RESPONSE TO BIOSECURITY AUSTRALIA'S REVISED DRAFT IMPORT RISK ANALYSIS ON NEW ZEALAND APPLES – DECEMBER 2005. FIRE BLIGHT SECTION

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Introduction

The response presented here by the stakeholder is confined only to the fire blight section in the Revised Draft Import Risk Analysis on New Zealand Apples – December 2005 (RDIRA – 2005). In Parts B and C the Import Risk Analysis Team (IRAT) of Biosecurity Australia (BA) has presented a fairly well balanced review of the literature on the relevant aspects of the disease. However, it was quite apparent that there was an element of bias in the interpretation of some of the results presented in a few of the papers cited. In some cases IRAT has under-rated the significance of some of the findings in the papers while in other cases it has over-rated the likely effects stated in the papers. In the treatment of the subject of "Unrestricted Risk" it was very commendable to note that IRAT had covered almost every conceivable scenario from a plant pathological point of view. However, the stakeholder found it difficult to fully agree with many of the points made with respect to these scenarios.

The areas where IRAT has downplayed the significance and relevance of research findings reported in the literature to the assessment of unrestricted risk include: (a) The epiphytic phase of the life cycle of the fire blight pathogen *Erwinina amylovora* (*Ea*). (b) The occurrence of endophytic populations of *Ea* in fruit. (c) The evidence for the occurrence of viable but non-culturable (VBNC) *Ea*; when the bacteria are in the VBNC state an absence of the pathogen may be recorded in or on apple tissue tested although the pathogen is present. (d) the evidence that in, surface infestations, *Ea* consist of both attached and planktonic cells, and that it is almost impossible to harvest the attached bacteria; attachment is known to occur only after a certain period of time, usually exceeding 24 hours, following colonization of the host surface. This would result in concluding that natural populations of *Ea* on plant surfaces including in the calyx sinus are low or absent. The harvesting techniques used (washing method) only capture the planktonic bacteria as nearly 90% of the bacteria are known to be attached. (e) Certain areas of new science like biofilm formation, multicellular behaviour, sigma factor and quorum sensing, which are implicated in the attachment of bacteria to host surfaces referred to under (d). The end result of (a), (b), (c), (d) and (e) is that the likelihoods of the Importation Steps that assess the Unrestricted Risk are rated well below realistic values.

IRAT has over-rated the effects in the following two areas: (i) The effect of "areas free from disease symptoms" and (ii) The effect of "disinfection treatment". The end result of (i) and (ii) is that the Restricted Risk is assessed well below the values.

Stakeholder,s comments on the significance of epiphytic *Ea* (including calyx infestations), endophytic *Ea* in fruit, VBNC and the effectiveness of *Ea* harvesting techniques in the assessment of Unrestricted Risk

(a) Epiphytic *E. amylovora* on buds, stems, leaves and in fruit calyces:
In some of the earlier research done in the USA, when commercial trade in apples with other countries was not an issue, **the importance of epiphytic bacteria in the epidemiology of fire blight and, therefore, of the disease cycle, was consistently emphasized;** this is clearly illustrated in the monograph titled "FIRE BLIGHT, A Bacterial Disease of Rosaceous Plants" published by the USDA in 1979 (van der Zwet and Keil 1979). In fact, the monograph gives the epiphytic phase of *Ea* a central role in the life cycle of the pathogen. The occurrence of epiphytic *Ea* on buds, stems and leaves have been reported by several workers (Baldwin and Goodman, 1963; Keil and van der Zwet, 1969; Keil and van der Zwet, 1972; Dueck and Morand, 1975; Sholberg *et al*, 1988; McManus and Jones, 1995). However, the current trend is to consider only the exponential growth of *Ea* on the stigma surface of flowers as epiphytic; to some extent the IRAT too seems to have gone along with this trend. Yet, as recently as 2001, late Dr Paul Steiner, one of the most respected fire blight researchers from the USA, stated as follows "*Erwinia amylovora* is a competent epiphyte capable of colonizing and multiplying on the surface of plants and it makes little difference whether the plants colonized are susceptible or resistant to fire blight" (Steiner, 2001).

The occurrence of **epiphytic *Ea* in the calyx sinus of apple fruit,** however, received attention largely as a result of the work published by Hale's group in New Zealand (Hale *et al*, 1987; Hale and Clark, 1990; Hale *et al*, 1996; Clark *et al*, 1993) and later by several other workers including van der Zwet *et al*, 1990, and McManus and Jones 1995.

A careful study of the events involved in Importation Steps 2, 3 and 5 show the extreme significance of the occurrence of epiphytic *Ea* on stems, buds, leaves and on fruit surface; even more significant is the occurrence in the calyx sinus of the fruit. A very important point to bear in mind with respect to epiphytic populations is that conventional methods of harvesting bacteria from the surfaces of infested tissue, by washing, are unlikely to give the actual levels of infestation. As stated earlier this is because of the firm attachment of the bacteria to the host surface with natural infestations. In recent years the scientific understanding of the relationship between bacteria and plant surfaces has developed. The importance of attachment is a central development in the understanding of epiphytic survival (Sapers *et al* 2000; Barak *et al*, 2005; Yap *et al*,. 2005; Mandrel *et al.*, 2006). A population of epiphytic bacteria is likely to be composed of two forms of bacteria: bacteria that are attached, and non-attached or planktonic bacteria. Bacterial colonization is associated with bacterial attachment to surfaces. The older research reported in the literature have used the conventional methods of harvesting the bacteria and thus assessed only the planktonic bacterial populations. The effectiveness of harvesting techniques is discussed later in more detail.

