease), it can be expected that new information will be published to further inform any future risk assessment. In anticipation of further information to emerge in the next few years, its seems unwise to speculate very much further than a few years into the future and taking a much longer term view is not appropriate. If the number of publications were declining or stabilising, it could suggest that sufficient information about the biology of the pest was available to permit a longer term time horizon to be considered perhaps.

**Figure 1.5:** Number of references cited in the datasheet for *Acidovorax citrulli* by year of publication



## 1.6 Introductions or interceptions (reported from EU or elsewhere)

*Provide information on interceptions or reports of pest spread and note whether the pest has a history of increasing its area of distribution.*

The disease, bacterial fruit blotch of cucurbits, was first reported in the USA in areas of commercial watermelon production in 1989 (Hopkins, 1989; Latin & Rane, 1990). However, reports of symptoms and damage to watermelons probably caused by what is now called *A. citrulli* date back to Georgia & Florida in the 1960s (Latin & Hopkins, 1995) although the identity of the causal agent could not be ascertained at that time. Only in 1990 was it concluded that the disease was seed borne (Wall *et al.,* 1990) by which time the disease was being reported in more US states and from other countries around the world. Table 1.6 shows a time line of published records reporting the first occurrence of bacterial fruit blotch of cucurbits or *A. citrulli* in US States and countries since 1965.

**Table 1.6:** Time line of reporting new and first occurrence of bacterial fruit blotch of cucurbits.

| Year of outbreak | Place of occurrence | Reference |
|---|---|---|
| 1965 | Georgia (USA) | Webb & Goth (1965) |
| 1967 | Florida (USA) | Crall & Schenck (1969) |
| 1978 | Queensland (Australia) | Queensland DPI (1978) |
| 1986 | Australia | Somodi *et al*. (1991) |
| 1987 | Mariana Islands (Guam, Tinian) | Wall & Santos (1988) |
| 1989 | USA (Iowa, Delaware, Maryland) | Latin & Hopkins (1995) |
| 1989 | USA (Indiana, Alabama) | Latin & Rane (1990); Sikora (2004) |
| 1989 | USA (Delaware, Florida) | Evans & Mulrooney (1991); Somodi et al. (1991) |
| 1989 | USA (North Carolina) | Bailey 1996 |
| 1991 | USA (Oklahoma) | Jacobs *et al.* (1992) |
| 1992 | USA (Georgia) | Hopkins *et al*. (2000) |
| 1993 | | |
| 1993 | USA (Texas) | Black *et al*. (1994) |
| 1994 | USA (Missouri) | Jett *et al*. (2002) |
| 1995 | | |
| 1995 | Turkey | Demir (1996) |
| 1996 | USA (Oregon ) | Hamm *et al*. (1997) |
| 1997 | Nicaragua* | Munoz & Monterroso (2002); |
| 1998 | China (Hainan) | Feng *et al*. (2009) |
| 1991 | Brazil | Assis *et al.* (1999); Oliveira *et al*. (2003) Robbs *et al*. 1991) |
| 1998 | Japan | Shirakawa *et al.* (2000) |
| 2001 | China (Xinjiang, Neimenggu) | Zhao *et al.* (2001) |
| 2001 | USA (Illinois); | Babadoost &Pataky (2002); |
| 2002 | Costa Rica | Mora-Umana & Araya (2002) |
| 2005 | China (Fujian) | Cai *et al.* (2005) |
| 2004 | Iran | Harighi (2007) |
| 2007 | Hungary | Palkovics *et al.* (2008) |
| 2005 | Greece | Holeva *et al*. (2009); |
| 2009 | Italy * | EPPO (2010) |
| 2008 | Taiwan | Deng *et al*. (2010) |
| 2011 | USA (Virginia, Delmarva Peninsula, Ohio, Kentucky) | Langston (2011) (radio interview) |

* under eradication or subsequently declared eradicated

Note that the disease has not necessarily persisted in all regions where it has been reported. For example the disease occurred in Iowa in 1989 and up to 1995 had not been seen again; similarly outbreaks in Delaware and Maryland in 1989 have not recurred, supporting the suggestion that in northern US States of America the disease relies on the pathogen being carried and introduced on infected planting material (Latin & Hopkins, 1995). This latter suggests that the pathogen is unable to persist outdoors in northern US climates. However, in southeastern US perennation of the pathogen may be possible as cucurbit weeds that survive in ditch banks and wooded areas may harbour the bacteria (Latin & Hopkins, 1995)

The finding of bacterial fruit blotch in Nicaragua followed the import of watermelon seed from Costa Rica in 1997 (EPPO, 2009b). In Turkey, the disease was first found in 1995 in the Edirne province on watermelon crops (Demir, 1996) and in 2005 it was in Adana Province (Mirik *et al.,* 2006). In Hungary, the disease was observed in watermelons in July 2007 during a hot summer period. Although the source of infection could not be determined, watermelon transplants had been imported from Turkey (Palkovics, 2008).

In Greece, the disease was found in July 2005 and September 2006, on mature watermelon fruits originating from the areas of Chryssoupoli (Macedonia, northern Greece) and Vagia (central Greece), respectively. Subsequently, the disease was found in May 2008, on young grafted watermelon plants from the area of Varda (Peloponnese, southern Greece) (Holeva *et al.*, 2010). Since then, there  has been no record of any other incidence of the disease in the country.

US watermelon seeds contaminated with *A. citrulli* were intercepted on a number of occasions by Israel between 1992 and 1994 (Assouline, 1996). The only report of diseased watermelon fruit being intercepted is from the Mariana Islands when diseased fruit moving from Tinian to Guam was intercepted by phytosanitary inspectors (Wall *et al*., 1990). Transfer from infected fruit to a growing crop seems unlikely unless the fruit is disposed of carelessly, very close to a site of production.

From the summary above, it is clear that there is recent history of reporting new occurrences of *A. citrulli* and bacterial fruit blotch. Most frequently it is suggested that the pathogen is spread via contaminated seed (*e.g.* interceptions by Israel and its entry into Nicaragua from Costa Rica via seed) or seedlings (*e.g.* occurrence in Hungary after importing seedlings from Turkey).

## Stage 2 - Pest Risk Assessment

(Outline approach)

This method for pest risk assessment involves first evaluating the likelihood of pest entry and transfer to a host within the risk assessment area. Likelihood of entry is assessed by considering five factors:

- (i) likelihood of association with commodity on the pathway at origin,
- (ii) pest survival during post harvest treatment,
- (iii) pest survival during storage and transport,
- (iv) pest survival during current phytosanitary procedures, and
- (v) the quantity of commodity imported.

The likelihood that sufficient numbers of pest will transfer from a pathway to a suitable host in order to initiate a new population is then considered. The combined likelihoods of entry and transfer via each pathway are then combined before likelihood of establishment is taken into account. Assessors then move onto assess consequences of establishment.

Each risk element or sub-element is divided into five categories. Assessors review data / evidence and allocate % likelihood to appropriate categories, either selecting a single category or spreading their judgment between categories. Guidance is provided to interpret the categories in order to provide some consistency.

Overall potential impact is determined via use of BBN software based on matrices that combine consequences of establishment with establishment potential **given entry and transfer**. Likelihood of entry and transfer is then combined with potential impact using the BBN software to estimate pest risk.

> Much of the current pest risk assessment uses the text from the earlier pest risk assessment where it was assumed that specific risk reduction options (RROs) (phytosanitary measures) were not in place.
>
> Where potential risk reduction options could act on a factor contributing to pest risk, the risk reduction option is identified and text commenting on the measure is provided to indicate how the measure affects the risk element. Such text informs the judgment of the assessor and should support how likelihoods are then distributed between the five categories for the risk element.
>
> Much of the data and information used in the original assessment (without RROs) are relevant to this assessment and are repeated here to avoid the need to refer back to the earlier risk assessment and also to allow this assessment to be used as a standalone document.

Acknowledgement

Method 4b has largely adopted questions from the USDA pathway initiated pest risk assessment method (USDA, 2000). However, the arrangement and structure of questions has been revised by Prima phacie so that the method is more aligned with EFSA needs.

Reference

USDA Guidelines for pathway initiated pest risk assessments v 5.02 (2000).

## Likelihood of pest entry and transfer to a host

**2.0 List and describe the pathways for pest entry into the risk assessment area**
A pathway is "*any means that allows the entry or spread of a pest*" (ISPM No. 5, IPPC, 2007*). Remember to consider potential pathways that are closed due to existing phytosanitary measures but which could be opened if the phytosanitary measures were changed.*

Entry is "*Movement of a pest into an area where it is not yet present, or present but not widely distributed and being officially controlled*" (ISPM No. 5, IPPC, 2007).

*Copy 2.1a to 2.7a for each pathway, and give responses for each individual pathway. Label questions 2.1b for the second pathway assessed, 2.1c for the third pathway etc.*

| Pathway | Pathway name | Summary description of pathway |
|---|---|---|
| **a.** | **Seeds of cucurbits** | Import of seeds of cucurbits, especially watermelon, from countries where *Acidovorax citrulli* occurs. |
| **b.** | **Seedlings (transplants) of cucurbits** | Import of seedlings of cucurbits for fruit production from countries where *Acidovorax citrulli* occurs. |
| **c.** | **Fruits of cucurbits** | Import of curcurbit fruit for consumer consumption from countries where *Acidovorax citrulli* occurs. |

*Note that on occasions risk assessments are initiated by a review of phytosanitary policy, e.g. for pests already present and perhaps widespread in an area and which therefore may no longer be suited to being dealt with using official phytosanitary measures. In such a situation, the likelihood of pest introduction is relevant only for limited parts of the area where the pest does not occur, or where it is not widely distributed and remains under official control. Assessment of pest entry could then be restricted to such areas.*

**Uncertainties (regarding pathways)**
*Following EFSA Guidance (EFSA, 2010), to ensure transparency in risk assessment, uncertainties should be identified, characterized and documented within all risk assessments. Identifying uncertainties can show not only which aspects of an assessment are uncertain but the degree of uncertainty and can help identify where further work could usefully reduce uncertainty.*

*Describe the uncertainties regarding the identification of pathways.*

There is a lack of detail regarding precise sources of plant material on each pathway, the time of year this material is moved, volumes, routes and modes of transport used. Assumptions had to be made about the environmental conditions in which commodities are transported.

In terms of additional pathways, there is one report from Israel suggesting that *A. citrulli* may be transmitted by solanaceous seeds – the pathogen has been isolated in Israel from imported tomato seeds and aubergine seedlings grown from imported seeds (Assouline *et al*., 1997). However, this pathway was not examined as such natural infection of solanaceous plants has not been reported elsewhere and its epidemiological significance remains to be determined. 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.

**Pathway 1: Seeds of cucurbits** (Seeds of cucurbits, especially watermelon, from countries where *Acidovorax citrulli* occurs)

**2.01a Likelihood of the pest being associated, spatially and temporally, with the pathway at origin**
(*There must be some likelihood of association otherwise there is no pathway*).

*Take into account pre-harvest cultivation and husbandry practices such as existing pest management measures, choice of cultivar, and applications of plant protection products. If phytosanitary measures (i.e. statutory risk reduction measures) are already in place against this or other pests at origin, specify whether these are being taken into account or not.*

**Information / evidence:** *Provide reasoning then give judgment*
The association of *A. citrulli* with watermelon seeds has been demonstrated (Rane & Latin, 1992) and the pathogen has been detected from cucurbit seeds in several cases (Rane & Latin, 1992; Assouline *et al*., 1997; Muñoz & Monterroso, 2002).
The EU does not provide data on exports and imports of specific vegetable seed hence there is very little data available about the EU import and trade of cucurbit seeds. However, anticipating the lack of statistics, the Prima phacie case study consortium developed a questionnaire for EU MS to address some anticipated information gaps (Annex 1). Part of the questionnaire was designed to determine which EU MS import cucurbit seed and from where such seed is obtained. A summary of responses is shown in Table 2.01a.

**Table 2.01a:** Third country sources of cucurbit seed imported by EU MS

| TC seed source | NL | DE | IT | PT | FI | CZ |
|---|---|---|---|---|---|---|
| USA * | x | x | x | x | x | |
| Israel * | x | x | x | x | | |
| Thailand * | x | x | | | | x |
| China * | x | | x | | | |
| South Korea * | | | | x | | |
| India * | x | | | | | |
| Japan * | x | | | | | |
| Taiwan * | x | | | | | |
| Chile | x | | | | | |
| Indonesia | x | | | | | |
| Peru | x | | | | | |
| Morocco | x | | | | | |
| Tanzania | x | | | | | |
| New Zealand | | x | | | | |

Source: Prima phacie questionnaire to EFSA PLH Network
Key: x = cucurbit seed has been imported from this Third Country
* = *Acidovorax citrulli* present in this county

Table 2.01a shows that at least six EU MS have sourced cucurbit seed from countries where *A. citrulli* occurs, with USA and Israel being the most common sources. Other EU MS may also use seed from these and other Third Countries but seed can be imported and repackaged within the EU and the origin of seed can then be lost. The same is true for seed imported from a Third Country via another Third Country, *e.g.* import of Indian seed to Israel via California (USA) (Assouline *et al.,* 1997).

It can be concluded that the pathway of seeds of cucurbits imported to the EU from countries where *A. citrulli* occurs is a real pathway.

Regarding likelihood of association at origin, recent studies have shown that infected fruit may contain *A. citrulli* infected seeds regardless of whether the fruit shows symptoms or not (Walcott *et al*., 2003; Lessl *et al*., 2007; Bahar *et al*., 2009). This causes problems for seed companies aiming to produce *A. citrulli* free seeds and means that it is very difficult to certify that seed is pest free, even if good field sanitation practices, such as removal of fruit debris, volunteers and wild hosts, have been applied at the place of production. No resistant cultivars have been identified, though some tolerance has been found in watermelons and melons (Hopkins & Thompson, 2002; Bahar *et al.,* 2009). There are no figures available for percentage infection amongst the seed being taken for growing stock, but the fact that money and effort is being put into post harvest treatments (Lovic & Hopkins, 2003) suggests that sourcing seed from countries where *A. citrulli* occurs does present a significant likelihood of association.

On the other hand, there have been only a few incidents of bacterial fruit blotch outbreaks on cucurbits in the EU despite a history of importing cucurbit seed from countries where the disease occurs. Nevertheless, an outbreak of bacterial fruit blotch in Nicaragua was linked to imported seed (EPPO, 2009b) as was an outbreak in Turkey (Demir, 1986; Nursen, 2008) so it is possible that there is association at origin.

The interception of infested seeds, *e.g.* i) watermelon seeds originating in USA have been intercepted several times in Israel between 1992 and 1994; ii) tomato seed from India intercepted in Israel, indicates that association of the pathogen with seed is possible (Assouline *et al*., 1997).

In addition, Zhao *et al*. (2009) reported that preliminary seed health testing in China revealed that up to 70% of the Hami melon seed lots in some areas was contaminated with the pathogen.

Most recently, it was shown that the seed inoculum level affects seedling transmission (16.6% up to 100% of seedlots transmitted the pathogen when inoculum level in one seed of each seedlot was 10 cfu up to $10^7$ cfu, respectively) as well as the spread of the disease, under greenhouse conditions (Dutta *et al.*, 2011). According to the same stydy, 100% of samples from seedlots (10,000 seeds/lot) with one seed containing $\geq 1 \times 10^5$ cfu tested positive for *A. citrulli*, 75% of samples from lots with one seed containing $1 \times 10^3$ cfu tested positive for the pathogen, and only 16.7% of samples with one seed containing 10 cfu tested positive using immunomagnetic separation (IMS) combined with a real-time PCR assay. Since disease transmission was observed for lots with just one seed containing 10 *A. citrulli* cfu, zero tolerance for seedborne *A. citrulli* is recommended for effective BFB management (Dutta *et al.*, 2011)

**Risk reduction options to consider**: *Require cucurbit seed to come from a pest free place of production or a pest free production site*

Effective control of the disease is currently best achieved through preventative measures. Of those, the most important is exclusion of *A. citrulli* by using pathogen-free seed and seedlings (Latin & Hopkins, 1995) for example, obtained from a production site free from *A. citrulli*. Crop inspection relies on identification of typical symptoms such as water-soaked lesions on the undersides of cotyledons; leaves can be inspected for small dark lesions and fruit show blotches on the upper surface. Thus, inspection is most valid if performed by trained personnel at several stages over the growing season (Lovic & Hopkins, 2003). However, simple visual inspections of seedlings, mature plants or fruit, from which seed is to be harvested may not always detect *A. citrulli.* For example, watermelon blossoms inoculated with *A. citrulli* can produce symptomless watermelon fruit although the seed of the fruit is infested. Thus infested seed could be produced in fields with no visible disease symptoms (Walcott *et al*., 2003). In testing seedlots from asymptomatic fruit, 44% of seedlots tested positive for *A. citrulli* using IMS-PCR. This was confirmed by bacteria isolation in 33% of the cases and by the 'seedling grow-out' test in 27% of the cases, under conditions conducive for disease expression (Walcott *et al.,* 2003). Thus, the inspection of fields or transplant facilities should be combined with laboratory testing (*e.g.* wash-PCR, IMS-real time PCR) of plant samples (including seeds) during the growing period and at harvest to check for latent infection.

Based on empirical evidence, Lovic & Hopkins (2003) reported that the seed production areas that originated the greatest number of known cases of contaminated seeds are harvested manually and sun-dried rather than those areas where seed harvesting, washing and drying equipment (*e.g.* mechanized seed harvesters, forced/heated-air dryers) was available to make these processes more reliable. Besides, Lovic & Hopkins (2003) suggest that is important for seed harvest to use only unblemished, surface decontaminated fruit and to clean and decontaminate periodically all seed processing equipment.*Conclusion regarding production / site freedom*

Sites of seed production should be inspected and samples taken to test for presence of *A. citrulli* in seed, seedlings and / or fruit. Specific inspection, sampling and testing procedures could be prescribed if the pest were to be listed as a harmful organism in the Plant Health Directive. Whilst sourcing seed from a site where *A. citrulli* is not known to occur will certainly reduce the likelihood of the pest being associated with the pathway at origin, it does not guarantee pest freedom given the difficulty in detecting the pest on site, the sensitivity and cost limitations of laboratory methods currently available, as well as the unavailability of highly specialised and expensive equipment for the seed harvesting phase in all production areas

**Uncertainties regarding likelihood of the pest being associated with the pathway at origin**
The pathogen is very difficult to detect and could occur at very low levels.

The number of seeds in a typical seed lot / consignment is unknown. Lot definition and seed sampling strategy are difficult to determine as they must reflect the variability of conditions under which individual seed crops are produced and harvested as related to the risk from *A. citrulli*.

| **2.01a: Likelihood of association with the pathway at origin if sourced from a pest free place of production / pest free production site** | | | |
|---|---|---|---|
| **Rating** | **Description** (likelihood of association | **Justification summary** | **Probability** |

| | is ....) | | **Assignment** [1] |
|---|---|---|---|
| **Very Low** | < 0.01% (less than one in ten thousand lots[2] of the commodity are likely to be contaminated / infested) | US seed suppliers no longer give guarantees that watermelon seed is free from *A. citrulli* (Anon., 2011) | 75% |
| **Low** | Between 0.01% and 0.1% (between one in ten thousand and one in one thousand lots[2] are likely to be contaminated / infested) | | 25% |
| **Medium** | Between 0.1% and 1% (between one in one thousand and one in one hundred lots[2] of the commodity are likely to be contaminated / infested) | Contamination at this level or above is assumed to be detected and lots prevented from being exported | - |
| **High** | Between 1% and 10% (between one in one hundred and one in ten lots[2] of the commodity are likely to be contaminated / infested) | | - |
| **Very High** | > 10% (more than one in ten lots[2] of the commodity are likely to be contaminated / infested) | | - |
| | | Check sum = | 100% |

[1] Spread your judgment according to your belief / evidence.
[2]**Lot**: a number of units of a single commodity, identifiable by its homogeneity of composition, origin etc., forming part of a consignment (ISPM no. 5, IPPC, 2007).
A consignment may be several lots or a single lot.

*The assessment of pest entry and transfer (2.01 -2.06) is based on considering "lots". However, if an alternative unit would be more appropriate then describe the chosen unit and ordinal scale using five categories.*

**2.02a Likelihood of surviving postharvest treatments / measures (before pest entry into risk assessment area)**

*Given that a proportion of lots/ consignments may be infested / contaminated, consider the proportion of infested/contaminated lots that are likely to remain infested/contaminated after any manipulation, handling or specific phytosanitary treatment to which the commodity is subjected. Examples of postharvest treatments include culling, washing, chemical treatment and cold storage. If there are no post harvest treatments the likelihood of survival should probably be considered "very high".*

*If post-harvest phytosanitary measures (i.e. statutory risk reduction measures) are already in place, specify whether these are being taken into account or not.*

**Information / evidence:** Provide reasoning then give judgment

**Risk reduction options to consider**: *Seed treatment to destroy A. citrulli*

A range of seed treatments (*e.g.* heat, NaOCl, HCl, Peroxyacetic acid and fermentation with $CaOCl_2$) have been investigated to try and eliminate *Acidovorax citrulli* but, although there has been success in reducing incidence in infected seeds, none have been found to completely eradicate the pathogen, without reducing seed quality (Rane & Latin, 1992; Hopkins *et al*., 2003; Feng *et al*., 2007; Hopkins *et al*., 1996; Sowell & Schaad, 1979; Wall, 1989). Assouline *et al.* (1997) reported detection of the pathogen from tomato seeds having a certificate stating to have been treated by hot water at 50$^{o}$C for 60 min. Rane & Latin (1992) reported the recovery of the pathogen from both the seed coats and the embryos of seeds from contaminated fruits, which indicates that seeds are both internally and externally contaminated. Similar observations were made by Silva Neto *et al.* (2006). Thus, the inability of the various treatments to eradicate the pathogen from the seed could be explained.

However, more recently, Chao *et al.* (2010) reported that treatment with chlorine dioxide solution ($ClO_2$, 50ppm for 30 min) of artificially or naturally infested watermelon seeds with *A. citrulli*, completely eradicated the pathogen from the seeds and controlled bacterial fruit blotch at the seedling stage without impairing seed germination rate.

