

To Technical and Administrative Services  
Plant Biosecurity  
Biosecurity Australia  
Canberra, ACT 2601

**Stakeholder comment on the Revised Draft Import Risk Analysis Report for Apples from New Zealand released December 2005.**

## **1. Introduction**

These comments refer to Part C of the report, Appendix 3 - Pest Data Sheets - Pathogens - Fire Blight.

second paragraph on p. 107 is as follows, with corrections to the text in parenthesis, bold lettering:

"In Japan. Goto (1992) described the disease affecting Asian pear (*Pyrus pyrifolia*) occurring in Hokkaido as 'bacterial shoot blight of pear (BSBP)'. The symptoms described by him were identical to those (of) fire blight and the causal agent (**was found to be**) nearly identical to *E. amylovora*, except for some specific but unidentified properties. The causal agent affecting pear was identified as *E. amylovora* (Tanii et al., 1976), but later (**reports showed that several isolates had a**) limited range of pathogenicity on certain Asian pear cultivars. A study on the characterization of bacterial isolates (Beer et al., 1996 showed that irrespective of the host tree specificity or bacterial strain involved, BSBP is identical to fire blight."

Beer et al. (1996) state that: "The existence of the fire blight pathogen in Japan and other countries of northeast Asia has been the subject of some controversy, conjecture and debate."

The purpose of this submission is to show that the paragraph quoted from the Report is out of date, does not reflect current thinking, and could be misleading. Work carried out in the past decade has shown that the bacterium causing BSBP in Japan is distinct from *E. amylovora*. This is no longer a subject of conjecture and controversy. The most recent paper on this subject states: ".....we conclude that the *Erwinia* pathogen of pear in Japan is closely related to *E. pyrifoliae* and that both of these pathogens are demonstrably distinct from *E. amylovora*." (Maxson-Stein et al., 2003).

## **2. Identity of the Causal Agent of Bacterial Shoot Blight of Pear in Japan**

### **(i) Reports from Japan**

Mizuno et al (2000) have compared the bacteriological properties and DNA-DNA homology values of the pathogen causing bacterial shoot blight of pear isolated in 1994-1996 with isolates of *Erwinia amylovora* obtained from outside Japan and other representatives of the *Amylovora* group. In most biochemical tests the BSBP bacterium was identical to *E. amylovora* except for distinct differences in hydrolysis of aesculin and acid production from salicin, etc. DNA homology among the strains of BSBP ranged from 85 to 103% and from 83 to 110% among strains of *E. amylovora*. In contrast, the values between BSBP strains and *E. amylovora* were 55 to 81%, while those between BSBP strains and other *Amylovora* group strains were 42% or less. In reaching the conclusion that the BSBP strains were sufficiently related to *E. amylovora* to be included in that species, the authors were influenced by the recommendation that members of a species should exhibit 70% or greater DNA homology in reciprocal DNA homology tests (Stackebrandt and Goebel, 1994). Some isolates of BSBP showed greater than 70% homology with *E. amylovora*, justifying the inclusion of BSBP in that species. The BSBP pathogen in Hokkaido was designated as a distinct biovar, biovar 4, distinct from *E. amylovora*

bv. 1,2 and 3 isolated in countries outside of Japan.

Mizuno et al (2002) made a detailed evaluation of the reports of fire blight in Japan and concluded that all were erroneous. Some reports were of apple canker (*Valsa canker*) caused by *Valsa ceratosperma*, and disease which was reported as fire blight of pear was either twig blight caused by *Diaporthe* sp., or bacterial shoot blight of pear.

## **(ii) Reports from outside Japan**

Momol et al. (1997) used a DNA-based method (random amplified polymorphic DNA or RAPD analysis) to assess the genetic diversity among 16 strains of *E. amylovora* representing different plant origins and geographical regions, including two isolates from BSBP in Hokkaido, Japan. Cluster analysis based on the number of RAPD fragments shared between strains showed that strains of *E. amylovora* isolated from subfamily Pomoideae formed a single group, whereas two isolates from *Rubus* (subfamily Rosoideae) formed a second group. Two strains isolated from Asian pear on Hokkaido, Japan, formed a third group.

These authors do not question the conclusions of Beer et al. (1996); however their work showed that the BSBP pathogen was distinct from strains of *E. amylovora* from North America.