If seed has been sourced from a site officially free from *A. citrulli* there should be no need to apply a seed treatment. However, as noted above, if it is not possible to give guarantees that production sites are pest free, or to compensate for possible failure of measures applied (*e.g.* inspection and lab testing), seed treatments could provide more reassurance that seed was less likely to be contaminated.

**Uncertainties regarding likelihood of the pest surviving postharvest treatments/ measures (before pest entry into the risk assessment area)**

It is unknown whether $ClO_2$, the apparently most efficient chemical reported in eradicating the pathogen from the seeds, is currently commercially used as a seed treatment. Previously there were no commercial treatments that are 100% effective.

*Conclusions*

Assuming a more effective seed treatment is developed and applied in the near future, the proportion of infested seeds in a lot will be reduced and infestations could perhaps be eliminated. However, it is likely that in the mean time seed treatments will help reduce the incidence of contamination in seed consignments without providing guaranteed pest freedom. Besides, since disease transmission is observed for lots with just one seed containing 10 *A. citrulli* cfu, zero tolerance for seedborne *A. citrulli* is recommended for effective disease management (Dutta *et al.*, 2011). More data is required to better inform how seed treatment will reduce the number of potentially infested consignments / lots.

| **2.02a: Likelihood that an infested/contaminated commodity remains infested/contaminated after postharvest treatments/measures** | | | |
|---|---|---|---|
| **Rating** | **Description** (likelihood of remaining infested/contaminated / pest survival is ..) | **Justification summary** | **Probability Assignment** [1] |
| **Very Low** | < 0.01% (less than one in ten thousand lots[2] of the original commodity are likely to remain contaminated / infested) | Some chemicals (*e.g.* $ClO_2$) appear very effective at eliminating *A. citrulli* from contaminated seed. However, it is not known whether the method is widely used. | 25% |
| **Low** | Between 0.01% and 0.1% (between one in ten thousand and one in one thousand lots[2] of the original commodity are likely to remain contaminated / infested) | | - |
| **Medium** | Between 0.1% and 1% (between one in one thousand and one in one hundred lots[2] of the original commodity are likely to remain contaminated / infested) | | - |
| **High** | Between 1% and 10% (between one in one hundred and one in ten lots[2] of the original commodity are likely to remain contaminated / infested) | Hot water or chemical treatment will reduce incidence within a lot but may not guarantee that all lots are pest free | 75% |
| **Very High** | > 10% (more than one in ten lots[2] of the original commodity are likely to remain contaminated / infested) | | - |
| | | Check sum = | 100% |

[1] Spread your judgment according to your belief / evidence

[2]**Lot**: a number of units of a single commodity, identifiable by its homogeneity of composition, origin etc., forming part of a consignment (ISPM no. 5, IPPC, 2007). A consignment may be several lots or a single lot.

**2.03a Likelihood of surviving storage and transport**

*Given that a proportion of lots/ consignments may still be infested / contaminated, estimate the proportion of lots that are likely to remain infested/contaminated because the pest can survive storage and transport; consider speed and conditions of transport and duration of the life cycle of the pest in relation to time in storage and transport, commercial procedures (e.g. refrigeration) applied to consignments in the country of origin, during shipping, and in the country of destination, that could affect the likelihood of pest survival. Take into account previous live interceptions on this or similar pathways (see 1.6).*

*If phytosanitary measures (i.e. statutory risk reduction measures) are already in place which act on the likelihood of pest survival during storage and transport, specify whether these are being taken into account or not.*

**Information / evidence:** *Provide reasoning then give judgment*

Contaminated seed is very likely to remain contaminated during transport. Survival of the pathogen in storage of up to at least 26 months is also very likely.

Hopkins *et al*. (1996) showed that the level of disease transmission was not reduced after contaminated seed had been stored at 12$^{o}$C for 12 months. Shirakawa *et al*. (2003) conducted trials on the survival of *A. citrulli* on artificially contaminated seeds at fixed temperatures between 4 and 30$^{o}$C over 26 months. The bacterium was able to survive at all temperatures. Long term storage of seed, if stored in conditions suitable for seed survival, *i.e.* in a cool and dry environment, is not likely to eliminate any contaminated seed (Hopkins *et al*., 1996). Furthermore, reports that the bacterium can be transmitted to seedlings from infested seeds stored for more than 38 years prompted Dutta & Walcott (2010) to compare the ability of *A. citrulli* to survive on seeds with that of *Xanthomonas campestris* pv. *campestris*, *Ralstonia solanacearum* and *Pantoea stewartii* subsp. *stewartii.* They found that the first three bacterial species are more tolerant to desiccation than *Pantoea stewartii* subsp. *stewartii.* They suggested that the ability of *A. citrulli* to survive for 38 years in stored seed is due to the location of the bacterium in the seed rather than some unique characteristic of the bacterium.

**Risk reduction options to consider**: None (seed drying is considered an already applied standard step in current cucurbit seed production)

**Uncertainties regarding likelihood of the pest surviving storage and transport**

It is unknown how long cucurbit seed is usually kept in storage on a commercial scale. As reviewed by Nerson (2007), cucurbit seed storability is affected by culture protocol, seed age at fruit harvest, seed moisture and environmental conditions in storage. However, storage of over 26 months (a time for which *A. citrulli* can survive) is unlikely on a commercial scale.

*Conclusions*
Assuming no specific measures are in place against *A. citrulli* the likelihood that infested seed remains infested in very high.

| 2.03a: Likelihood of surviving storage and transport | | | |
|---|---|---|---|
| **Rating** | **Description** (likelihood of remaining contaminated / pest survival is ....) | **Justification summary** | **Probability Assignment** [1] |
| **Very Low** | < 0.01% (less than one in ten thousand lots[2] of the original commodity are likely to remain contaminated / infested) | | - |
| **Low** | Between 0.01% and 0.1% (between one in ten thousand and one in one thousand lots[2] of the original commodity are likely to remain contaminated / infested) | | - |
| **Medium** | Between 0.1% and 1% (between one in one thousand and one in one hundred lots[2] of the original commodity are likely to remain contaminated / infested) | | - |
| **High** | Between 1% and 10% (between one in one hundred and one in ten lots[2] of the original commodity are likely to remain contaminated / infested) | | - |
| **Very High** | > 10% (more than one in ten lots[2] of the original commodity are likely to remain contaminated / infested) | Without specific measures applied there is no reason to expect contaminated seed to become pest free | 100% |
| | | Check sum = | 100% |

[1] Spread your judgment according to your belief / evidence

[2]**Lot**: a number of units of a single commodity, identifiable by its homogeneity of composition, origin etc., forming part of a consignment (ISPM no. 5, IPPC, 2007).
A consignment may be several lots or a single lot.

**2.04a Likelihood of pest surviving specific phytosanitary procedures at the point of entry or elsewhere in the risk assessment area**
*Given that a proportion of lots may still be infested / contaminated, estimate the proportion of lots that are likely to remain infested/contaminated because the pest survives existing phytosanitary procedures e.g. it is not detected at entry and/ or it can survive any existing phytosanitary procedures within the pest risk assessment area. Take into account the intensity of sampling and inspection and ease of detecting and distinguishing the pest from other organisms.*

**Information / evidence:** Provide reasoning then give judgment

**Risk reduction options to consider**: Seed testing.

To check whether a seedlot is likely to be contaminated, the USA National Seed Health System (NSHS) recommends the seedling grow-out test (SGO) and a laboratory test based on PCR on washings of seed samples. In the SGO test, thousands of seeds are grown for 3 weeks under conditions favourable for symptom development (RH: 55%, T: 24-38$^{o}$C) and seedlings with symptoms are analysed further for pathogen confirmation. Due to the significant crop devastation potential of the pathogen, a very high threshold has been set, where one infected seed in 30,000 is sufficient to reject the seedlot. Currently, it is recommended that the US seed industry test 10,000–50,000 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 to the seedling grow-out assay, is the 'sweatbox' method (Ha *et al.*, 2009), where seeds are incubated in transplant boxes under environmental conditions conducive to disease. This method allows seedlings with symptoms to be identified easily, because infected seedlings contact healthy seedlings and become infested themselves. Hence over time more seedlings in the box become infected and show symptoms which are more easily detected.

Seed lots can therefore be tested to check whether they are likely to be free from *A. citrulli*. However, the tests that could be used are very expensive in relation to the cost of seed. In the USA seed suppliers do conduct tests but do not give guarantees that marketed seed is entirely free from *A. citrulli* (Latin & Hopkins, 1995). In Israel, where the pathogen is regulated, there have been reports of interceptions of infested seeds (Assouline *et al.,* 1997).

*Conclusions regarding seed testing*

Comprehensive seed testing would be effective but as noted above, it is expensive and time consuming. Large numbers of seed are required to conduct tests.

**Uncertainties regarding likelihood of the pest surviving current phytosanitary procedures**
The number of seeds in a consignment / seedlot is unknown. The proportion of seedlots that escape detection is unknown.

| 2.04a: Likelihood of pest surviving phytosanitary procedures *i.e.* seed testing | | | |
|---|---|---|---|
| **Rating** | **Description** (likelihood of remaining contaminated/ pest survival is ....) | **Justification summary** | **Probability Assignment** [1] |
| **Very Low** | < 0.01% (less than one in ten thousand lots[2] of the original commodity are likely to remain contaminated / infested) | Assumed that the seed testing is quite effective | 75% |
| **Low** | Between 0.01% and 0.1% (between one in ten thousand and one in one thousand lots[2] of the original commodity are likely to remain contaminated / infested) | | 25% |
| **Medium** | Between 0.1% and 1% (between one in one thousand and one in one hundred lots[2] of the original commodity are likely to remain contaminated / infested) | Seed testing is assumed to perform sufficiently well as to not fail at this level or higher | - |
| **High** | Between 1% and 10% (between one in one hundred and one in ten lots[2] of the original commodity are likely to remain contaminated / infested) | | - |
| **Very High** | > 10% (more than one in ten lots[2] of the original commodity are likely to remain contaminated / infested) | | - |
| | Check sum = | | 100% |

[1] Spread your judgment according to your belief / evidence

[2]**Lot**: a number of units of a single commodity, identifiable by its homogeneity of composition, origin etc., forming part of a consignment (ISPM no. 5, IPPC, 2007). A consignment may be several lots or a single lot.

**2.05a Quantity of commodity imported annually**

*Quantity of commodity imported annually: The likelihood that a pest will enter depends on the amount of the potentially-infested commodity that is imported. For qualitative pest risk assessments, the amount of commodity imported is estimated in units of tonnes, or other metric such as standard 40 foot long shipping containers.*

*If the quantity of commodity imported is better described using alternative units, such as the number of plants for planting, assessors should devise a 5 level scale and provide some reasoning to support use of the scale.*

**Risk reduction options to consider**: None.

**Information / evidence:** *Provide reasoning then give judgment*

The EU does not provide data on exports and imports of specific vegetable seed hence there is very little data available about the EU import and trade of cucurbit seeds. However, some countries such as the US, India and Chile break their vegetable seed exports down by crop and country.

For tariff and tax reasons customs authorities record the quantity of many commodities and goods that are traded internationally. Such commodities can be described using a six digit number within a system called the Harmonised System (HS). The HS is a high-level international nomenclature owned and developed by the World Customs Organisation. For example, within the HS, 060240 refers to “rose plants, whether grafted or not”. However, within the EU a more detailed code system of 8 or 10 digits is used. This divides the HS codes into ever more detailed descriptions of goods. Thus whilst 06024 refers to rose plants, whether grafted or not, 06020411 refers to roses with a stock diameter of up to 10mm, whilst 06024019 refers to roses with a stock diameter of more than 10mm. The EU system is called the Combined Nomenclature (CN).

Many fruit and vegetable commodities are described within the HS and CN systems. Unfortunately the trade in seeds of plants are not well described within the HS or CN systems. Nevertheless, the International Seed Federation (ISF)[5] does provide basic statistics about fruit, flower and field crop seed exports and imports. It is not known whether seeds of watermelons, melons, cucumbers, pumpkins and other cucurbits are regarded as vegetable seeds or field crops. It is assumed that field crops are mainly arable crops, *e.g.* cereals, and that cucurbits are regarded as vegetables. Thus Table 2.05a 1 show EU imports of vegetable seeds. In 2010 almost 100,000 tonnes of vegetable seeds were imported by EU countries. However, only *a small proportion* of the seeds are likely to be cucurbit seeds.

EU MS do import cucurbit seed from a number of countries where *A. citrulli* occurs (see Table 2.01a previous). Table 2.05a 2 shows the quantity of all vegetable seed exported by countries where *A. citrulli* occurs, or where the status of the pathogen in unconfirmed (South Africa) or where outbreaks of the disease have been reported. As with EU imports, only a small proportion of the export seeds are assumed to be cucurbit seeds. A relationship between EU imports and countries exports cannot be made using this data. However, there is undoubtedly an international trade in cucurbit seeds as evidenced by responses to the Prima phacie questionnaire (Table 2.01a).

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[5] http://www.worldseed.org/isf/home.html

| **Table 2.05a 1:** EU imports of vegetable seeds for planting, 2010 (Tonnes) | | | | |
|---|---|---|---|---|
| EU MS | Tonnes | | EU MS | Tonnes |
| Belgium | 21,434 | | Ireland | 764 |
| Netherlands | 18,672 | | Sweden | 700 |
| Austria | 16,587 | | Lithuania | 577 |
| Italy | 9,976 | | Slovakia | 400 |
| Germany | 5,766 | | Czech Republic | 379 |
| UK | 5,654 | | Denmark | 343 |
| France | 4,450 | | Cyprus | 275 |
| Spain | 2,790 | | Slovenia | 256 |
| Hungary | 2,541 | | Bulgaria | 215 |
| Greece | 2,342 | | Malta | 200 |
| Poland | 2,220 | | Finland | 180 |
| Romania | 1,504 | | Latvia | 100 |
| Portugal | 770 | | Estonia | 50 |
| | | | Sum | 99,145 |

**Source:** http://www.worldseed.org/isf/seed_statistics.html

Note that only a fraction of the quantity of seed imported into the EU is likely to be cucurbit seed.

| **Table 2.05a 2:** Exports of vegetable seeds for planting from countries where *A. citrulli* occurs or where its status is unconfirmed, 2010. | | |
|---|---|---|
| Country | Quantity (tonnes) | Value (US $'000,000) |
| USA * | 21,603 | 485 |
| Italy (*) | 10,453 | 106 |
| Israel * | unknown | 106 |
| China * | 5,742 | 88 |
| Hungary * | 2,039 | 17 |
| Japan * | 1,406 | 98 |
| South Africa (*?) | 1,253 | 17 |
| Australia * | 1,036 | 22 |
| Turkey * | 644 | 13 |
| Korea, Republic of * | 397 | 20 |
| China*, Taiwan * | unknown | 11 |
| Costa Rica * | 0 | 10 |
| Greece (*) | 312 | 1 |
| | 44,885 + unknowns | 994 |

**Source:** http://www.worldseed.org.isf/seed_statistics.html

(*) = outbreaks      * = present      (*?) = unconfirmed

Note that only a fraction of the quantity of seed exported by the countries in Table 2.05a 2 is likely to be cucurbit seed. Note also that the table shows total exports, not all of which would be destined for the EU.

Seed import statistics available for the USA show that over the past 20 years, the majority of imported watermelon seeds for planting in the US have come from Egypt or Israel. US data since 2005 is shown in Table 2.05b 3, below.

**Table 2.05a 3:** US import sources and quantities of watermelon seed for planting (tonnes), 2005-'10

| | **2005** | **2006** | **2007** | **2008** | **2009** | **2010** | **6 year mean** | **% of 6 year mean** |
|---|---|---|---|---|---|---|---|---|
| Egypt | 137.5 | 78.0 | 43.3 | 61.7 | 74.6 | 62.0 | 76.2 | 29.6 |
| Israel * | 183.4 | 114.5 | 32.3 | 71.6 | 7.9 | 5.3 | 69.2 | 26.9 |
| China * | 18.3 | 23.3 | 19.6 | 31.0 | 40.9 | 16.5 | 24.9 | 9.7 |
| Chile | 26.0 | 37.0 | 28.0 | 10.8 | 10.2 | 27.8 | 23.3 | 9.1 |
| Taiwan * | 8.2 | 14.0 | 6.6 | 16.1 | 24.3 | 9.3 | 13.1 | 5.1 |
| Peru | 7.8 | 7.0 | 6.9 | 6.3 | 6.1 | 11.1 | 7.5 | 2.9 |
| Mexico | 16.5 | 3.6 | 1.3 | 1.8 | 5.6 | 4.0 | 5.5 | 2.1 |
| Thailand* | 3.6 | 3.7 | 4.0 | 7.9 | 3.3 | 3.5 | 4.3 | 1.7 |
| Japan * | 0.1 | 0.0 | 0.5 | 0.8 | 1.0 | 1.0 | 0.5 | 0.2 |
| All others | 41.2 | 55.0 | 28.9 | 9.2 | 29.0 | 32.2 | 32.6 | 12.7 |
| Sum | 401.4 | 281.2 | 142.3 | 208.1 | 173.9 | 140.5 | 257.1 | 100.0 |

Source: Extracted from Table 19, Watermelon seed US Imports by country 1980 – 2010. Available at USDA Economics, Statistics and Market Information System http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1399

* *A. citrulli* present

Since 2005, on average, the USA has imported just over 110 tonnes of watermelon seed from countries where *A. citrulli* occurs (Israel, China, Taiwan, Thailand, Japan). Although no data could be found regarding the quantity of cucurbit seed imported by all EU MS, the Dutch Plant Protection service was able to provide some information (Table 2.05a 4) which shows that over 85% of cucurbit seed imported by the Netherlands was sourced from countries where *A. citrulli* is present.

**Table 2.05a 4**: NL imports of cucurbit seed from Third countries 2008-2010 (kg)

| Year | 2008 | 2009 | 2010 | 3 year mean | Mean annual % |
|---|---|---|---|---|---|
| China * | 16,209 | 11,065 | 21,608 | 16,294 | 84.4 |
| USA * | 51 | 75 | 122 | 83 | 0.4 |
| Israel * | 1 | 192 | 35 | 76 | 0.4 |
| Japan * | 100 | | | 33 | 0.2 |
| Taiwan * | 37 | | | 12 | 0.1 |
| Thailand* | 209 | 939 | | 383 | 2 |
| *Sub total* | *16,607* | *12,271* | *21,765* | *16,881* | *87.4* |
| Chile | 640 | 2,294 | 2,106 | 1,680 | 8.7 |
| Peru | 264 | 1,135 | 485 | 628 | 3.3 |
| | | | | | |
| Morocco | 155 | 186 | 16 | 119 | 0.6 |
| Tanzania | 5 | 18 | | 8 | 0 |
| Indonesia | 1 | | | 0 | 0 |
| *Sub total* | *1,065* | *3,633* | *2,607* | *2,435* | *12.6* |
| sum | 17,672 | 15,904 | 24,372 | 19,316 | 100 |

Source: NL Plant Protection Service, pers. comm.. * = *Acidovorax citrulli* present

Table 2.05a 4 shows that between 2008 and 2010 the Netherlands imported between 12.3 tonnes (2009) and 21.8 tonnes (2010) of cucurbit seed from countries where *A. citrulli* occurs.

The Royal Botanic Gardens at Kew has compiled a database of seed biological trait data from published and unpublished sources (http://data.kew.org/sid/). The primary purpose is as an internal source of a variety of seed biological information; for use in large scale analysis and decision support for seed conservation operations. The database provides the 1,000 seed weight for many plant species and varieties, including watermelons, melons and cucumbers (Table 2.05a 5). The 1,000 seed weight for watermelons, melons and cucumbers was used to estimate the number of cucurbit plants that could potentially develop from 10 tonnes of imported seed, or 25 tonnes of imported seed. Such quantities are within the order of trade occurring in recent years between NL and Third Countries.

**Table 2.05a 5:** Estimating the number of cucurbit seeds imported by NL based on 1,000 seed weight

| Crop plant | 1000 seed weight (g) | Approximate number of seeds in 1 kg | Approximate number of seeds (millions) in 10 tonnes | 25 tonnes |
|---|---|---|---|---|
| watermelon | 85.0 | 12,000 | 120 | 300 |
| cucumber | 16.6 | 60,000 | 600 | 1,500 |
| melon | 7.2 | 140,000 | 1,400 | 3,500 |

In recent years, in the order of 10 to 25 tonnes of cucurbit seed have been imported by the Netherlands from countries where *A. citrulli* occurs (Table 2.05a 4). This equates to approximately between 120 million seeds of watermelons (if all cucurbit seed imports were watermelon) to 3,500 million seeds of melon (if all cucurbit seed imports were other melons). Of all cucurbit seed imported, only a proportion of it would have been watermelon seed, the suspected primary source for introduction of *A. citrulli*. Assuming that across the whole of the EU between 2 and 8 tonnes of watermelon seed is imported from countries where *A. citrulli* occurs, this would equate to between approximately 24 million and 96 million seeds.

In terms of the number of consignments imported by NL, at a very minimum each cell in Table 2.05a4 represents at least one consignment. Thus in 2008 there would have been at least 6 consignments of cucurbit seeds from countries where *A. citrulli* occurs (one from each of China, Thailand, USA, Israel, Japan and Taiwan). And in 2010 there was a minimum of 3 consignments (China, USA and Israel). Whilst in 2008 the imports from USA, Taiwan and Israel could well have been single consignments of between 1 kg and 51 kg, the import from China is less likely to have been a single consignment of over 16 tonnes. Equally it is very unlikely that the Chinese imports were 16,000 consignments of just over 1kg each.
Taking the weight of the cucurbit seed imported by NL from Thailand, USA, Israel, Japan and Taiwan between 2008 and 2010, then ranking the data in order gives an ordered list of: 1, 35, 37, 51, 75, 100, 122, 192, 209 and 939kg. Excluding the outliers of 1 and 939, the mean weight of seed from these countries is approximately 102kg. Assuming that the typical seed consignment (lot) of imported cucurbit seed weighs approximately 100kg, and that records of more than 100kg imports represent multiple consignments, then we can estimate for example that approximately 162 consignments of cucurbit seed were imported by NL

from China in 2008. Applying such an approach across Table 2.05a 4 suggest that between 2008 and 2010 an average of approximately 170 consignments of cucurbit seed were imported by NL from countries where *A. citrulli* occurs. Recognising that NL is very likely to be the major EU importer, importing the majority of consignments, it can be estimated that the EU as a whole imports perhaps around 200 consignments of cucurbit seed per year from countries where *A. citrulli* occurs.

**Uncertainties regarding the quantity of commodity imported annually**

Although in the EU, and generally elsewhere in the world, there is very little statistical information collected specifically about the trade in cucurbit seeds, we can be confident that the magnitude of EU imports is a fraction of total vegetable seed imports of up to 100,000 tonnes per year (Table 2.05a 1) and from countries where *A. citrulli* occurs is a fraction of 44,000 tonnes (Table 2.05a 2), assuming that the EU does source cucurbit seed from such countries.

Specific information from the Netherlands enabled approximations to be made regarding the number of cucurbit seeds imported. However, the proportion of cucurbit seed that is watermelon, and the most likely source of the pathogen, is unknown.

Based on NL data for 2008 – 2010, crude calculations have been used to estimate that approximately 200 consignments of cucurbit seed are imported into the EU each year from countries where *A. citrulli* occurs

As awareness of bacterial fruit blotch increases within the industry there is potential for seed importers to switch sources of supply and buy seed from countries where *A. citrulli* is not known to occur. The volume of seed imported could therefore change. However, this is not a phytosanitary measure and so is not taken into account in this assessment.

*Conclusions*

The quantity of watermelon seed imported into EU MS from countries where *A. citrulli* occurs is assumed to be primarily in the order of 10 million to 100 million seeds per year.

The table below uses a log scale for categorising the volume of commodity imported / number of seeds / plants imported into the EU.

| **2.05a Quantity of annual imports** (Examples provided based on a log scale of millions of seeds). | | | |
|---|---|---|---|
| **Rating** | **Number of seeds imported per year** | **Justification summary** | **Probability Assignment** [1] |
| **Very low** | < 1 million | - | |
| **Low** | 1 to 10 million | There is some uncertainty around the calculations | 9% |
| **Medium** | 10 to 100 million | Assuming 2 to 8 tonnes of susceptible seed is imported, this equates to 24 to 96 million seeds | 90% |
| **High** | 100 to 1,000 million | There is some uncertainty around the calculations | 1% |
| **Very high** | >1,000 million | - | |
| | | Check sum = | 100% |

[1] Spread your judgment according to your belief / evidence

**2.06a Likelihood of transfer via pathway**

*Consider the likelihood that the commodity will be distributed and subsequently allow the pest to transfer to a suitable host. For example, consider the geographic location of likely markets and the proportion of the commodity that is likely to move to locations where the pest could transfer to a host. Even if infested commodities are shipped to areas where environmental factors allow establishment, unless the pest can locate a host, establishment will not be possible. Consider the intended use of the commodity, e.g. plants for planting or produce for processing and consumption; likelihood of transfer from by-products of processing, or disposal of the commodity in the vicinity of suitable hosts; the pests ability to disperse and whether vectors provide a route from the pathway to a host; the time of year at which import takes place.*

*If possible consider the likelihood that sufficient numbers of the pest will transfer from the pathway to a suitable host in order to potentially initiate a new population. The reproductive strategy of the pest should be taken into account. Alternatively consider the potential number of "transfer events" that could occur per infested/contaminated consignment that has entered. See table for descriptions.*

**Information / evidence:** Provide reasoning then give judgment

The commodity is seed for planting so there is a very high likelihood that infected seed would transfer to a growing crop if distributed to the grower.

**Risk reduction options to consider**: None.

**Uncertainties regarding likelihood of transfer**

None

*Conclusions*

If a seedlot is infected there is a very high likelihood of transfer.

| 2.06a: Likelihood pest will transfer in sufficient numbers to a host | | | |
|---|---|---|---|
| **Rating** | **Description** (likelihood of pest transfer is ....) | **Justification summary** | **Probability Assignment** [1] |
| **Very low** | < 0.01% (less than one in ten thousand contaminated lots will provide transfer opportunities) | | % |
| **Low** | Between 0.01% and 0.1% (between one in ten thousand and one in one thousand contaminated lots will provide transfer opportunities) | | % |
| **Medium** | Between 0.1% - 1% (between one in one thousand and one in one hundred contaminated lots will provide transfer opportunities) | | % |
| **High** | Between 1% and 10% (between one in one hundred and one in ten contaminated lots will provide transfer opportunities) | | % |
| **Very high** | > 10% (more than one in ten contaminated lots will provide transfer opportunities) | Pathway is seeds for planting. If contaminated transfer highly likely | 100% |
| | Check sum = | | 100% |

[1] Spread your judgment according to your belief / evidence

### 2.07a Likelihood of entry and transfer via pathway "a" (Seeds of cucurbits, especially watermelon, from countries where *Acidovorax citrulli* occurs)

*Use the BBN to combine the scores to questions 2.01 to 2.06, which all relate to the likelihood of pest entry then transfer. Present it as Figure 2.07.*

The result of combining scores to individual questions 2.01(a) to 2.05(a), that relate to likelihood of entry is combined with score for likelihood of transfer 2.06(a) using a BBN to provide an assessment of potential for entry and transfer for the pathway and is shown in Figure 2.07 a. It suggests that with risk reduction options in place the potential for entry and transfer via the pathway "Seeds of cucurbits, especially watermelon, from countries where *Acidovorax citrulli* occurs" is towards to lower end of the likelihood scale "very low" to "medium". Within this uncertainty the highest likelihood category is "low".

A previous assessment without risk reduction options in place suggested that entry and transfer on this pathway was spread between "low" and "high" and most likely "medium". Whilst the model shows that risk reduction options have lowered the likelihood of entry and transfer, the degree to which risk reduction options have lowered the likelihood does not accord with assessors' judgment, *i.e.* assessors would have expected likelihood of transfer to have been lowered much more with the measures described above in place.

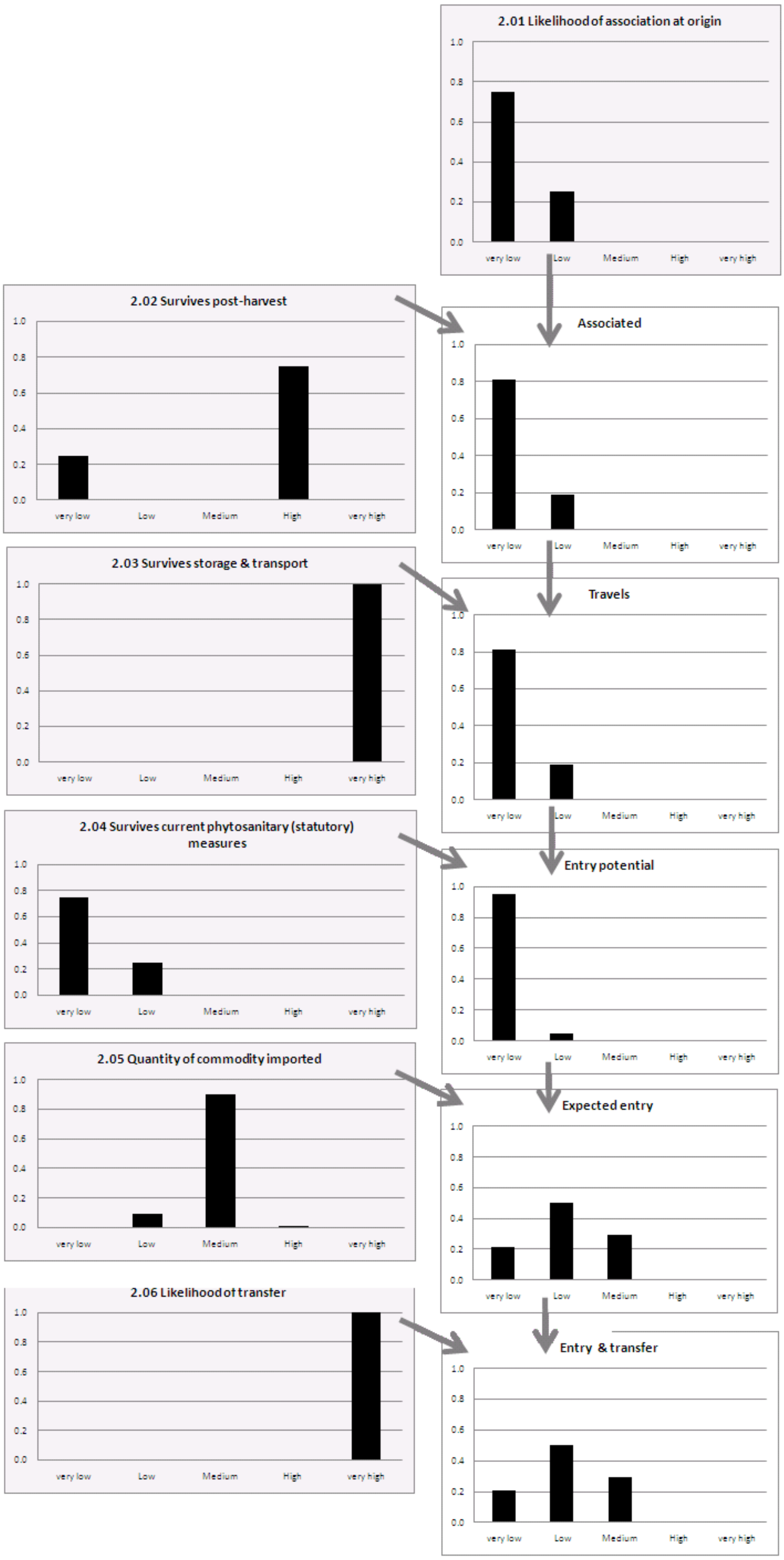
However, despite that within the US watermelon industry the disease is a major concern (Langston, 2011) and growers source seed from suppliers not known to have had the disease in seed crops, and with seed testing in place, new incidents of the disease still continue to be found (Langston, 2011).

**Uncertainties regarding likelihood of entry and transfer**
There is a lack of detail regarding the species of cucurbit seed imported into the EU and of its origin. As noted previously the time of year material is moved, volumes, routes and modes of transport used are also unknown. Whether or not cucurbit seed is tested for the presence of *A. citrulli*, and whether *A. citrulli* has been intercepted in the EU on seed previously is unknown. The efficacy of seed treatment at decontaminating whole lots is unknown.

*Conclusions*
With risk reduction options in place the likelihood of *A. citrulli* entering and transferring to a host is lower than without such risk reduction options in place. The BBN model (described elsewhere) does not suggest that the RROs are very effective.

*The model prediction is not in line with assessors' judgment. The wording of questions relating to risk elements considered in test Method 4b, the rating guidance provided for each category and how they operate within the BBN would benefit from further study.*

**Figure 2.07a:** Model output from BBN indicating likelihood of entry of *Acidovorax citrulli* via seeds of cucurbits, especially watermelon, from countries where *Acidovorax citrulli* occurs with Risk reduction options in place.



**Pathway 2: Seedlings of cucurbits** (especially watermelon, from countries where *Acidovorax citrulli* occurs).

**2.01b Likelihood of the pest being associated, spatially and temporally, with the pathway at origin**
(*There must be some likelihood of association otherwise there is no pathway*).

*Take into account pre-harvest cultivation and husbandry practices such as existing pest management measures, choice of cultivar, and applications of plant protection products. If phytosanitary measures (i.e. statutory risk reduction measures) are already in place against this or other pests at origin, specify whether these are being taken into account or not.*

**Risk reduction options to consider**: *Source seedlings from pest free place of production or pest free production site.*

**Information / evidence:** Provide reasoning then give judgment
The EU does not provide data on exports and imports of cucurbit seedlings (plants for planting) hence there is very little data available about the EU import and trade of cucurbit transplants. However, anticipating the lack of statistics, the Prima phacie case study consortium developed a questionnaire for EU MS to address some anticipated information gaps (Annex 1). Part of the questionnaire was designed to determine which EU MS import seedlings and from where such transplants are obtained.

Only three of the 27 EU MS replied stating that cucurbit seedlings had indeed been imported. The Czech Republic had sourced cucurbit seedlings from China, a country where *A. citrulli* is known to occur. Italy has imported seedlings but did not know their origin and Germany had received seedlings from the Netherlands. Although the later movement of seedlings from the Netherlands to Germany is not strictly an “import”, since it was within the single market, it is possible that the seedlings had come from a Third Country via the Netherlands.

The pathogen has been found in association with seedlings of curcubits in trade; the first report of what is now called *Acidovorax citrulli* was in 1965, in Georgia, USA when an unidentified seedborne bacterium was isolated from necrotic watermelon cotyledons originating in Turkey (Webb & Goth, 1965; Bahar & Burdman, 2010). Hungary had an outbreak of *A. citrulli* in July 2007. The source of infection was unclear, but it was noted that grafted watermelon transplants had been imported from Turkey (Palkovics *et al*., 2008). Latin & Hopkins (1995) reported that the outbreak in Iowa state was linked to purchase of transplants from an Indiana grower who suffered outbreak of the disease in his transplant facilities and in his fields.

The source of infection for most seedlings is the seed itself. It was shown in by Shirakawa *et al.* (2003) that under high humidity infested seeds with only 1cfu of *A. citrulli* have a high probability of resulting in diseased germinated seedlings. Glasshouse conditions, where seedlings are likely to be grown include high temperatures and high relative humidity, which alongside high populations of plants is conducive to seedling transmission and rapid disease spread (Walcott, 2005). As few as 1-2 seedlings can lead to widespread disease (Schaad *et al*., 2003).

Effective prevention of bacterial fruit blotch is best achieved through preventative measures. Of those, the most important is exclusion of *A. citrulli* by using pathogen-free seedlings (Latin

& Hopkins, 1995) for example, obtained from a production site free from *A. citrulli*. Providing assurance that seedlings are disease free is not easy. Crop inspection relies on identification of typical symptoms such as water-soaked lesions on the undersides of cotyledons; leaves can be inspected for small dark lesions and fruit should be examined for blotches on the upper surface. However, simple visual inspections of seedlings may not always detect *A. citrulli.* Tests such as the 'seedling grow-out' test would help give assurance that batches of seedlings are free of the pathogen (Walcott *et al.,* 2003). However, this would not guarantee a whole site is pest free.

**Uncertainties regarding likelihood of the pest being associated with the pathway at origin**

Sites of seedling production should be inspected and samples taken to test for presence of *A. citrulli*. Specific inspection, sampling and testing procedures could be prescribed if the pest were to be listed as a harmful organism in the Plant Health Directive.

*Conclusion regarding production / site freedom*

Whilst sourcing seedlings from a site where *A. citrulli* is not known to occur (proved by survey) will certainly reduce the likelihood of the pest being associated with the pathway at origin, it does not guarantee pest freedom given the difficulty in detecting the pest.

| **2.01b: Likelihood of association with the pathway at origin if sourced from pest free place of production or pest free production site** | | | |
|---|---|---|---|
| **Rating** | **Description** (likelihood of association is ....) | **Justification summary** | **Probability Assignment** [1] |
| **Very Low** | < 0.01% (less than one in ten thousand lots[2] of the commodity are likely to be contaminated / infested) | Despite great awareness in the US industry incidents are still occurring and seedlings have been implicated in EU outbreaks | 75% |
| **Low** | Between 0.01% and 0.1% (between one in ten thousand and one in one thousand lots[2] are likely to be contaminated / infested) | Seedlings have been implicated in EU outbreaks | 25% |
| **Medium** | Between 0.1% and 1% (between one in one thousand and one in one hundred lots[2] of the commodity are likely to be contaminated / infested) | Seedlings lots infested at this level or higher would be detected and lots prevented from being exported. | - |
| **High** | Between 1% and 10% (between one in one hundred and one in ten lots[2] of the commodity are likely to be contaminated / infested) | - | - |
| **Very High** | > 10% (more than one in ten lots[2] of the commodity are likely to be contaminated / infested) | - | - |
| | | Check sum = | 100% |

[1] Spread your judgment according to your belief / evidence.
[2]**Lot**: a number of units of a single commodity, identifiable by its homogeneity of composition, origin etc., forming part of a consignment (ISPM no. 5, IPPC, 2007).
A consignment may be several lots or a single lot.

*The assessment of pest entry and transfer (2.01 -2.06) is based on considering "lots".*

*However, if an alternative unit would be more appropriate then describe the chosen unit and ordinal scale using five categories.*

**2.02b Likelihood of surviving postharvest treatments / measures (before pest entry into risk assessment area)**

*Given that a proportion of lots/ consignments may be infested / contaminated, consider the proportion of infested/contaminated lots that are likely to remain infested/contaminated after any manipulation, handling or specific phytosanitary treatment to which the commodity is subjected. Examples of postharvest treatments include culling, washing, chemical treatment and cold storage. If there are no post harvest treatments the likelihood of survival should probably be considered "very high".*

*If post-harvest phytosanitary measures (i.e. statutory risk reduction measures) are already in place, specify whether these are being taken into account or not.*

**Risk reduction options to consider**: *Visual check of seedlings and test a sample for presence of pathogen.*

**Information / evidence:** *Provide reasoning then give judgment*

Infected seedlings may show symptoms of infection, especially if grown in warm conditions of high humidity. Infected plants that can be detected macroscopically are likely to be rejected before being sent to customers; however, not all infected seedlings will show symptoms. Hence additional testing for latent infection of consignments using, for example, the seedling grow out test would provide more assurance as to whether seedlings were contaminated.

**Uncertainties regarding likelihood of the pest surviving postharvest treatments/ measures (before pest entry into the risk assessment area)**

The level of seedlings in which infection may remain undetected depends on the sensitivity of the screening method used and needs to be determined.

*Conclusions*

Visual inspections and laboratory testing of seedlings will provide opportunities to detect then reject infested consignments. Nevertheless symptoms may not show in all cases and some seedlings could remain infested.

**2.02b: Likelihood that an infested/contaminated commodity remains infested/contaminated after visual inspection and testing**

| Rating | Description (likelihood of remaining infested/contaminated / pest survival is ....) | Justification summary | Probability Assignment [1] |
|---|---|---|---|
| **Very Low** | < 0.01% (less than one in ten thousand lots[2] of the original commodity are likely to remain contaminated / infested) | Many infected seedling lots are likely to be rejected by finding infected seedlings, but not all will show symptoms or proved positive in lab tests and hence some will remain on the pathway | 75% |
| **Low** | Between 0.01% and 0.1% (between one in ten thousand and one in one thousand lots[2] of the original commodity are likely to remain contaminated / infested) | | 25% |
| **Medium** | Between 0.1% and 1% (between one in one thousand and one in one hundred lots[2] of the original commodity are likely to remain contaminated / infested) | Seedling lots infested at this level or higher would be detected and lots prevented from being exported. | - |
| **High** | Between 1% and 10% (between one in one hundred and one in ten lots[2] of the original commodity are likely to remain contaminated / infested) | | - |
| **Very High** | > 10% (more than one in ten lots[2] of the original commodity are likely to remain contaminated / infested) | | - |
| | | Check sum = | 100% |

[1] Spread your judgment according to your belief / evidence

[2]**Lot**: a number of units of a single commodity, identifiable by its homogeneity of composition, origin etc., forming part of a consignment (ISPM no. 5, IPPC, 2007).
A consignment may be several lots or a single lot.

**2.03b Likelihood of surviving storage and transport**

*Given that a proportion of lots/ consignments may still be infested / contaminated, estimate the proportion of lots that are likely to remain infested/contaminated because the pest can survive storage and transport; consider speed and conditions of transport and duration of the life cycle of the pest in relation to time in storage and transport, commercial procedures (e.g. refrigeration) applied to consignments in the country of origin, during shipping, and in the country of destination, that could affect the likelihood of pest survival. Take into account previous live interceptions on this or similar pathways (see 1.6).*

*If phytosanitary measures (i.e. statutory risk reduction measures) are already in place which act on the likelihood of pest survival during storage and transport, specify whether these are being taken into account or not.*

**Risk reduction options to consider**: *None*

**Information / evidence:** Provide reasoning then give judgment

There is no data available on the length of time taken for transport and storage of this commodity. However it is likely to be short given the fragile nature of young plants and the need for them to be planted and grown on. No known reason why pest could not survive.

Using artificial inoculations of melon plants, Silva *et al*. (2006), reported an epiphytical survival of *A. citrulli* on leaves of 54 days, in greenhouse as well as in the field. The epiphytic population increased initially and decreased after a certain period of time. In a glasshouse, the bacterial population on the roots and rhizosphere decreased with time.

**Uncertainties regarding likelihood of the pest surviving storage and transport**

None

*Conclusions*

Young plants are likely to be shipped and stored for only a short time. *A. citrulli* highly likely to survive. No phytosanitary measures are applied at this point.

| **2.03b: Likelihood of surviving storage and transport** | | | |
|---|---|---|---|
| **Rating** | **Description** (likelihood of remaining contaminated / pest survival is ....) | **Justification summary** | **Probability Assignment** [1] |
| **Very Low** | < 0.01% (less than one in ten thousand lots[2] of the original commodity are likely to remain contaminated / infested) | | % |
| **Low** | Between 0.01% and 0.1% (between one in ten thousand and one in one thousand lots[2] of the original commodity are likely to remain contaminated / infested) | | % |
| **Medium** | Between 0.1% and 1% (between one in one thousand and one in one hundred lots[2] of the original commodity are likely to remain contaminated / infested) | | % |
| **High** | Between 1% and 10% (between one in one hundred and one in ten lots[2] of the original commodity are likely to remain contaminated / infested) | | % |
| **Very High** | > 10% (more than one in ten lots[2] of the original commodity are likely to remain contaminated / infested) | Storage and shipping time likely to be short | 100% |
| | | Check sum = | 100% |

[1] Spread your judgment according to your belief / evidence

[2]**Lot**: a number of units of a single commodity, identifiable by its homogeneity of composition, origin etc., forming part of a consignment (ISPM no. 5, IPPC, 2007).