A shoot necrosis of Asian pear (*Pyrus pyrifolia*) in South Korea was described by Rhim et al. (1999). The bacterium causing this shoot necrosis was shown to be a novel species in the genus *Erwinia* related to but distinct from *E. amylovora*. The pathogen was later fully described as a new species, *Erwinia pyrifoliae* (Kim et al. 1999). Sequencing of the 16S rDNA and the adjacent intergenic region of the Asian pear pathogen was done in comparison with other members of the Enterobacteriaceae. The 16S rDNA of the Asian pear pathogen was almost identical with the sequence of *E. amylovora*, in contrast to the 16S-23S rRNA intergenic transcribed spacer region of both species. Dendrograms derived from 121 biochemical characteristics placed the Asian pear pathogen close to *E. amylovora* and more distantly to other members of the genus *Erwinia*, *Pantoea* and *Enterobacter*. DNA hybridization data showed that the Asian pear strains constituted a tight DNA hybridization group (89-100%) distantly related to strains of *E. amylovora* with which there was 40-50% hybridization. The designation of a new species was therefore justified on the basis of a variety of DNA-based and biochemical properties.

The description of a new species, *Erwinia pyrifoliae*, for the bacterial pathogen of Asian pear in South Korea has stimulated interest in reexamining the identification of *Erwinia* strains that cause bacterial shoot blight of pear in Japan. The symptoms produced by both pathogens on pear are virtually identical to those of fire blight caused by *E. amylovora*, but their host range is more restricted than the host range for typical *E. amylovora* (Maxson-Stein et al., 2003). On inoculation to apple both BSBP and *E. pyrifoliae* produce a localised necrotic reaction unlike that typical of fire blight infection.

Kim et al (2001a,2001b) compared the BSBP agent with *E.pyrifoliae* and *E.amylovora*. Plasmid profiles, protein patterns and genomic DNA analysed by pulsed field gel electrophoresis (PFGE) after *Xba*1 and *Spe*1 digestion were different from *Erwinia amylovora*. The BSBP agent was not identical to *E. pyrifoliae* but had many features in common with the Asian pear blight pathogen, including in nucleotide sequence of a DNA fragment preceding the gene cluster for exopolysaccharide synthesis. In the latter the BSBP agent was again more similar to *E. pyrifoliae* than to *E. amylovora*. Kim et al (2001a) concluded ".....that pathogenic bacteria isolated in Japan from pear trees with symptoms resembling fire blight are possibly different from *Erwinia amylovora*."

*E. pyrifoliae* was shown to be more closely related to *E.amylovora* than to any other species of the genus *Erwinia* on the basis of the relatedness of chromosomal and plasmid DNAs including partial sequencing of the *groEL* gene. A phylogenetic dendrogram based on a comparison of partial *groEL* gene sequences, clearly shows that *E. pyrifoliae* and *E. amylovora* are distinct from

all other

species and at a higher level of similarity distinctly different from each other (McGhee et al. 2002). Extension of this work to include representative isolates of the BSBP agent from Japan confirmed that they were closely related to *E. pyrifoliae*. The authors concluded that "... a wider array of strains must be examined with techniques such as total DNA-DNA homology to establish whether the pathogen is *E. pyrifoliae* or a novel species." (Maxson-Stein et al. 2003).

### **(iii) Proposed Nomenclature for the Pathogen Causing Bacterial Shoot Blight of Pear in Hokkaido, Japan**

The common name Bacterial Shoot Blight of Pear (BSBP) has entered the scientific literature and has not been challenged.

The BSBP pathogen has not yet been formally described as a separate species or subspecies. In view of the close relationship between the BSBP bacterium and *Erwinia pyrifoliae*, and the clear difference between both pathogens and *E. amylovora*, a logical step would be to describe the BSBP pathogen as either a separate species or as a subspecies of *E. pyrifoliae*.

Since 1992 the BSBP pathogen has been identified as one of the following:

as a pathovar of *Erwinia amylovora* (*E. amylovora* pv. *pyri*) (Goto, 1992)

as *Erwinia amylovora* (Beer et al., 1996)

as a distinct biovar (biovar 4) of *Erwinia amylovora* (Mizuno et al. 2000)

None of these proposals is satisfactory in view of recent evidence establishing the separate identity of the BSBP pathogen (Maxson-Stein et al., 2003), and the closer similarity of this agent to *E. pyrifoliae* than to *E. amylovora*.

### **3. Conclusions**

1. There is no evidence that fire blight of apple and pear occurs in Japan.
2. Bacterial shoot blight of pear in Japan is a disease identical in symptoms on pear to fire blight but the causative organism is different and shows greater similarity to *E. pyrifoliae* than to any other *Erwinia* species.
3. The BSBP agent has not yet been formally described as a separate taxon. It may be a new species of *Erwinia* or a subspecies of *E. pyrifoliae*.
- 4..The Pest Data Sheet for Fire Blight should be amended to include work published since Beer et al. (1996).

### **4. References**

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