**2.04b Likelihood of pest surviving current phytosanitary procedures at the point of entry or elsewhere in the risk assessment area**

*Given that a proportion of lots may still be infested / contaminated, estimate the proportion of lots that are likely to remain infested/contaminated because the pest survives existing phytosanitary procedures e.g. it is not detected at entry and/ or it can survive any existing phytosanitary procedures within the pest risk assessment area. Take into account the intensity of sampling and inspection and ease of detecting and distinguishing the pest from other organisms.*

**Risk reduction options to consider**: *Import inspections of seedlings and lab testing*

**Information / evidence:** *Provide reasoning then give judgment*

*Citrullus lanatus* (watermelon) plants for planting have been implicated in previous outbreaks of bacterial fruit blotch. For example watermelon seedlings from Turkey were linked with the original outbreak in Georgia in the 1960s and the more recent outbreak in Hungary also followed the import of watermelons seedlings from Turkey (Palkovics *et al.*, 2008).

If *Acidovorax citrulli* was listed as a harmful organism in EU phytosanitary legislation, the phytosanitary certificate which is already required (since these are plants for planting from Third Countries) would need to indicate that the seedlings were free from any EU listed quarantine pests, including *A. citrulli*. Inspections would be made on arrival in the EU to check compliance with the certificate.

As noted above, visual inspection of plants is no guarantee of ensuring any infected seedling would be detected. Some seedlings may be asymptomatic. In cases of symptomatic seedlings, the pathogen could be identified using lab *e.g.* ELISA, PCR-based assays. However, such checks are time-, space- and labour-consuming, and seedlings moderately infected or symptomless could be overlooked (*i.e.* the method is not sensitive) (Walcott *et al.*, 2006; Bahar *et al.*, 2008). A fast and reliable screening lab method has to be developed if the pathogen is going to be listed as a quarantine pest.

**Uncertainties regarding likelihood of the pest surviving current phytosanitary procedures**

Although general phytosanitary checks would be conducted on seedling samples at import, it could be difficult to detect an infected consignment at low levels of infestation.

*Conclusions*

Only basic phytosanitary procedures would be required on material that is already certified free from quarantine pests, therefore some consignments with low level infection could remain on the pathway.

Despite vigilance in the USA, new outbreaks occur which could be seed or seedling related.

| 2.04b: Likelihood of pest surviving current phytosanitary procedures | | | |
|---|---|---|---|
| **Rating** | **Description** (likelihood of remaining contaminated/ pest survival is ....) | **Justification summary** | **Probability Assignment** [1] |
| **Very Low** | < 0.01% (less than one in ten thousand lots[2] of the original commodity are likely to remain contaminated / infested) | | - |
| **Low** | Between 0.01% and 0.1% (between one in ten thousand and one in one thousand lots[2] of the original commodity are likely to remain contaminated / infested) | Some infected seedlings likely to be weeded out, but not all will show symptoms or test positive in the lab checks and hence remain on the pathway. | 45% |
| **Medium** | Between 0.1% and 1% (between one in one thousand and one in one hundred lots[2] of the original commodity are likely to remain contaminated / infested) | | 45% |
| **High** | Between 1% and 10% (between one in one hundred and one in ten lots[2] of the original commodity are likely to remain contaminated / infested) | | 10% |
| **Very High** | > 10% (more than one in ten lots[2] of the original commodity are likely to remain contaminated / infested) | | - |
| | Check sum = | | 100% |

[1] Spread your judgment according to your belief / evidence

[2]**Lot**: a number of units of a single commodity, identifiable by its homogeneity of composition, origin etc., forming part of a consignment (ISPM no. 5, IPPC, 2007). A consignment may be several lots or a single lot.

**2.05b Quantity of commodity imported annually**

*Quantity of commodity imported annually: The likelihood that a pest will enter depends on the amount of the potentially-infested commodity that is imported. For qualitative pest risk assessments, the amount of commodity imported is estimated in units of tonnes, or other metric such as standard 40 foot long shipping containers.*

*If the quantity of commodity imported is better described using alternative units, such as the number of plants for planting, assessors should devise a 5 level scale and provide some reasoning to support use of the scale.*

**Information / evidence:** Provide reasoning then give judgment

Prima phacie case study consortium developed a questionnaire for EU MS to address some anticipated information gaps (Annex 1). Part of the questionnaire was designed to determine which EU MS import seedlings and from where such transplants are obtained. There are no figures for quantities, but very few countries responded that seedlings were imported and quantities are likely to be less than for seed. Countries are more likely to grow their own plants from seed.

The Netherlands have imported cucurbit seedlings from Israel (Dirk Jan van der Gaag, pers. comm.).

**Uncertainties regarding the quantity of commodity imported annually**

No data is available to support import assumptions.

*Conclusions*

The quantity of seedlings import is assumed to be less than that of seed, and is assumed mainly “low” as indicated in Table 2.05 b below. We assume most EU countries grow cucurbits using EU or imported seed. There is considerable uncertainty around the number of plants / seedlings imported. However, use of a log scale facilitates uncertainty being accounted for.

| **2.05b Quantity of annual imports** (Examples provided based on a log scale of millions of plants / seedlings). | | | |
|---|---|---|---|
| **Rating** | **Number of plants / seedlings imported per year** | **Justification summary** | **Probability Assignment [1]** |
| **Very low** | < 1 million | | 9% |
| **Low** | 1 to 10 million | | 90% |
| **Medium** | 10 to 100 million | | 1% |
| **High** | 100 to 1,000 million | | - |
| **Very high** | >1,000 million | | - |
| | | Check sum = | 100% |

[1] Spread your judgment according to your belief / evidence

**2.06b Likelihood of transfer via the pathway of cucurbit seedlings, especially watermelon, from countries where *Acidovorax citrulli* occurs**.

*Consider the likelihood that the commodity will be distributed and subsequently allow the pest to transfer to a suitable host. For example, consider the geographic location of likely markets and the proportion of the commodity that is likely to move to locations where the pest could transfer to a host. Even if infested commodities are shipped to areas where environmental factors allow establishment, unless the pest can locate a host, establishment will not be possible. Consider the intended use of the commodity, e.g. plants for planting or produce for processing and consumption; likelihood of transfer from by-products of processing, or disposal of the commodity in the vicinity of suitable hosts; the pests ability to disperse and whether vectors provide a route from the pathway to a host; the time of year at which import takes place.*

*If possible consider the likelihood that sufficient numbers of the pest will transfer from the pathway to a suitable host in order to potentially initiate a new population. The reproductive strategy of the pest should be taken into account. Alternatively consider the potential number of "transfer events" that could occur per infested/contaminated consignment that has entered. See table for descriptions.*

**Risk reduction options to consider**: *None*
**Information / evidence:** *Provide reasoning then give judgment*
The commodity is plants for planting, therefore transfer to growing host is highly likely.

**Uncertainties regarding likelihood of transfer**
None.

*Conclusions*
No restrictions on transfer to host.

| **2.06b: Likelihood pest will transfer in sufficient numbers to a host** | | | |
|---|---|---|---|
| **Rating** | **Description** (likelihood of pest transfer is ....) | **Justification summary** | **Probability Assignment** [1] |
| **Very low** | < 0.01% (less than one in ten thousand contaminated lots will provide transfer opportunities) | | % |
| **Low** | Between 0.01% and 0.1% (between one in ten thousand and one in one thousand contaminated lots will provide transfer opportunities) | | % |
| **Medium** | Between 0.1% - 1% (between one in one thousand and one in one hundred contaminated lots will provide transfer opportunities) | | % |
| **High** | Between 1% and 10% (between one in one hundred and one in ten contaminated lots will provide transfer opportunities) | | % |
| **Very high** | > 10% (more than one in ten contaminated lots will provide transfer opportunities) | Infected seedlings are hosts themselves. | 100% |
| | Check sum = | | 100% |

[1] Spread your judgment according to your belief / evidence

**2.07b Likelihood of entry and transfer via pathway "b", cucurbit seedlings, especially watermelon, from countries where *Acidovorax citrulli* occurs**.

*Use the BBN to combine the scores to questions 2.01 to 2.06, which all relate to the likelihood of pest entry then transfer. Present it as Figure 2.07.*

The result of combining scores to individual questions 2.01(b) to 2.05(b), that relate to likelihood of entry with risk reduction options in place are combined with scores for likelihood of transfer 2.06(b) using a BBN to provide an assessment of potential for entry and transfer for the pathway and is shown in Figure 2.07 b. It suggests that the potential for entry and transfer via cucurbit seedlings, especially watermelon, from countries where *Acidovorax citrulli* occurs is most likely "low" or "very low".

**Uncertainties regarding likelihood of entry and transfer**

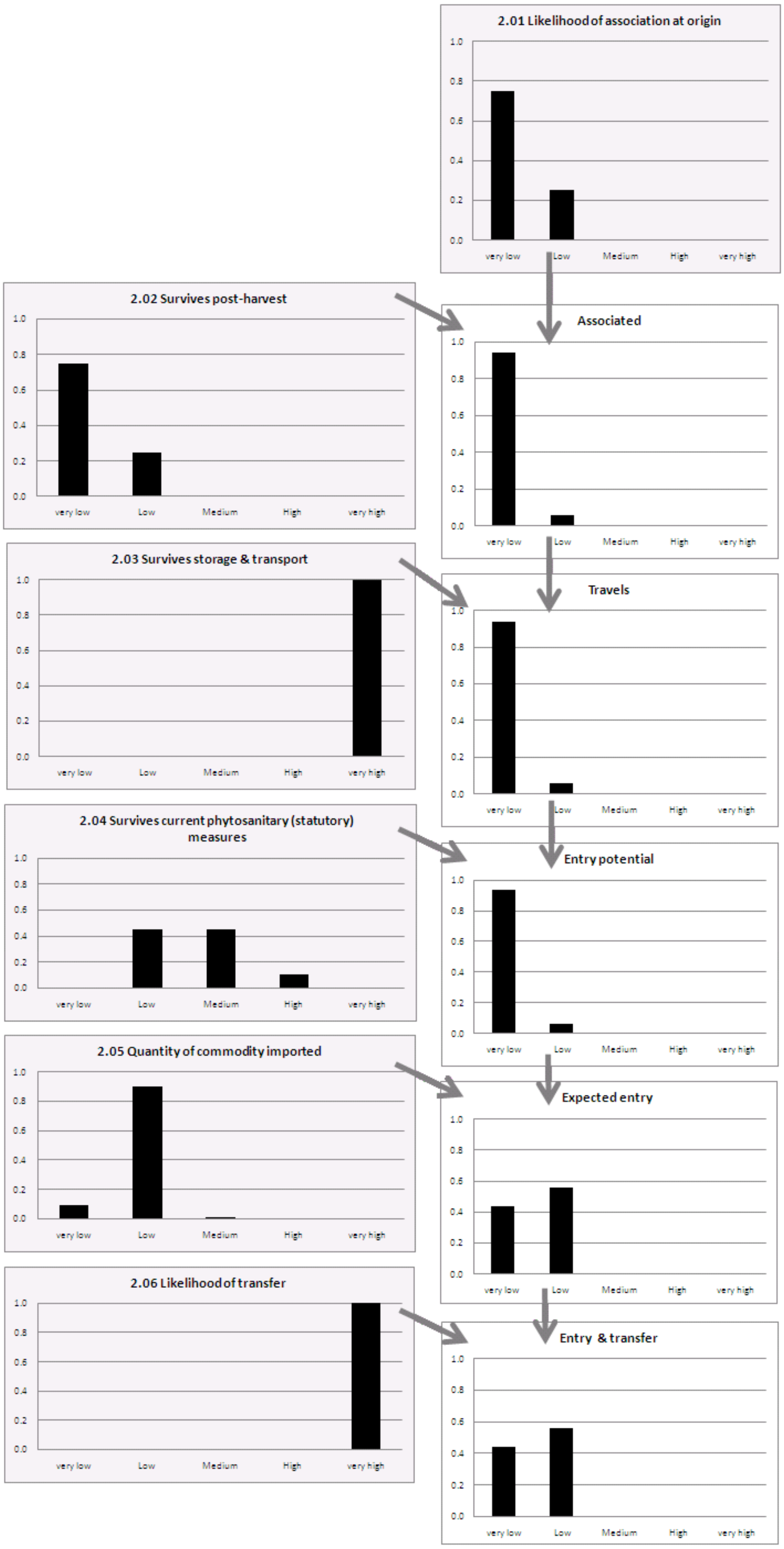
There is a lack of detail regarding the species of cucurbit seedlings imported into the EU and of its origin. As noted previously the time of year material is moved, volumes, routes and modes of transport used are also unknown. The effectiveness of risk reduction options are also uncertain.

*Conclusions*

The BBN model (described elsewhere) suggests that the likelihood of entry and transfer via seedlings is most likely considered "very low" to "low". Without risk reduction options in place the likelihood of entry and transfer via this pathway was previously assessed as "very low" to "medium", hence the model shows that risk reduction options have lowered the likelihood of entry and transfer somewhat. However, the degree to which risk reduction options have lowered the likelihood does not accord with assessors' judgment*, i.e.* assessors would have expected likelihood of transfer to have been lowered much more if the measures described above were in place.

*The model prediction is not in line with assessors' judgment. The wording of questions relating to risk elements considered in test Method 4b, the rating guidance provided for each category and how they operate within the BBN would benefit from further study.*

**Figure 2.07b:** Model output from BBN indicating likelihood of entry of *Acidovorax citrulli* via seedlings of cucurbits, especially watermelon, when risk reduction options are in place from countries where *Acidovorax citrulli* occurs.



**Pathway 3: Fruit of cucurbits (especially watermelon, imported from countries where *Acidovorax citrulli* occurs)**

The previous risk assessment that considered the likelihood of *A. citrulli* entering and transferring to a host via imported cucurbit fruit concluded that the import of cucurbit fruit did not add to the likelihood of the pest transferring to a susceptible host. Risk reduction options are therefore not considered for cucurbit fruit.

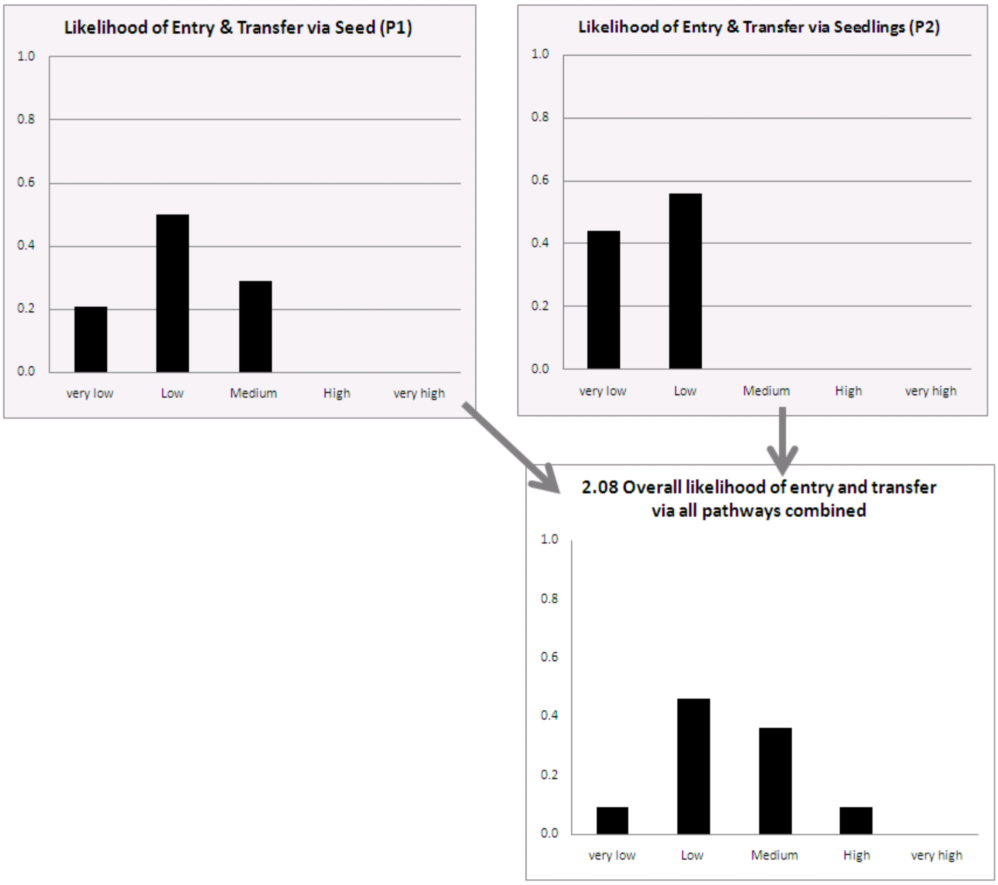
*If there are multiple pathways, repeat steps 2.01 to 2.07 for each pathway*.

There are no more pathways to consider.

### 2.08 Overall likelihood of entry and transfer via all pathways assessed

The likelihood of entry and transfer from all pathways is combined in the BBN, results of which are shown in Figure 2.08 below.

**Figure 2.08:** Overall likelihood of entry and transfer via major pathways




*Conclusions*

Figure 2.08 shows the BBN model output from combining scores of entry and transfer for the two pathways assessed, cucurbit seed (2.07a) and cucurbit seedlings (2.07b). Within individual pathways, the range of likelihoods spreads across up to three of the possible five categories, the maximum of which is “medium” (for the seed pathway). However, when pathways are combined the likelihood increases up to “high”. Whilst additional pathways will add additional likelihood of entry, the assessors view was that in this case it would increase the likelihood of “medium” rather than shift likelihood to include a higher rating.

*Whilst Method 4b does address several of the issues identified by EFSA in their guidance document on conducting pest risk assessment (EFSA, 2010) the mechanism by which risk elements are combined, including how pathways combine, in the BBN deserves further study.*

## 3.00 Potential for pest establishment[6] and extent of spread given entry and transfer

*Having transferred to a host we next consider whether the pest will survive and reproduce to initiate a population that will establish.*

> The assessment of the potential for pest establishment, used to identify the endangered area, is not affected by phytosanitary risk reduction options identified above. The following text from the risk assessment without risk reduction options being considered is presented here to avoid the need to refer back to the original pest risk assessment.

**3.01 Environmental suitability** (particularly considering climate and hosts)

*When introduced to new areas, pests can be expected to behave as they do in their native areas if host plants and climates are similar. Ecological zonation and the interactions of the pests and their biotic and abiotic environments are considered here, with a focus on hosts and climate so that an assessment is based on availability of both host material and suitable climatic conditions.*

*If a pest's distribution is likely to be limited by frosts and/ or low winter temperatures first consider which hardiness zones the pest currently occurs in outside of the risk assessment area. Next consider the area occupied by hosts in relevant hardiness zones within the risk assessment area.*

*If a pest's distribution is likely to be limited by a lack of accumulated temperature, e.g. low summer temperatures, first consider where the pest occurs in terms of accumulated degree day zones outside of the risk assessment area. Next consider the area occupied by hosts in relevant degree day zones within the risk assessment area.*

*Recall that hardiness maps and accumulated degree day maps are based on 10 or 30 year averages. In reality the areas of each zone vary year to year.*

*Taking into account the area of suitable climate and availability of host plants judge what area the pest could potentially establish in.*

*In addition to climate and host, many other factors can be taken into account when assessing likelihood of establishment. ISPM 11 lists other factors to consider, e.g. biotic factors such as the reproductive strategy of the pest, whether the species is polymorphic and the degree to which the pest has demonstrated the ability to adapt to conditions like those in the risk assessment area; the minimum population needed for establishment; competition and natural enemies. Abiotic factors in the environment such as soil type could also be important.*

*Where applicable, practices employed during the cultivation/production of the host crops should be compared to determine if there are differences in such practices between the risk assessment area and the origin of the pest that may influence its ability to establish. Pest control programs already in the risk assessment area which reduce the probability of establishment should be taken into account. Pests for which control is not feasible should be considered more likely to establish than those for which treatment is easily accomplished.*

---

[6] *Establishment; Perpetuation, for the foreseeable future, of a pest within an area after entry* (ISPM No. 5, IPPC, 2007).

*The probability of establishment in a protected environment, e.g. in glasshouses, should also be considered.*

*Contracting parties to the IPPC recognise the necessity for preventing the international spread of plant pests and their introduction into endangered areas (FAO, 1997). The IPPC defines "endangered area" as "an area where ecological factors favour the establishment of a pest whose presence in the area will result in economically important loss". For the purposes of EFSA, assessors are advised to interpret endangered area as "the area where ecological factors favour the establishment of a pest whose presence in the area will result in harmful consequences to cultivated and managed plants[7] and/ or the environment".*

*If the risk assessment has been initiated by a review of phytosanitary policy where the pest is already present and perhaps widespread in an area, the likelihood of pest establishment should focus on those parts of the area where the pest does not occur.*

*Fill out table 3.01 by considering how much of the HOST area within the pest risk assessment area is suitable for the pest's establishment taking into account relevant factors such as where host distribution overlaps with suitable climatic conditions (e.g. plant hardiness zones or accumulated degree day zones). For example an assessor could judge that it is 75% likely that 1/3 – 2/3 of the host area is suitable for establishment but 25% likely that between 2/3 and 90% of host area is suitable.*

*In Table 3.01 distinguish between the host area suitable for establishment and the ENDANGERED AREA which is, for the purposes of EFSA, the area where ecological factors favour the establishment of a pest whose presence in the area will result in harmful consequences to cultivated and managed plants and/ or the environment. In making a judgment regarding the endangered area, the rate of pest population development and any threshold required for harmful pest consequences to materialize within cultivated and managed plants could be taken into account. The magnitude of consequences is considered in Q 3.03 and 3.04.*

*Sophisticated quantitative environmental modelling could be used to more precisely identify the area where establishment is most likely and to identify the endangered area.*

**Information / evidence:** *Provide reasoning then give judgment.*
*Acidovorax citrulli* occurs on all continents although a suggestion that it occurs in Africa (South Africa) has not been confirmed. Whilst the pathogen has been shown to survive on watermelon seeds at 4$^{o}$C for 26 months (Shirakawa *et al*., 2003), the disease is generally seen in humid spring or summer months (Bahar & Burdman, 2010). Optimum conditions for disease expression are 55% relative humidity and temperatures between 24$^{o}$C and 38$^{o}$C. Hamm *et al.* (1997) considered that the disease may have a significant impact on watermelon production in the southern Columbia Basin, which is dry (<5 cm rainfall), has low relative humidity (46-57%) and high temperatures (29-41$^{o}$C).

Figure 3.01a shows the world plant hardiness zones (NAPPFAST, 2007; Magarey *et al.*, 2008) with dotted ellipses showing the approximate occurrence of *Acidovorax citrulli* in relation to hardiness zones, based on the map in Figure 3.01 b showing *A. citrulli*

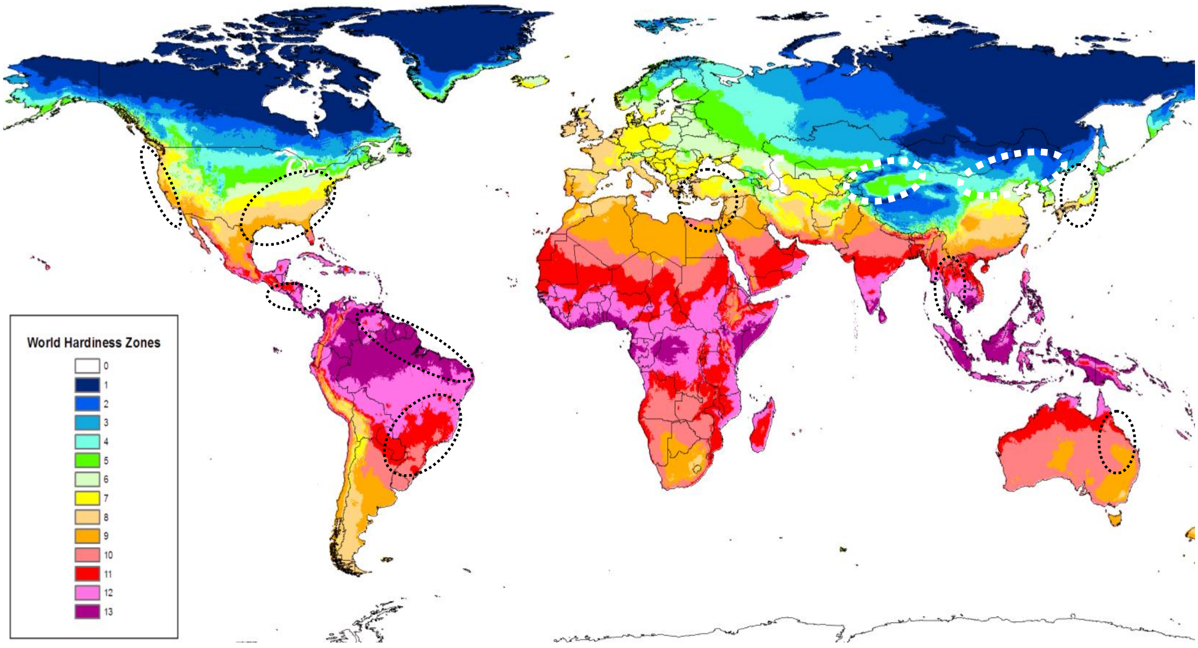
[7] Managed plants are those plants appreciated / valued/ desired by man, whose growth and spread / distribution are modified by human intervention. It would include plants grown in private gardens.

distribution. The pathogen appears to occur in plant hardiness zones 5 or 6 up to plant hardiness zones 10 or 11.

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$^{o}$C (Figure 3.01c). However, there is insufficient detailed information for the distribution of *A. citrulli* to accurately determine the accumulated temperature required by *A. citrulli* to cause disease symptoms. When outbreaks of bacterial fruit blotch are reported in the literature, there is often mention of hot and humid conditions. Map 3.01d shows the approximate occurrence of the pathogen in relation to Köppen-Geiger climate classification.

Maps in figures 3.01e to 3.01h show the approximate occurrence of *A. citrulli* in relation to global distribution and production of various cucurbit crops (3.01e watermelons; 3.01f cantaloupe and other melons; 3.01g cucumbers and gherkins; 3.01h and pumpkins, squash & gourds).

**Figure 3.01a:** Wold plant hardiness zones




Source: NAPPFAST (2007) http://www.nappfast.org/Plant_hardiness/ph_index.htm

Dotted ellipses show approximate occurrence of *Acidovorax citrulli* in relation to hardiness zones (based on map 3.01b, below).

**Figure 3.01b:** Distribution of *Acidovorax citrulli* Source: EPPO PQR (2011)

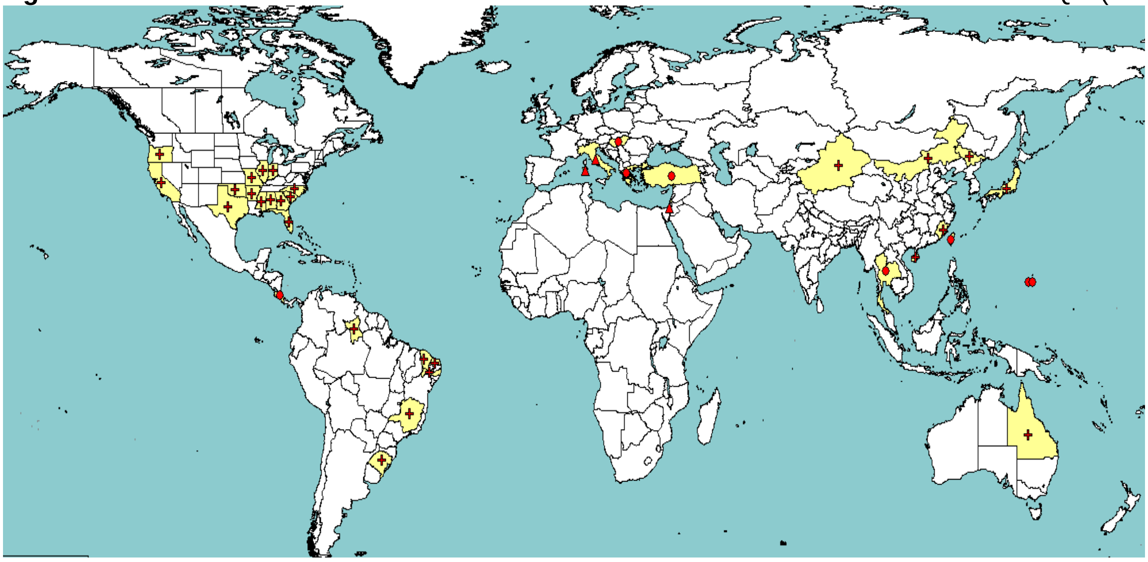


**Figure 3.01c:** Approximate occurrence of *Acidovorax citrulli* in relation to global accumulated temperature above a base of 10°C

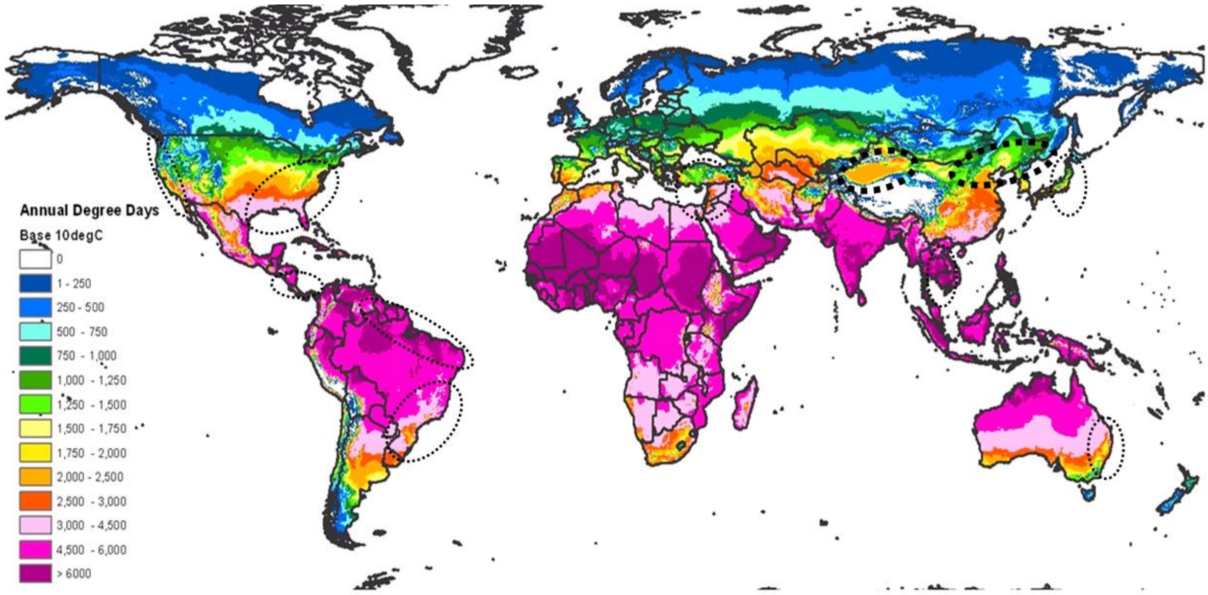
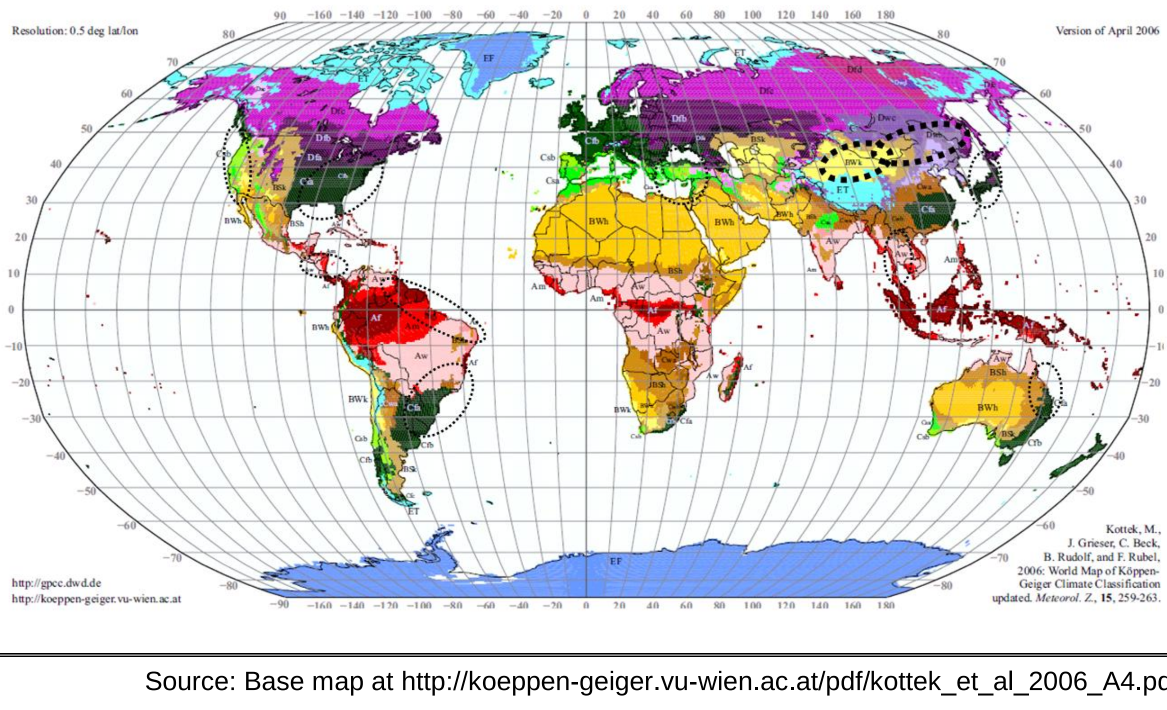



**Figure 3.01d:** Approximate occurrence of *Acidovorax citrulli* in relation to Köppen-Geiger climate classification




Source: Base map at http://koeppen-geiger.vu-wien.ac.at/pdf/kottek_et_al_2006_A4.pdf

It is noteworthy that bacterial fruit blotch occurs in warm temperate and warm tropical humid zones with either hot or warm summers.

**Figure 3.01e:** Global distribution of watermelon production with approximate occurrence of *A. citrulli*

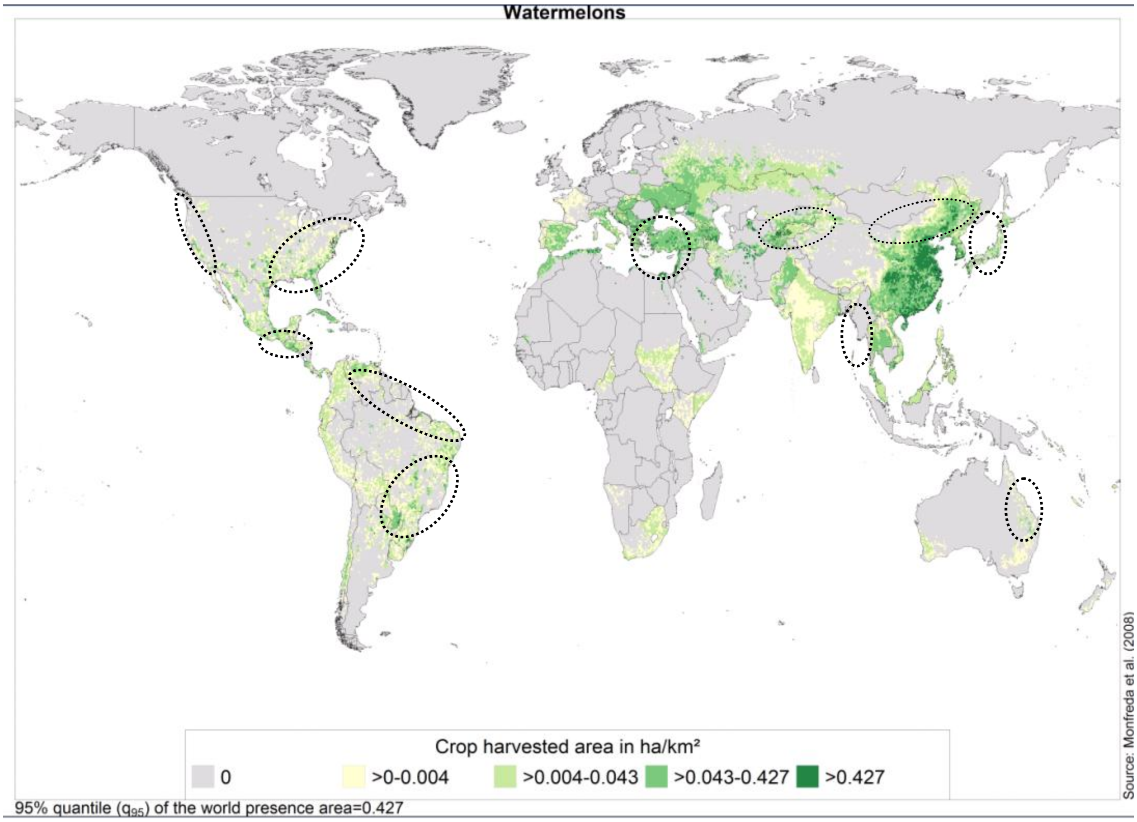



**Figure 3.01f:** Global distribution of production of cantaloupes and other melons with approximate occurrence of *A. citrulli*

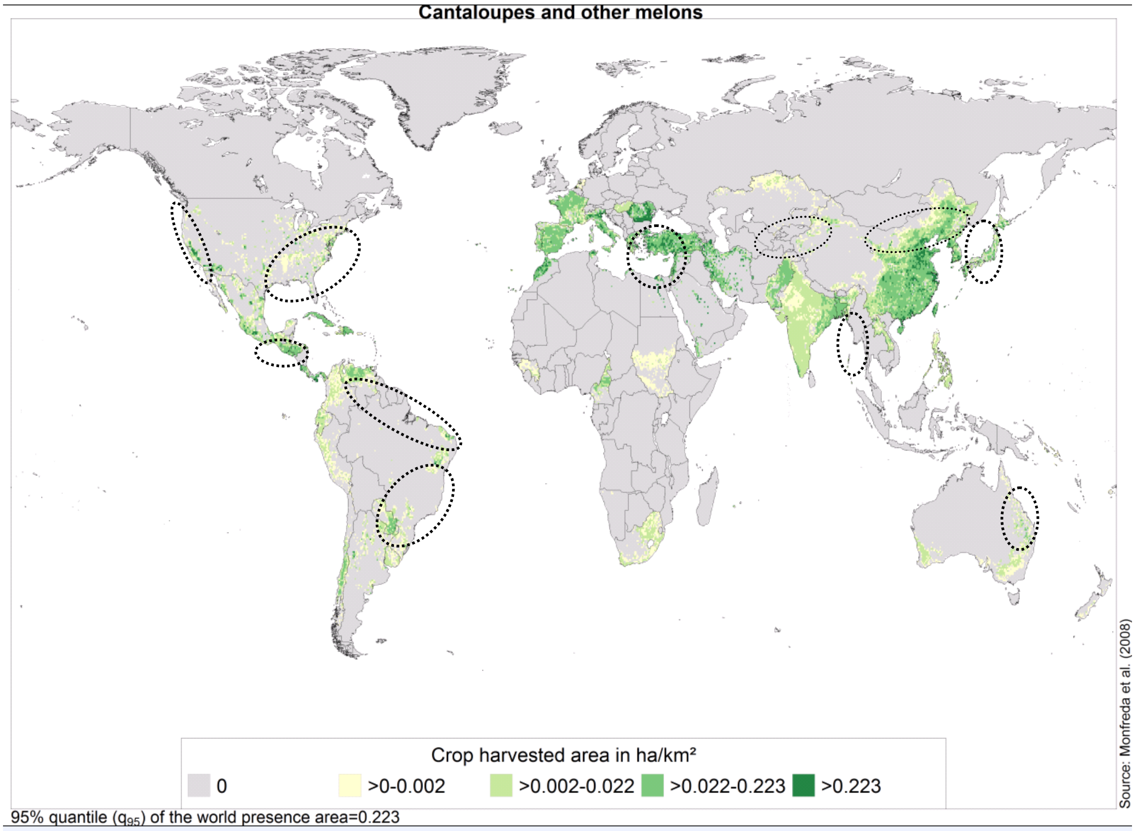


**Figure 3.01g:** Global distribution of production of cucumbers and gherkins with approximate occurrence of *A. citrulli*

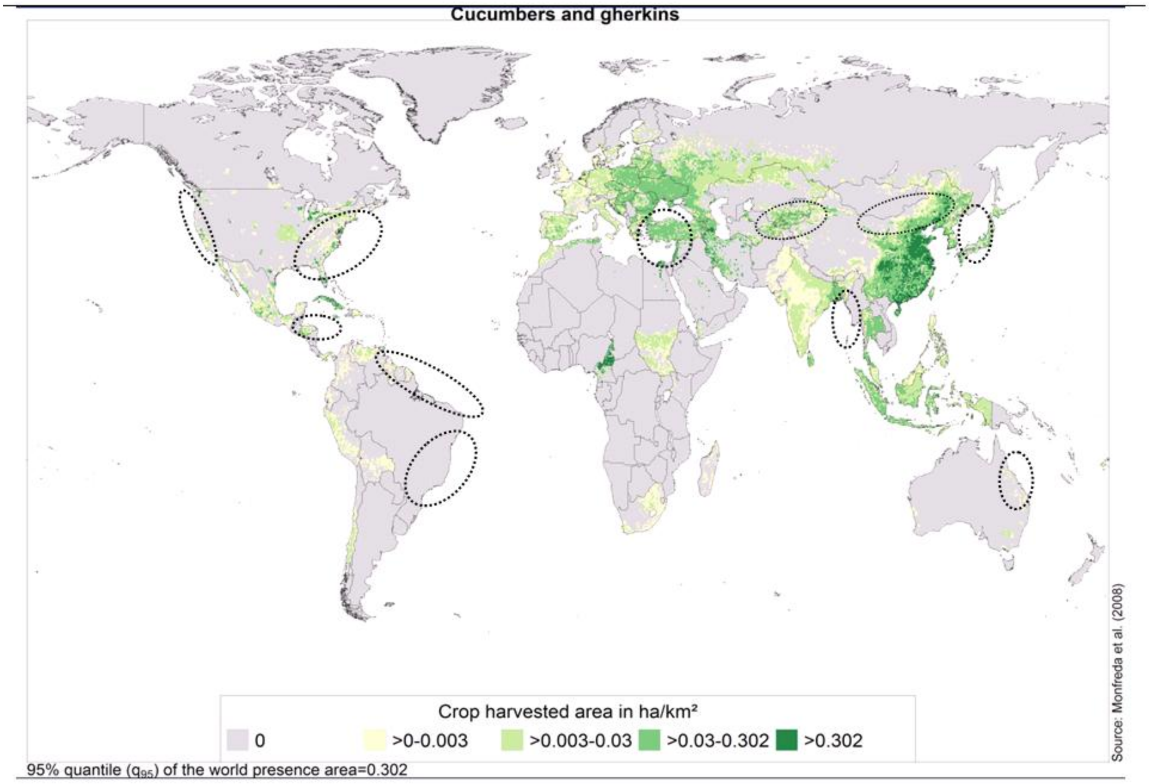
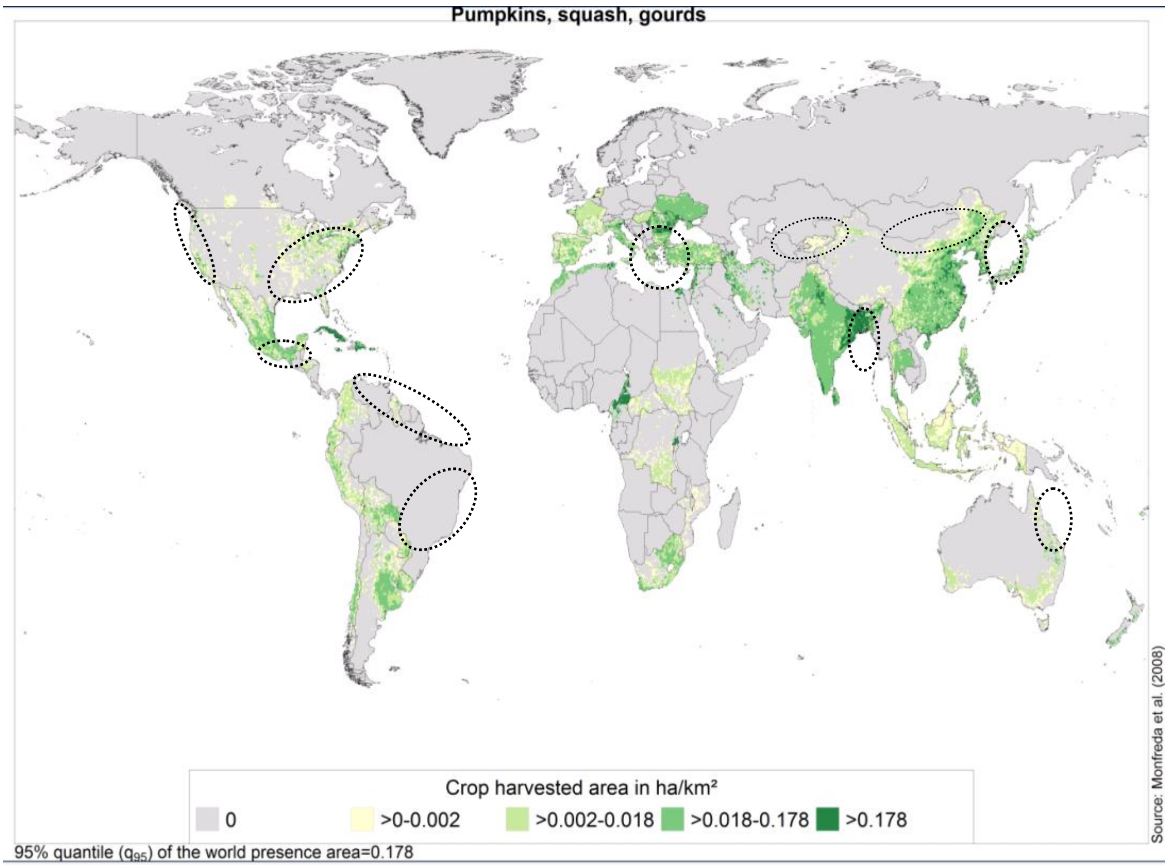
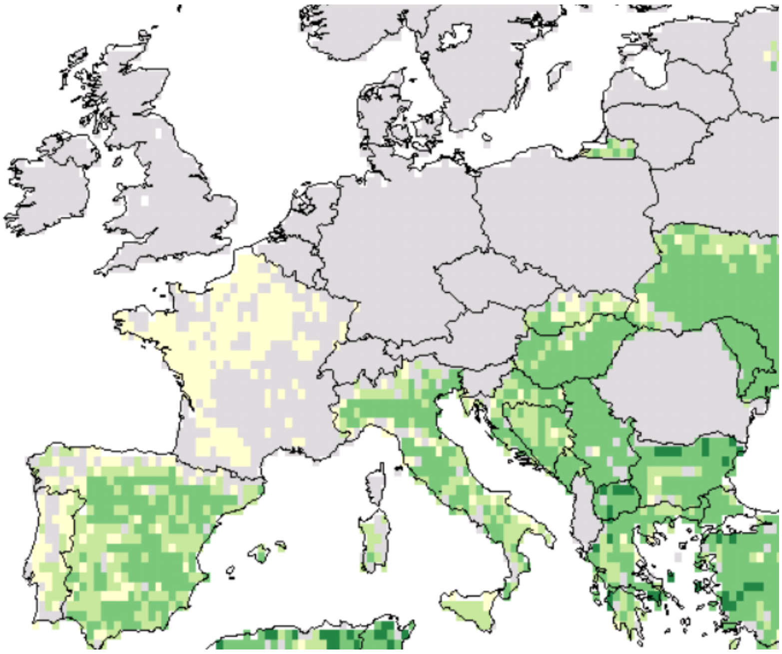



**Figure 3.01h:** Global distribution of production of pumpkins, squash and gourds with approximate occurrence of *A. citrulli*



To estimate the area of potential establishment in Europe, the area where potential hosts are grown is considered. Maps 3.01 i to 3.01 l show EU production of cucurbits.

**Figure 3.01 i:** EU area of watermelon production



Crop harvested area in ha/km²

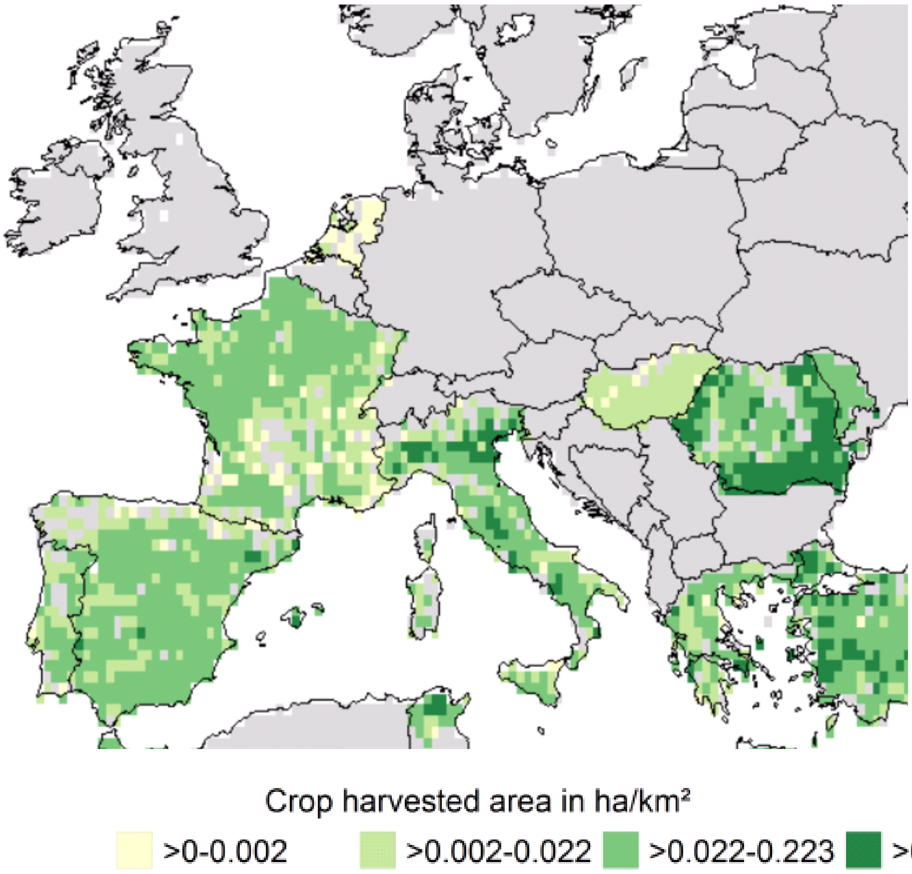
0 | >0-0.004 | >0.004-0.043 | >0.043-0.427 | >0.427

95% quantile of world presence area = 0.427 ha /$km^2$

| **Table 3.01 i:** EU area of harvested watermelon 2007 – 2009 (ha) | | | | | |
|---|---|---|---|---|---|
| EU MS | 2007 | 2008 | 2009 | 3 year mean | % of EU area |
| Romania | 25,224 | 25,930 | 28,491 | 26,548 | 31.1 |
| Greece | 17,108 | 17,000 | 17,955 | 17,354 | 20.4 |
| Spain | 16,861 | 15,674 | 17,600 | 16,712 | 19.6 |
| Italy | 11,141 | 11,091 | 11,000 | 11,077 | 13.0 |
| Hungary | 7,900 | 7,870 | 6,918 | 7,563 | 8.9 |
| Bulgaria | 4,572 | 4,749 | 5,593 | 4,971 | 5.8 |
| Slovakia | 393 | 334 | 339 | 355 | 0.4 |
| Portugal | 350 | 338 | 370 | 353 | 0.4 |
| France | 182 | 186 | 200 | 189 | 0.2 |
| Malta | 125 | 117 | 124 | 122 | 0.1 |
| Austria | 10 | 13 | 8 | 10 | 0.0 |
| **sum** | 83,866 | 83,302 | 88,598 | 85,255 | 100.0 |

Source: FAO Stat Crop production (Area harvested)

**Figure 3.01 j:** EU area of cantaloupe and other melon production

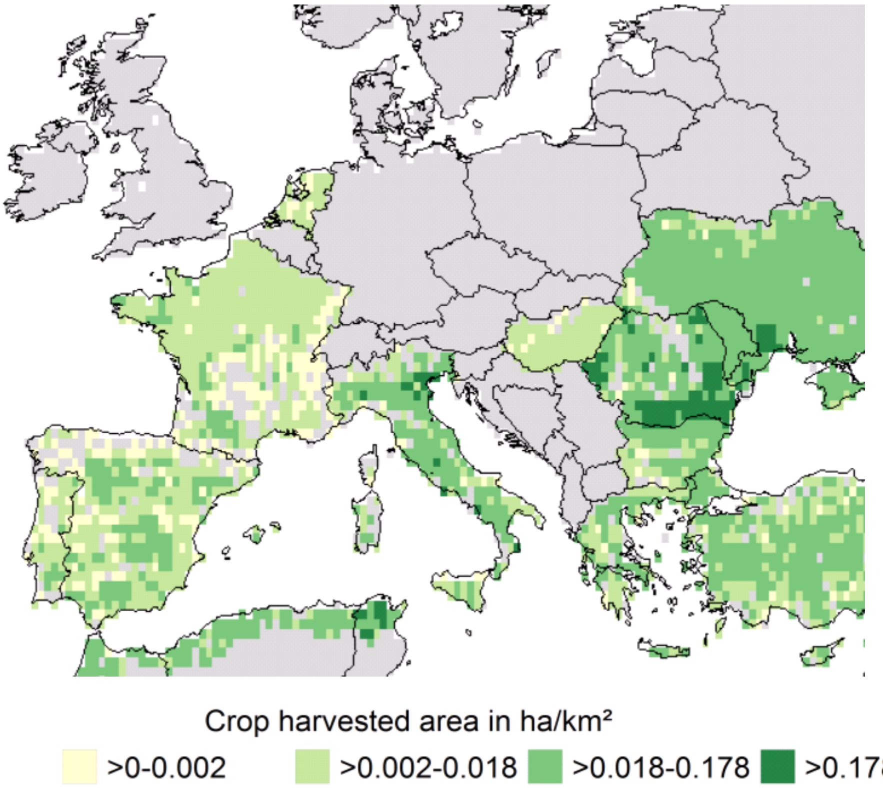



95% quantile of world presence area = 0.223 ha /km$^2$

| **Table 3.0 j:** EU area of harvested canteloupe and other rmelon 2007 – 2009 (ha) | | | | | |
|---|---|---|---|---|---|
| EU MS | 2007 | 2008 | 2009 | 3 year mean | % of EU area |
| Spain | 38,688 | 33,388 | 31,400 | 34,492 | 37.5 |
| Italy | 25,947 | 28,199 | 22,300 | 25,482 | 27.7 |
| France | 14,948 | 15,603 | 15,504 | 15,352 | 16.7 |
| Greece | 8,359 | 8,400 | 7,296 | 8,018 | 8.7 |
| Romania | 3,430 | 3,672 | 4,328 | 3,810 | 4.1 |
| Portugal | 3,100 | 3,150 | 3,699 | 3,316 | 3.6 |
| Hungary | 1,100 | 878 | 955 | 978 | 1.1 |
| Malta | 232 | 224 | 195 | 217 | 0.2 |
| Slovakia | 146 | 126 | 106 | 126 | 0.1 |
| Netherlands | 70 | 73 | 67 | 70 | 0.1 |
| **sum** | 96,020 | 93,713 | 85,850 | 91,861 | 100.0 |

Source: FAO Stat Crop production (Area harvested)

**Figure 3.01 k:** EU area of pumpkin, squash and gourd production



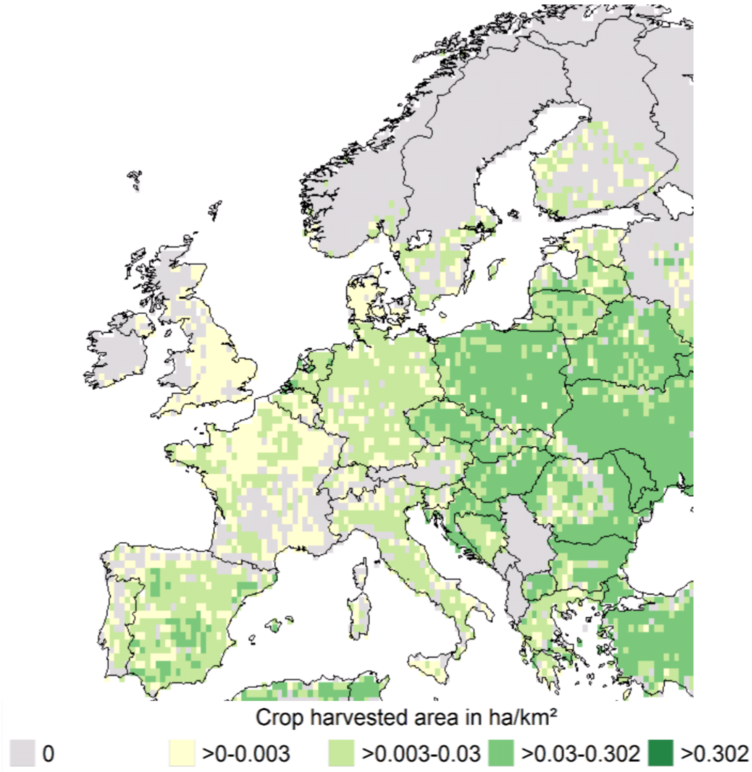

95% quantile of world presence area = 0.178 ha /km$^2$

**Table 3.01 k:** EU area of harvested pumpkin, squash & gourd 2007 – 2009 (ha)

| EU MS | 2007 | 2008 | 2009 | 3 year mean | % of EU area |
|---|---|---|---|---|---|
| Italy | 17,023 | 16,582 | 13,500 | 15,702 | 34.0 |
| Spain | 8,500 | 8,000 | 5,500 | 7,333 | 15.9 |
| Romania | 5,603 | 5,278 | 5,462 | 5,448 | 11.8 |
| France | 4,714 | 5,100 | 5,054 | 4,956 | 10.7 |
| Greece | 4,804 | 3,900 | 3,211 | 3,972 | 8.6 |
| Germany | 2,238 | 2,671 | 2,728 | 2,546 | 5.5 |
| Slovakia | 1,831 | 1,744 | 1,949 | 1,841 | 4.0 |
| Poland | 1,528 | 1,547 | 1,883 | 1,653 | 3.6 |
| Hungary | 500 | 530 | 1,413 | 814 | 1.8 |
| Portugal | 700 | 710 | 730 | 713 | 1.5 |
| Austria | 365 | 367 | 391 | 374 | 0.8 |
| Bulgaria | 272 | 260 | 226 | 253 | 0.5 |
| Latvia | 400 | 74 | 128 | 201 | 0.4 |
| Netherlands | 200 | 200 | 200 | 200 | 0.4 |
| Malta | 70 | 66 | 54 | 63 | 0.1 |
| Finland | 46 | 43 | 46 | 45 | 0.1 |
| Denmark | 20 | 19 | 18 | 19 | 0.0 |
| Lithuania | 20 | 13 | 16 | 16 | 0.0 |
| **sum** | 48,834 | 47,104 | 42,509 | 46,149 | 100.0 |

Source: FAO Stat Crop production (Area harvested)

**Figure 3.01 l:** EU area of cucumbers and gherkin production




95% quantile of world presence area = 0.302 ha /km$^2$

**Table 3.01 l:** EU area of harvested cucmbers & gherkins 2007 – 2009 (ha)

| EU MS | 2007 | 2008 | 2009 | 3 year mean | % of EU area |
|---|---|---|---|---|---|
| Poland | 21,036 | 19,960 | 20,144 | 20,380 | 34.6 |
| Romania | 12,590 | 12,986 | 13,122 | 12,899 | 21.9 |
| Spain | 7,466 | 8,286 | 8,500 | 8,084 | 13.7 |
| Germany | 3,224 | 3,086 | 3,015 | 3,108 | 5.3 |
| Greece | 2,765 | 2,100 | 2,069 | 2,311 | 3.9 |
| Italy | 2,145 | 2,065 | 2,000 | 2,070 | 3.5 |
| Slovakia | 2,249 | 2,191 | 1,198 | 1,879 | 3.2 |
| Czech Repub | 1,457 | 1,655 | 1,641 | 1,584 | 2.7 |
| Lithuania | 1,187 | 1,102 | 1,050 | 1,113 | 1.9 |
| Hungary | 1,117 | 978 | 1,149 | 1,081 | 1.8 |
| Bulgaria | 850 | 371 | 876 | 699 | 1.2 |
| France | 642 | 631 | 617 | 630 | 1.1 |
| Netherlands | 600 | 600 | 600 | 600 | 1.0 |
| Austria | 405 | 365 | 426 | 399 | 0.7 |
| Portugal | 350 | 338 | 397 | 362 | 0.6 |
| Latvia | 746 | 166 | 77 | 330 | 0.6 |
| Finland | 354 | 324 | 288 | 322 | 0.5 |
| Sweden | 300 | 300 | 300 | 300 | 0.5 |
| Estonia | 283 | 261 | 270 | 271 | 0.5 |
| Slovenia | 119 | 115 | 154 | 129 | 0.2 |
| Denmark | 120 | 93 | 120 | 111 | 0.2 |
| UK | 103 | 103 | 103 | 103 | 0.2 |

| **Table 3.01 l:** EU area of harvested cucmbers & gherkins 2007 – 2009 (ha) | | | | | |
|---|---|---|---|---|---|
| EU MS | 2007 | 2008 | 2009 | 3 year mean | % of EU area |
| Belgium | 100 | 46 | 43 | 63 | 0.1 |
| Malta | 26 | 27 | 27 | 27 | 0.0 |
| Ireland | 15 | 12 | 11 | 13 | 0.0 |
| | 60,249 | 58,161 | 58,197 | 58,869 | 100.0 |

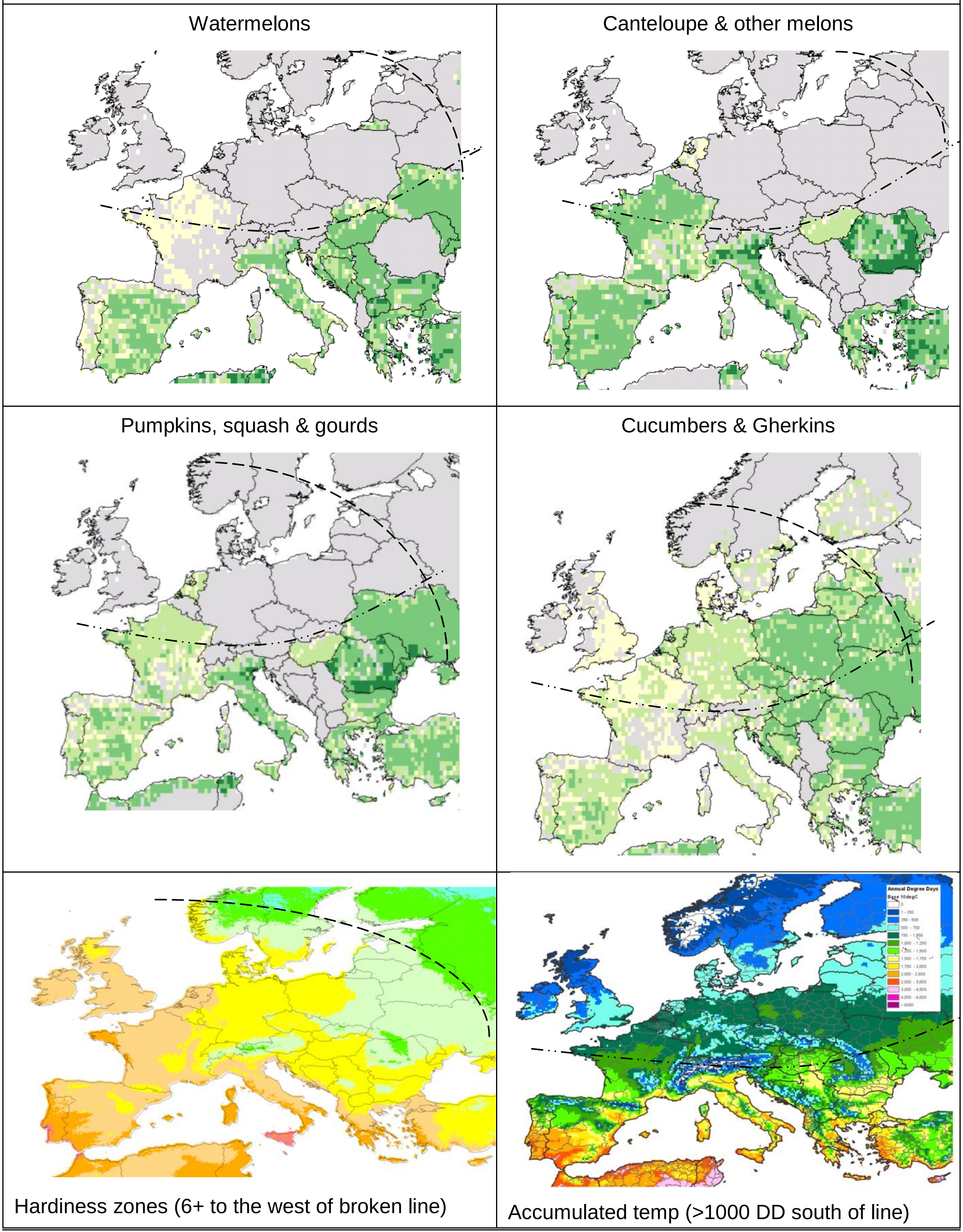
Source: FAO Stat Crop production (Area harvested)

As noted above there is no very detailed information regarding the environmental conditions favoured by *A. citrulli*. It has been reported that disease outbreaks are favoured by heavy rainfall and windy conditions (which spreads the pathogen) and symptoms are expressed during humid warm periods of summer or spring (Wall & Santos, 1988; Schaad *et al*., 2008).

The temperature requirements for hosts could be considered as proxies for the temperature requirements for the pathogen. Watermelon germinates best between 21°C and 35°C. Ideal growing temperatures for transplants are between 21°C and 27°C during the day and between 16°C and 22°C at night (CABI CPC, 2010 ).

Combining plant hardiness zone information, accumulated temperature maps, EU cucurbit host maps and information about the (little described) known distribution of the pathogen and disease, the pathogen could probably establish outdoors in southern and central Europe (Figure 3.01 m). 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. However, whether the weather conditions prior to outbreaks developing were typical for the areas is unknown.

**Figure 3.01 m:** EU cucurbit production, plant hardiness zones and accumulated temperature




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.

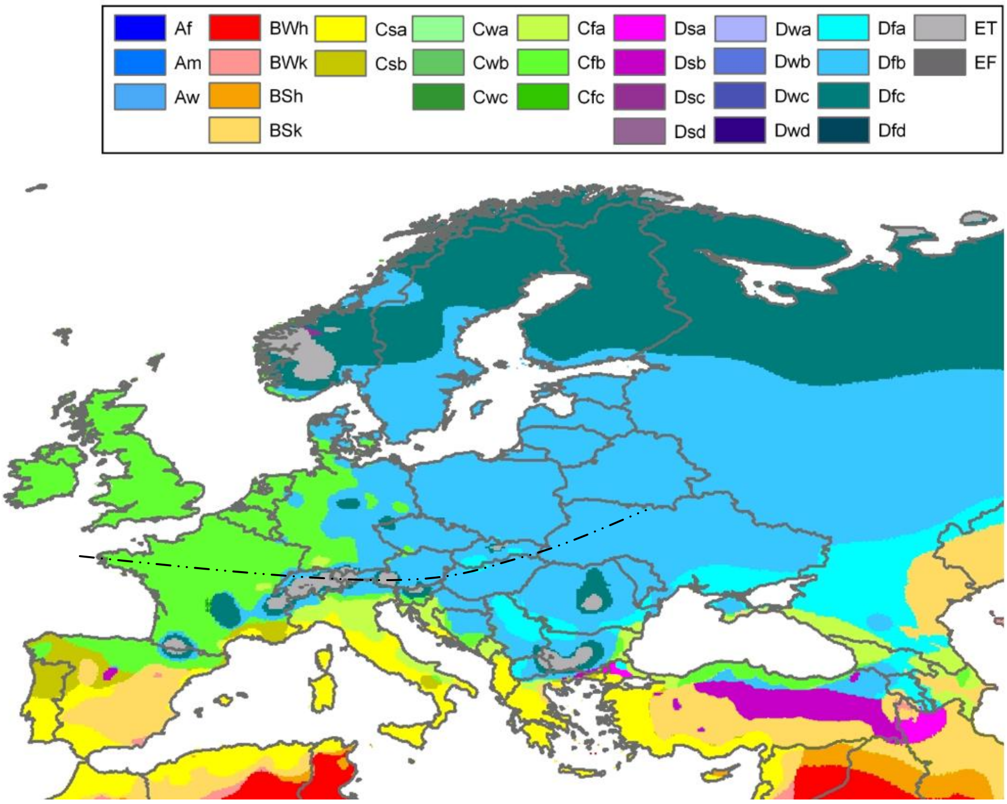
**Uncertainties regarding environmental suitability**

A combination of appropriate temperature and relative humidity are critical in determining whether *A. citrulli* is able to cause bacterial fruit blotch of cucurbits. The range of temperature and relative humidity within which disease symptoms appear are not known although optimum conditions have been described as 55% relative humidity between 24$^{o}$C and 38°C.

*Comment regarding the endangered area*
The current assessment considers the endangered area as those watermelons growing regions of the EU where accumulated temperature exceeds 1,000 day degrees above a threshold of 10$^{o}$C (broadly south of the broken line shown in Figure 3.01n).

Detailed modelling using GIS could be performed to identify where exactly in Europe hosts, relative humidity and temperatures combine to favour disease expression.

**Figure 3.01 n:** Köppen-Geiger climate map for Europe.




*Conclusions*
Large areas of watermelon production in the EU fall within climate zones assumed to be potentially suitable for <u>establishment</u> of *A. citrulli*. However, whether the conditions over the large areas are suitable for <u>disease expression</u> is more uncertain. It is noteworthy that although the disease has been reported from many US States, in most years the disease occurs in relatively few fields, perhaps due to the particular environmental requirements to stimulate outbreaks.

| **3.01: Environmental suitability** (particularly climate and host) | | | | |
|---|---|---|---|---|
| **Rating** | **Pest is likely to be able to establish in ...** | **Justification summary** | **Probability for suitable area** [1] | **Probability for endangered area %** |
| **Very low** | Less than 10% of host area | | - | - |
| **Low** | Between 10% and 1/3 of host area | | - | - |
| **Medium** | Between 1/3 and 2/3 of host area | | - | - |
| **High** | Between 2/3 and 90% of host area | Some outdoor crops occur in areas where accumulated temp is below 1,000 DD | 15 % | 15 % |
| **Very high** | More than 90% of host area | Outdoor cucurbits in southern EU occur in areas where pathogen could establish | 85 % | 85 % |
| | Check sum = | | 100% | 100 % |

[1] Spread your judgment according to your belief / evidence

**3.02 Extent of spread**

*Having found a host and established in the PRA area, a pest will need to spread / disperse after introduction. Consider how quickly the pest could spread. For example, take into account its reproductive potential, suitability of the environment and inherent powers of movement. Assessors should take into account the likelihood that spread may not be contiguous and satellite populations may develop at significant distances from the original point of establishment. Such dispersal could occur via biotic or abiotic vectors, wind, water, or, for example, be facilitated via trade or transport links or via others forms of human assistance, such as movement of infected/infested plant material for propagation purposes (seedlings, scions, budwood)*.

*Taking into account the time horizon considered within this assessment (see Initiation, 1.5) estimate the area likely to be occupied by the pest at that time[8]. Fill out table 3.02a by estimating the likelihood that the pest would have spread to occupy the given proportion of the host area suitable for establishment within the time period / time horizon considered by this assessment. Then fill out table 3.02b by estimating the likelihood that the pest would have spread to occupy the given proportion of endangered area within the time period / time horizon considered by this assessment.*

*The tables are used to consider the extent of spread in relation to the area where establishment is suitable. Hence it is recognised that fast moving pests with a large area suitable for establishment may be rated below slower moving pests with much smaller areas suitable for establishment. Quantitative spread modelling could be considered to examine spread more precisely.*

*Specify the time period / time horizon considered by this assessment (refer to 1.5).*

**Risk reduction options to consider**: Destruction of crops where outbreaks are detected and imposition of hygiene measures to inhibit local spread and "carry-over" of disease.

**Information / evidence:** *Provide reasoning then give judgment*

If *Acidovorax citrulli* was made a quarantine pest for the EU, then if outbreaks are found infested crops ought to be destroyed. To minimize local spread of the pathogen, work should not be conducted in the infested field when foliage is wet. Irrigation and mechanical equipment should be decontaminated before moving from an infested to a non infested field. In glasshouses spread of the bacterium could be minimized by low humidity (increased air flow), low temperature, bottom watering and by practices that reduce or eliminate long periods of wet foliage (Latin *et al*., 1995).

To inhibit the likelihood of recurrence or in field carry-over, the crop should undergo deep plowing. There should also be removal of volunteer plants that emerge the next season. There should also be crop rotation with non-cucurbit hosts in outbreak fields, for at least 3

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[8] When assessing the extent of spread, be *clear about the scenario being considered e.g. you could be considering a scenario without risk reduction options in place, or a scenario with specific phytosanitary measures that inhibit spread (risk reduction options) in place.*

years. Wild cucurbits should be removed to prevent possible inoculum sources from re-infesting crops. Without such action *A. citrulli* could spread between commercial crops, from crops to other host plants as well as between other host plants. To estimate how quickly the pest can spread, the following were taken into account: the population dynamics (reproductive potential) of the pest, the influence of the environment and the movement of commodities.

Regarding the population dynamics of the pest, Shirakawa *et al.* (2003) reported that when infested seeds with only 1 cfu of *A. citrulli* were sown and grown under high humidity, there was a high probability of the germinated seedlings being diseased. The percentage of diseased plants was higher under high humidity than low. Besides, when watermelon seedlings were inoculated with $10^5$ cfu *A. citrulli* /ml (spray inoculation) and then grown at high humidity, the population of the pest on seedlings increased from less than 10 cfu/g fresh weight to $10^6$-$10^7$ cfu/g fresh weight, and seedlings were diseased within 2 days of inoculation. When the inoculated seedlings were incubated at low humidity, the rate of multiplication was lower, reaching $10^4$-$10^5$ cfu/g fresh weight by 2 or 3 days, and no seedling was diseased (Shirakawa*et al.*, 2003). Transmission from seed to seedling is high, and concentration of the pest on seedlings is higher than on seeds (Latin and Hopkins, 1995).

Hamm *et al*. (1997) reported that when symptomatic test plants were transplanted to fields, maturing melons developed bacterial fruit blotch symptoms.

Regarding plant colonization dynamics by *A. citrulli*, studies on melon plants showed that the bacterium colonized different parts of the melon plant over time, depending on its initial location: leaves or seed (Alves *et al.,* 2010).

The rate of spread depends on inoculum level, aggressiveness of the strains, environmental conditions and presence of host plants.

Hopkins (1993) evaluated the rate of spread from a point-source of inoculum in a watermelon field and found that one infected seedling planted during spring in the centre of a 15m x 15m plot (50 plants) resulted in 80% of the plants showing foliar symptoms before harvest, and 45% of fruit infected. The same severity of foliar symptom incidence occurred in another 7.5m x 30m experimental field plot when three plants were initially artificially inoculated. In the latter case, symptoms developed earlier possibly due to higher starting inoculum level (three infected plants instead of one), but fruit infection reached only 5%, possibly due to dry weather conditions. Regarding inoculum level, Rane & Latin (1992) reported that the bacterial fruit blotch epidemic in Indiana in 1989 was most likely introduced through contaminated seed, and the initial level of contamination was estimated to be 1 infected seed in 9,000.

Similarly, Dutta *et al.* (2011) showed that a) temporal spread of the disease in 128-cell seedling trays increased linearly with inoculum load, b) the frequency of spatial spread of the disease from an inoculated seedling in the center of a planting tray to adjacent healthy seedlings over one-, two-, or three-cell distances was greater for lots with one seed infested with at least $1 \times 10^5$ cfu than for lots with one seed infested at lower inoculum loads ($1 \times 10^1$ and $1 \times 10^3$ cfu/seed). Regarding aggressiveness of the strains, as described in the relevant datasheet (Table 1) one group (Group I) of strains is considered moderately to highly aggressive to a wide range of cucurbit hosts, while another group (Group II) highly aggressive on watermelon and weakly aggressive on other cucurbits (Walcott *et al.,* 2004; Burdman *et al.,* 2005).

Once infection is established, the pest spreads in intercellular spaces of the seedling and initiate water-soaked lesions. In the transplant facilities, the seedlings are raised under warm and humid conditions, usually in a soilless potting medium. These environmental conditions and the presence of susceptible plant tissue in high density (high plant population) favour disease and the rate of its spread can be especially high (Latin & Hopkins, 1995).

Splashing water from rain or overhead irrigation spread the bacteria from infected seedlings (the source of secondary inoculum) to neighbouring seedlings (Hopkins, 1994; Latin & Hopkins, 1995; Latin *et al*., 1995). More specifically, in glasshouses, the rate of spread of the disease has been linked to the type of irrigation applied (Hopkins, 1994; Latin *et al.*, 1995). Overhead irrigation resulted in higher disease incidence compared to watering plants from the bottom. Besides, in the first case, symptoms occurred in plants located several cm from another diseased seedling, while in the second case symptoms only spread to plants adjacent to the inoculum source (Hopkins, 1994; Latin *et al.*, 1995). However, with the overhead irrigation, daily low humidity was suggested as a critical limiting factor for the spread of the disease (Hopkins, 1994). Subirrigation has been suggested as a viable option for managing splash-dispersal (Latin *et al*., 1995).

Reduced relative humidities after overhead irrigation diminished disease spread (Latin *et al*., 1995). However, successful infection can take place with leaf wetness periods of only 30 min at temperatures around 26$^{o}$C (Latin, 1996).

The rate of disease spread depends also on the developmental stage of the plants. Young fruits, 2 to 3 weeks after flowering, are most susceptible to infection (possibly though splashing water during rain or irrigation) through stomata and blotch symptoms appear rapidly on fruit 3-7 days later and generally shortly before they ripen (Frankle *et al.,* 1993). After this period, stomata become blocked by the deposition of waxes on the fruit surface and spread is prevented (Frankle *et al.,* 1993; Walcott, 2005). The surface lesions on mature fruits are an additional source of secondary inoculum in the field and they expand rapidly. Fruit may eventually rot in the field and their seeds, infested by contact with contaminated tissues, slip to the soil (Latin & Hopkins, 1995; Bahar & Burdman, 2010).

The role of wind-driven rain and mechanical means in the local spread of the pest has been acknowledged (Hopkins *et al.*, 2000), but no studies have been made to estimate distances of dispersal by wind-driven rain, as it has been done with other pathogens. For instance: a) *Xanthomonas citri* is readily spread by wind and rain within trees or to neighboring trees (up to 32 meters from infected trees in Argentina), but there is also evidence for much longer dispersals cases associated with meteorological events, such as severe tropical storms, hurricanes and tornadoes, where spread reached up to 56 kms in Florida (Stall *et al.,* 1980; Pruvost *et al.*, 1999; Gottwald *et al.,* 2001; Pruvost *et al*., 2002; Bock *et al.,* 2010), b) *Pseudomonas syringae* pv. *actinidiae* has shown a spread capacity of around 10 km from the initial infected orchards in Italy (Vanneste *et al.* 2011 in press). The only relevant information found in the literature for *A. citrulli* is that a) honeydew fruits (*Cucumis melo* var. *inodorus*) infected with *A. citrulli* were collected in a field in Texas at 0.8 km from a watermelon field where the disease had been reported 9 weeks earlier (Isakeit *et al.,* 1997), b) citronmelon fruits (*Citrullus lanatus* var. *citroides*) infected with *A. citrulli* were collected in a cowpea filed in Texas that was nearby watermelon fields where the disease was present, with the closest field at 50 m from the cowpea field (Isakeit *et al.*, 1998).

No vector has been implicated in the dispersal of the pest (besides humans) (Latin & Hopkins, 1995; Bahar & Burdman, 2010). However, the role of honeybees in watermelon seed infestation through blossom inoculation has been suggested (Fessehaie *et al.,* 2005) but further research is needed to clarify this issue.

It is known that the bacterium can overwinter in volunteer seedlings from seeds from infected fruits, infested rind or other infested crop residues as well as cucurbit weed hosts (especially wild citron), all of them constituting a local source of inoculum that may contribute to outbreaks in subsequent watermelon crops (Latin & Hopkins, 1995). However, according to the NPPOs from 13 EPPO countries (NPPO questionnaires), the citron weed is not known to be present in their countries.

Latin *et al.* (1995) studied the survival of the bacterium on plastic transplants production trays and reported that the bacterium survived 63 days on trays containing potting substrate and root debris. Its longevity decreased with increasing storage temperature and it was not detected after treatment of the trays for 5 min with sodium hypochlorite (10% household bleach).

Spread of the pest can also occur through uncontrolled movement of plant propagation material, *e.g.* through exchanges of seeds and seedlings between homeowners, especially as latent infections are possible. This is generally a common practice. Although such propagation material can constitute a primary source of inoculum, the volume of this material is very to be likely small. However, if commercial crops are in proximity to such sites (*i.e.* there is host continuity), the risk of spreading the disease is likely. This has been observed with other bacterial pathogens of vegetable crops, *e.g. Ralstonia solanacearum* infecting potato and tomato plants or *Xanthomonas citri* spreading from backyard trees to commercial orchards (Gottwald *et al.,* 2001).

As for other bacterial pathogens, the use of non-disinfected tools and equipment, and generally failure to keep sanitized measures in field crops or transplant facilities result in spreading the pest.

In addition, as for all bacterial plant diseases, no curative management measures exists, and it is difficult to contain the pest, especially as even few infected seeds in a lot could initiate a disease outbreak and latent infections may escape inspections.

From the maps (Figure 3.01) showing distribution of major commercial crops in EU that can host the pathogen and the maps on hardiness zones and accumulated temperature, it can be concluded that hosts occur outdoor in plant hardiness zones 6 (Romania, Poland) to 10 (Southern Portugal, Spain, Italy). It has been estimated that the possible establishment area is where accumulated temperatures are above 1,000 degree days above a base of 10$^{o}$C. (Presence of host plants grown under protection (glasshouses) have not been related to the hardiness zones)

*Experience from outbreaks in USA*: the pathogen was first observed in commercial watermelon production areas in 1989 and since then it has been reported in many states, for examp, Iowa (1989), Delaware (1989), Maryland (1989), Indiana (1990), Oklahoma (1992), Texas (1994), Oregon (1997), Georgia (1999), Illinois (2002). Outbreaks reported in Maryland and Iowa in 1989 have not recurred, suggesting the introduction of the pathogen with infested or infected propagation material is essential for outbreaks occurrence (Latin & Hopkins, 1995). Thus, the magnitude of threat posed by local inoculum is probably much lower than that of infested or infected propagation material (Latin & Hopkins, 1995).

*Experience from Greece:* The pathogen was detected in 2005 and 2006 on watermelon fruits from fields in very distant areas (Macedonia in Northern Greece and Voiotia in Central Greece, respectively), as well as in 2008 on young watermelon plants from a nursery in the area of Ilia in Southern Greece (Holeva *et al*., 2010). No further detection of the pathogen has been reported ever since in these areas or any other place (Holeva personal communication).

*Experience from Italy:* The pathogen has been detected in Emilia Romagna (Northern Italy) in 2009 and in Sardinia (Southern Italy) in 2011, regions of Italy separated by approximately 500km and the Tyrrhenian Sea (that part of the Mediterranean sea between mainland Italy, Sardinia and Sicily). These outbreaks could have resulted from separate introduction events rather than the result of *A. citrulli* spread from Emilia Romagna to Sardinia.

**Uncertainties regarding extent of spread**

1) spread capacity studies are not available,
2) the role of pollinators in watermelon seed infestation through blossom inoculation has not been clarified,
3) systematic monitoring (surveys) of reported disease outbreaks has not been always available as the pathogen is not currently regulated.
4) No detailed data are available for the presence of wild host plants in the risk assessment area.

**Conclusions**

With swift action taken to destroy infested crops and strict hygiene measures implemented to prevent local spread, effectively eradicating the pathogen at a local scale, spread would be much reduced. Outbreaks in the USA and elsewhere suggest that occurrence of the disease is very much based on the use of contaminated seed or seedlings. There is not much spread from sites of initial introduction.

| **3.02a: Extent of spread in area of potential establishment at time horizon** | | | |
|---|---|---|---|
| **Rating** | **Within the time horizon considered the pest is likely to have spread to ...** | **Justification summary** | **Probability that given area will be occupied at time horizon [1]** |
| **Very low** | Less than 10% of the area suitable for establishment | Spread in localised area may be fast by natural means (rain splash & wind-blown) but mostly will not spread very far | 90% |
| **Low** | Between 10% and 1/3 of the area suitable for establishment | Where outbreaks are not detected or action is not effective spread could lead to a wider area being occupied. | 10% |
| **Medium** | Between 1/3 and 2/3 of the area suitable for establishment | | - |
| **High** | Between 2/3 and 90% of the area suitable for establishment | | - |
| **Very high** | More than 90% of the area suitable for establishment | | - |
| | Check sum = | | 100% |

[1] Spread your judgment according to your belief / evidence

| **3.02b: Extent of spread in endangered area at time horizon** | | | |
|---|---|---|---|
| **Rating** | **Within the time horizon considered the pest is likely to have spread to ...** | **Justification summary** | **Probability that given area will be occupied at time horizon[1]** |
| **Very low** | Less than 10% of the endangered area | As 3.02a above | 90% |
| **Low** | Between 10% and 1/3 of the endangered area | As 3.02a above | 10% |
| **Medium** | Between 1/3 and 2/3 of the endangered area | | - |
| **High** | Between 2/3 and 90% of the endangered area | | - |
| **Very high** | More than 90% of the endangered area | | - |
| | Check sum = | | 100% |

[1] Spread your judgment according to your belief / evidence

**Consequences of pest establishment and spread**

**3.03 Crop consequences (yield and quality)**

*Introduced pests are capable of causing a variety of direct and indirect impacts. The remit of EFSA limits assessors to consider the consequences of pest introduction on crop yield and quality (crop consequences / impacts) (3.03) and environmental consequences /impacts (3.04) e.g. impacts on ecosystem services or biodiversity itself. We recognise that other types of impacts, listed in ISPM 11, may also occur.*

*Fill out table 3.03 by taking into account the extent of pest spread within the endangered area up to the time horizon of the assessment, and other factors such as the rate of pest population development and any threshold required for harmful pest consequences to materialize within cultivated and managed plants. Consequences should be estimated taking into account the current situation in the endangered area with respect to the control efforts undertaken by growers /nurserymen/ producers etc. against other pests. Although we recognise that growers may respond by increasing pest management efforts to minimize impacts of a new pest, such additional efforts are not taken into account.*

*If the risk assessment has been initiated by a review of phytosanitary policy where the pest is already present and action is being taken against it, specify whether consequences are being assessed assuming that action is stopped.*

**Risk reduction options to consider:** None

**Information / evidence:** *Provide reasoning then give judgment*

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 one of watermelon and melon crops and is a major threat to these industries around the world (Bahar & Burdman, 2010). In warm and wet conditions (optimally 55% relative humidity and temperatures between 24°C and 38°C), the consequences to the most susceptible crops such as watermelons and melons can be severe.

<u>Losses in watermelons and melons in the USA</u>

Following the initial reports of losses from whole fields in the Mariana Islands (Wall *et al*., 1990), losses of up to 50% of marketable fruit have been reported in Florida (Somodi *et al.,* 1991) and other US States (south eastern, mid-Atlantic, and mid-Western states) as the season progressed. More than 500 ha of watermelons in south western Indiana were affected in summer 1989, with nearly 100 ha sustaining losses approaching 90% (Latin & Rane, 1990). In Texas, when there was above-average precipitation in May and early June 1993, watermelon fields in Frio County were affected with approximately 50% of fruit showing symptoms in affected parts of fields (Black *et al.,* 1994). Fields near to severely affected crops can incur 5 to 50% losses according to environmental conditions and the crop growth stage when infection occurs (Latin & Hopkins, 1995).

Disease incidence of 5% on cantaloupe fruit was reported in Georgia (Walcott *et al.,* 2000) and more than 50% of honeydew fruit loss was reported in Texas (Isakeit *et al.,* 1997). In Oklahome, in 1991 yield losses in marketable watermelon fruit ranged from 10 to 15% in an affected field (Jacobs *et al.*, 1992).

Although the disease has been reported from many US states in most years the disease occurs in relatively few fields, but where it has occurred it has been devastating in many of them. Bacterial fruit blotch was especially widespread in the USA in 1994 when it caused losses across thousands of hectares distributed over at least 10 States. According to most recent information (Langston 2011), major watermelon production states in US, like Georgia, had outbreaks in greenhouses and fields that made 2011 one of the 'worst years' regarding this disease.

<u>Losses in watermelons and melons elsewhere</u>
When the disease was found in a watermelon production area of 35,000 ha in the Adana Province in the eastern Mediterranean Region of Turkey in 1995, it caused "serious" yield reductions (Nursen, 2008). In 13 commercial watermelon fields surveyed in the area, 30 – 45% of fruit showed symptoms of bacterial fruit blotch (Mirik *et al*., 2006). In Brazil, crop losses on melon were estimated to be 40 – 50%, and up to 100% in some crops.

In China (Xinjiang Province), during an outbreak, the disease incidence on edible seed watermelon fruit was 30% and on seedlings 20% (Ren *et al.,* 2006).

<u>Impacts on other hosts</u>
Although primarily regarded as a pathogen of watermelon and melon, *A. citrulli* has been reported causing lesions on pumpkins leading to total collapse of infected fruit in a commercial pumpkin field in Terrell County, Georgia, USA in September 1998 (Langston *et al.*, 1999). In March 1999, in northern Queensland, Australia, *A. citrulli* caused foliar disease symptoms in a commercial crop of cucumber with more than 20% of the crop affected. No symptoms were observed on plant stems or fruits (Martin *et al.*, 1999). In March 2001, foliar symptoms were observed on gramma seedlings in a commercial nursery (*Cucurbita moschata*) (Martin and Horlock, 2002). In 2008, *A. citrulli* was reported from betel vine in Taiwan where it caused losses of 30-70% (Deng *et al*., 2010). This is most significant given that betel vine is not a cucurbit.

**Uncertainties regarding crop consequences**
Bacterial fruit blotch has been reported from many locations around the world. However, there is no evidence of the disease recurring year after year at the same location. This could be due to variation in use of contaminated seed or seedlings at locations where outbreaks have previously occurred, or due to the lack of the specific (unknown) environmental conditions occurring in the same locations each year. Losses can vary from 5% to 100% depending on when plants or fruit become infected and environmental conditions. Most reports suggest that when conditions are suitable for the disease, consequences are usually "devastating" for watermelon producers.

## *Conclusions*

| 3.03: Potential consequences on crops and managed plants | | | |
|---|---|---|---|
| **Rating** | **Description** (if established in the endangered area, the pest ....(descriptions within categories provide guidance, not all descriptions need to be satisfied in each category) | **Justification summary** | **Probability Assignment** [1] |
| **Very low** | Under existing pest management regimes, the pest is likely to have **negligible or no impact** on a standing crop and/or stored products.<br><br>Yield and/or quality losses would be **negligible and within the range of natural variation**. | | - |
| **Low** | Under existing pest management regimes, the pest is likely to have **minimal impact** on a standing crop and/or stored products.<br><br>Yield / quality losses would be **minimal.** | | - |
| **Medium** | Under existing pest management regimes, the pest is likely to have a **minor to moderate impact** on a standing crop and / or stored products.<br><br>Yield / quality losses would be **moderate.** | | - |
| **High** | Under existing pest management regimes, the pest is likely to have a **moderate to severe impact** on a standing crop and / or stored products. Thus the pest will not be effectively controlled by actions already applied against other pests by growers.<br><br>Yield / quality losses would be **moderate to severe**. | | 50 % |
| **Very high** | Under existing pest management regimes, the pest is likely to have a **severe impact** on a standing crop and / or stored products. Thus the pest will not be effectively controlled by actions already applied against other pests by growers.<br><br>Yield / quality losses would be severe. | Most reports suggest that when conditions are suitable for the disease, consequences are usually “devastating” for watermelon producers. | 50 % |
| | | Check sum = | 100% |

[1] Spread your judgment according to your belief / evidence

### 3.04 Environmental Consequences

*The assessment of the potential of a pest to cause environmental damage proceeds by considering the following factors:*

- *can the introduction of the pest cause permanent (irreversible) significant, direct environmental impacts, e.g. reduced biodiversity, ecological disruption.*
- *can the pest have direct impacts on endangered/threatened species by infesting/infecting a plant listed in Annex II or IV of the EC Habitats Directive[9] or infesting / infecting a plant which is a key component of a habitat listed in Annex I of the EC Habitats Directive? If the pest attacks other species within the genus or other genera within the family, and preference/no preference tests have not been conducted with the listed plant and the pest, then the plant is assumed to be a host.*
- *Can the pest have indirect impacts on species that are listed in Annex II or IV of the EC Habitats Directive or on species that are key components of habitats listed in Annex I of the EC Habitats Directive?*
- *Would the introduction of the pest stimulate chemical or biological control programmes which would disrupt existing biological or integrated systems for control of other pests or have negative effects on the environment e.g. biodiversity (at various levels), reduce population sizes, or increase their fragmentation.*

*Fill out table 3.04 by considering the likely magnitude of the above impacts, taking into account the extent of pest spread within the endangered area up to the time horizon of the assessment, and other factors such as the rate of pest population development and any threshold required for the pest to cause environmental harm in the environment.*

**Risk reduction options to consider:** None

**Information / evidence:** *Provide reasoning then give judgment*
Although there are wild cucurbit hosts, bacterial fruit blotch does not cause any significant harm in the wider environment or cause any impacts that need to be considered here.

**Uncertainties regarding environmental consequences**
None.

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[9] Council Directive 92/43/EEC (as amended) on the Conservation of natural habitats and of wild fauns and flora. Available at
http://www.central2013.eu/fileadmin/user_upload/Downloads/Document_Centre/OP_Resources/HABITAT_DIRECTIVE_92-43-EEC.pdf

*Conclusions*

| 3.04: Potential environmental consequences | | | |
|---|---|---|---|
| **Rating** | **Description** | **Justification summary** | **Probability Assignment** [1] |
| **Very low** | None of the above would occur; the pest is only able to establish on crops grown in protected cultivation such as glasshouses or shade houses. Nevertheless, it is assumed that introduction of a non-indigenous pest will have some environmental impact (by definition, introduction of a non-indigenous species affects biodiversity). | No environmental impacts are reported elsewhere.<br><br>This is a pest of commercial crops. | 100 % |
| **Low** | None of the above would occur; nevertheless the pest could establish outdoors and it is assumed that introduction of a non-indigenous pest will have some environmental impact (by definition, introduction of a non-indigenous species affects biodiversity). | | - |
| **Medium** | One of the above would occur.<br><br>*However, if effects are relatively small, the potential consequences can be rated Low instead of Medium.* | | - |
| **High** | Two of the above would occur.<br><br>*However, if effects are relatively small, the potential consequences can be rated Medium* | | - |
| **Very high** | Three or more of the above would occur.<br><br>*However, if effects are relatively small, the potential consequences can be rated High* | | - |
| | | Check sum = | 100% |

[1] Spread your judgment according to your belief / evidence

### 3.05 Potential impact

*The potential impact is assessed assuming entry has occurred and takes into account the endangered area occupied at the given time horizon (3.02b) with consequences to crops (3.03) and the environment (3.04) within the endangered area.*

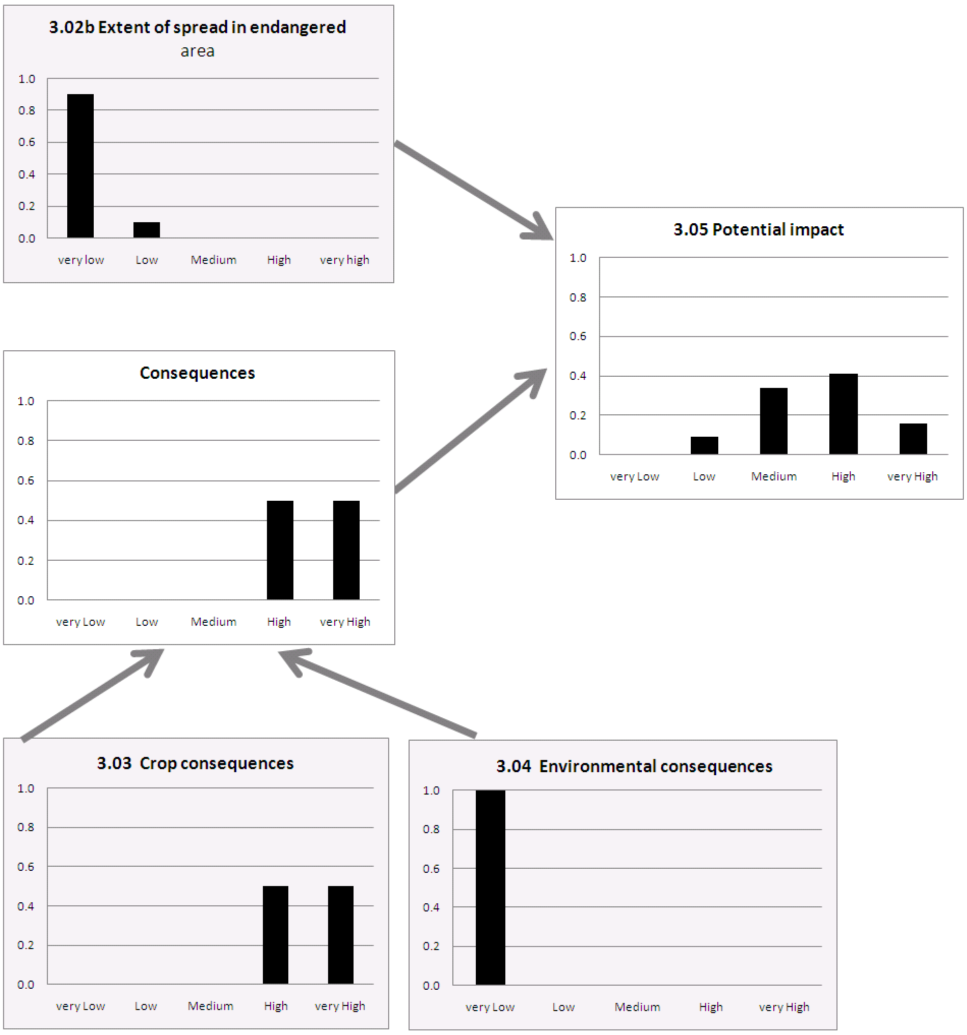
Figure 3.05 shows the BBN model output from combining scores of consequences with the area of the endangered area that could be occupied within the time horizon considered by this assessment.

*Conclusion*

There is considerable uncertainty associated with the estimate of potential impact. Where disease occurs it could have potentially severe consequences (as seen in USA and Turkey) but that impact may well occur in only a small area. The consequences are therefore diluted over the area which is shown as more uncertainty.

*Further study of how risk elements are combined in the BBN model is justified.*

**Figure 3.05:** Graphical representation of potential impact, combining consequences of pest introduction with the area occupied by the pest at the time horizon with risk reduction options in place.



### 3.06 Pest Risk

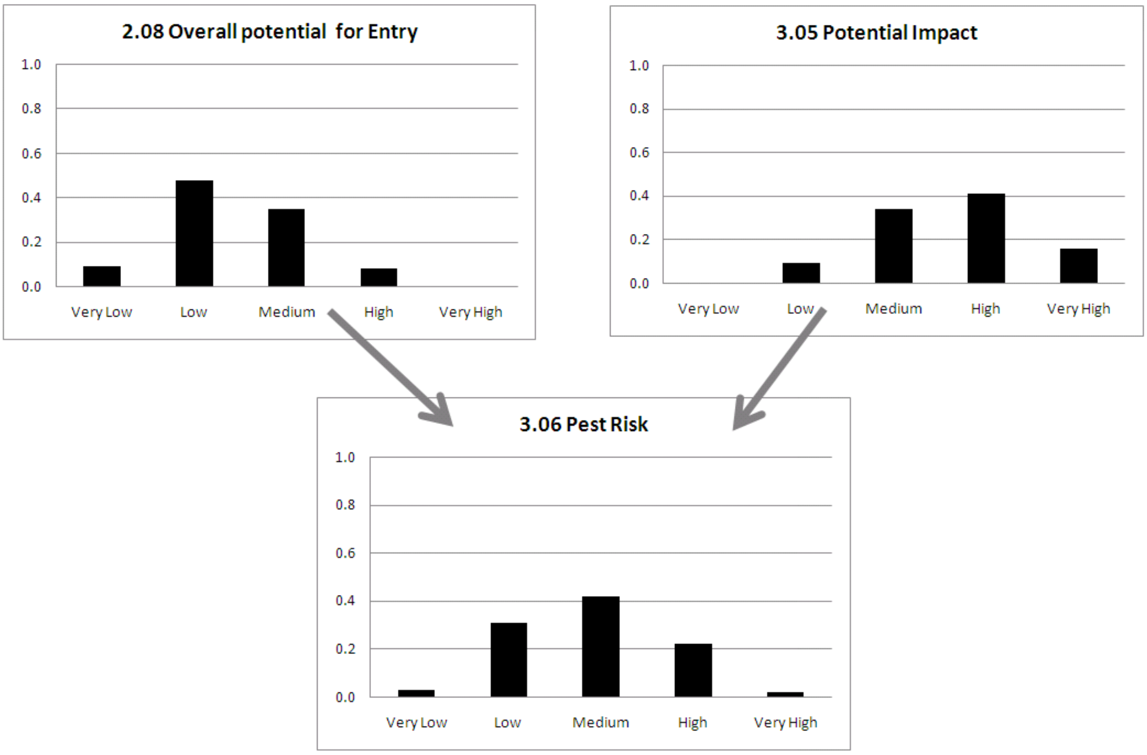
*To assess the risk for a pest that has not yet entered the PRA area, the potential impact (3.05) which assumes entry has occurred, must be combined with likelihood of entry and transfer (2.08).*

Figure 3.06 shows the BBN model output from combining scores for likelihood of entry and transfer with potential impact to give an overall pest risk profile. The profile shows much uncertainty with some likelihood of risk being minimal *(i.e.* very low) up to major (very high). Most likely the pest risk is actually medium. This risk profile is fairly similar to that without RROs in place, suggesting that the RROs are not effective, or that the BBN model does not combine risk elements in the correct manner. As noted at various points in this assessment, further study of the mechanism in the BBN which combine risk elements is justified.

*Conclusion*
*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.

An experimental protocol based on a BBN, which combines elements of pest risk in a systematic way, expresses overall pest risk as a bar chart using the categories “very low” to “very high”. Further research would be required to provide robust interpretations for what each of the categories in the ordinal scale mean. With risk reduction options in place the likelihood that pest risk is high or very high is reduced but still remains possible.

**Figure 3.06:** Graphical representation of Pest Risk (3.06), combining overall potential for pest entry and transfer (2.08) with potential impact (3.05) with risk reduction options in place.



### 4.0 Uncertainties

*Following EFSA Guidance (EFSA, 2010), to ensure transparency in risk assessment, uncertainties should be identified, characterized and documented within all risk assessments. This can show not only which aspects of an assessment are uncertain but the degree of uncertainty and can help identify where further work could usefully reduce uncertainty.*

**Table 4: Summary of uncertainties identified and further work that could be undertaken to reduce uncertainties**

| **Section of risk assessment** | | **Uncertainties** | **Research that would reduce uncertainty** |
|---|---|---|---|
| 2.0 | Pathways | | |
| **2.01a** | Pest associated | | Survey seed production sites for presence of *A. citrulli*. Studies to increase the sensitivity of laboratory screening methods. |
| 2.02a | Survive post harvest | | Study practices used and survey for pest. |
| 2.03a | Survive storage | | Monitor survival of the pest on/in seed in store. Collect data on usual storage practices in commerce |
| 2.04a | Survives measures | | Survey seed screening checks |
| 2.05a | Quantity imported | | Collect import data (quantity, time of the year, routes, modes of transport, number of seeds in typical seed lots/consignments) from trade |
| 2.06a | Transfer | | Conduct experiments using contaminated seed |
| **2.01b** | Pest associated | | Survey seedling production sites for presence of *A. citrulli*. Studies to increase the sensitivity of laboratory screening methods. |
| 2.02b | Survive post harvest | | Study practices used and survey for pest. |
| 2.03b | Survive storage/transport | | Monitor survival of contaminated seedlings during transport |
| 2.04b | Survives measures | | Survey seedling screening checks |
| 2.05b | Quantity imported | | Collect import data (quantity, time of the year, routes, modes of transport, number of seedlings in typical consignments) from trade |
| 2.06b | Transfer | | Conduct experiments using contaminated seedlings |
| 3.01 | Environmental suitability | | Conduct studies in controlled atmosphere chambers to determine the range of conditions required for disease expression. |
| 3.02 | Extent of spread | | Detailed climate mapping based on findings from 3.01 with models of spread via trade networks to determine extent of spread. |
| 3.03 | Crop consequences | | Test broad range of EU cucurbit crops, including different varieties. |
| 3.04 | Environmental consequences | | Survey for wild cucurbit hosts and potential impacts thereon. |

### Conclusion

The bacterial plant pathogen *Acidovorax citrulli* has the characteristics of a quarantine pest for the EU. Production of cucurbit propagation material through an internationally recognised **certification scheme** that includes elimination of *A. citrulli*, would be crucial to reduce the risk presented from this pest.

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(see also Prima phacie datasheet for *Acidovorax citrulli*)


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## Supporting documentation

Annex 1: Template questionnaire distributed to EFSA Plant Health Network

**Annex 1:** Template questionnaire distributed to EFSA Plant Health Network

Prima phacie

Request for Information to support EFSA project CFP/EFSA/PLH/2009/01

## *Acidovorax avenae* subsp. *citrulli*

**EU Member State:** Please indicate which EU Member State you are providing information from

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Belgium | | Greece | | Luxembourg | | Romania | |
| Bulgaria | | Spain | | Hungary | | Slovenia | |
| Czech Republic | | France | | Malta | | Slovakia | |
| Denmark | | Italy | | Netherlands | | Finland | |
| Germany | | Cyprus | | Austria | | Sweden | |
| Estonia | | Latvia | | Poland | | United Kingdom | |
| Ireland | | Lithuania | | Portugal | | | |

Please give the total number of regions/departments/provinces/federal states in your country: 1 Department on Plant Health
(For example: 12 provinces in The Netherlands, 9 federal states in Austria)

### Surveys

**a)** Do you perform surveys to confirm the pest status (presence or absence) of *Acidovorax avenae* subsp. *citrulli* ?
No

| Region(s) where surveys are performed | Type of places included in the survey [1] | Survey frequency [2] | Plant species and plant parts included in the survey [3] | Type of survey performed [4] |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |
| | | | | |

[1] Indicate the type of places where surveys are performed, by using the following codes: (N) nursery; (F) field crop.
[2] Indicate the frequency of surveys for each region, e.g. every year since 2001, 2 times between 2004 and 2010.
[3] Indicate the plant species and plant parts surveyed, *e.g.* watermelon (seeds, seedlings, fruits).
[4] Indicate the type of survey performed, by using the following codes: (V) visual inspections; (L) laboratory testing.

### Outbreaks

**b)** Has an outbreak of *Acidovorax avenae* subsp. *citrulli* ever occurred in your country?

YES ☐ NO ☐ NO DATA AVAILABLE ☐

If YES, please complete the table below:

| Region(s) / First year of detection [1] | Current distribution in the region(s) [2] | Type of places where outbreaks occurred [3] | Host plants/ plant parts[4] | Source of data [5] |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

[1] List all regions/departments/provinces/federal states, etc, where the pest was found to occur and the year of first detection.
[2] Indicate the current distribution of the pest in each region, using the following codes: (W) widespread; (L) localized; (O) occasionally present; (D) reported in the past, but no longer present; (E) eradicated; (U) under eradication.
[3] Indicate for each region if the pest occurred in nurseries or field crops, using the following codes: (N) nursery, (F) filed crop.
[4] Indicate the plant species and the plant parts found to be infected, *e.g.* watermelon/seedlings.
[5] Indicate the source of data, by using the following code: (S) survey results; (Gs) general surveillance, which includes data from local government agencies, research institutions, universities, scientific societies, producers, consultants, museums and the general public.

# **Annex 1** (contd.)

Prima phacie

Request for Information to support EFSA project CFP/EFSA/PLH/2009/01

**Wild hosts**

**c)** Does the weed Citron (*Citrullus lanatus* var. *citroides*) occur in your country?

YES ☐ NO ☐ NO DATA AVAILABLE ☐

If YES, which is its prevalence in the country?

WIDESPREAD ☐ LOCALIZED ☐ OCCASIONALLY PRESENT ☐ NO DATA AVAILABLE ☐

Has Citron been found in proximity to cultivated cucurbits?

YES ☐ NO ☐ NO DATA AVAILABLE ☐

**Imports**

**d)** Does your country import cucurbits (propagation plant material and plant products)?

YES ☐ NO ☐

If YES, please complete the following table:

| Type of plant material of cucurbits imported | Countries of origin | Number of interceptions [1] |
|---|---|---|
| Seeds | | |
| Seedlings | | |
| Fruits | | |
| other | | |

[1] Indicate the number of interceptions of imported material, due to *Acidovorax avenae* subsp. *citrulli* infection.

**Measures**

**e)** Are there any measures applied in your country to prevent introduction and spread of *Acidovorax avenae* subsp. *citrulli*? (*e.g.* phytosanitary checks, chemical treatment, cultural practices, etc)

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**Contact details**

**f)** Please provide your contact details should we need to contact you again.

| | |
|---|---|
| Name: | |
| e-mail: | |
| Phone: | (international code) , area code, number |

**Many thanks for completing this questionnaire.**

**Your contribution to Prima phacie is greatly appreciated.**

**Please return this completed form to alan.macleod@fera.gsi.gov.uk by July 5th 2010**