(b) Endophytic *E. amylovora* in fruit. When *Ea* occurs in the internal tissues of a plant, including fruit, without causing any symptoms it is said to exist endophytically. Apples carrying endophytic infections cannot be distinguished externally from healthy fruit. However, they may begin to show symptoms of fruit blight several weeks after harvest under conditions favourable for disease development and may even act as potent sources of inoculum as it happens in Missouri (Goodman, 1954).

Several researchers have reported the occurrence of endophytic *E. amylovora* in apple fruit. While some of these endophytic infections have occurred naturally in the field others have occurred as a result of inoculation carried out in experiments. RDIRA-2005 has listed some of these papers (McLarty 1924, 1925 and 1926; van der Zwet *et al* 1990, Goodman 1954) but proceeds to conclude "…the lack of evidence of endophytic infection in mature fruit suggests that if endophytic infection does take place it must be a rare event." Likewise, RDIRA-2005 acknowledges the presence of *E. amylovora* in the vascular tissues in symptomless trees and cites several papers reporting such presence (Bogs *et al*, 1998; Vanneste and Eden-Green, 2000; Lewis and Goodman, 1965; Eden-Green and Billing, 1974), but does not acknowledge that the bacteria could move into the fruit. Very recently, Azegami *et al* (2006) experimentally demonstrated the systemic movement of fire blight bacteria from the stem into the fruit with mature apples attached to the tree. These authors inoculated fruit-bearing twigs of apple trees in the greenhouse with *E. amylovora* bacteria at *ca.* 10^5 cfu tagged with bioluminescence genes. The pathogen was recovered from 10.8% of the apples. These results show that bacteria can pass through the abscission layer into the fruit, even though the mature fruit may lack symptoms. The stakeholder believes that the systemic movement of *Ea* may occur with younger fruit with greater ease than with fully mature fruit. Thus, endophytic fruit infections are directly relevant to Imortation Steps 2, 4 and 6.

(c) VBNC state in *E. amylovora*: VBNC becomes problematic when isolation on culture media for the purpose of detection is attempted from bacterial infestations where the numbers are either low or where the bacteria are under some kind stress. *Ea* infestations in the calyces of apple fruit is a good example in this regard. The numbers of bacteria in the calyces may not only be relatively low but also they would be under nutrient stress. In addition to that if copper based chemicals have been applied a few days before flowering for the control of black spot (*Venturia inaequalis*) then that too would induce the bacteria to go into the VBNC state. The effect will be even more pronounced if conventional methods of washing the tissue are used to harvest the bacteria from the calyx. **Consequently, it will not be possible to grow the bacterium in culture and the conclusion will be that calyx is free of *Ea*.** With infections, where disease symptoms are apparent, bacterial numbers in tissues affected are relatively high and are thus easily isolated on culture media. The occurrence of the VBNC state with virulent *Ea* induced by low nutrient conditions and by copper ions has been demonstrated in extensive studies by Lopez and her co-workers (fire blight researchers) in Spain. Thus, Biosca *et al* (2004; 2006) have demonstrated that virulent *E .amylovora* cells in irrigation water, drinking water and deionized water enter the VBNC state under nutrient starvation. Biosca *et al* (2006) conclude that the existence of such viable but non-culturable (VBNC) cells of *E. amylovora* could lead to an understimation of the pathogen population from environmental sources when using only cultural methods. Ordax *et al* (2004; 2006a; 2006b) investigated the ability of *E. amylovora* cells to enter into the VBNC state in the presence of free-copper ions; this was followed by a study of the pathogenicity of the VBNC cells. Also, they studied the possible reversion or resuscitation from the non-culturable state, and whether the resuscitated cells retained their virulence. Copper compounds are commonly used in the control of many bacterial diseases of plants, and, along with antibiotics, they are used in the control of fire blight. In their study Ordax *et* al (2006a; 2006b) performed counts of the number of (a) total cells,

(b) viable cells and (c) culturable cells throughout a period of 4 months. They found that *E. amylovora* enters into the VBNC state induced by copper. Total and viable cell counts remained relatively constant at the initial levels (10^8-10^9 cells/ml) in all the cases, independently of the copper concentration assayed. However, the culturability of the bacterium decreased in different ways depending on the copper concentration. In the presence of copper ions, the culturability of *E. amylovora* went down quickly below the detection limit (<1 cfu/ml) and cells became nonculturable in spite of the high numbers of viable cells. Most of the bacterial population (87.5-94.4%) enter into VBNC state in the presence of the three copper concentrations assayed, with the time of entry much faster as concentration of this metal was increasing (days 36, 1 and 0 for 0.005, 0.01 and 0.05 mM Cu^{2+}, respectively). **Ordax *et* al (2006a; 2006b) found that the removal of copper ions with copper complexing agents was effective in all cases of restoring the culturability of copper-induced VBNC cells**, but their ability to recover such cells varied depending on the time after the entry of *E. amylovora* into the VBNC state. With further experiments it was shown that culturability achieved was a true resuscitation and not a regrowth.

Thus, results obtained by Biosca *et al* (2004; 2006) and Ordax *et al* (2005; 2006) clearly indicate the possibility of *E. amylovora* infestations in the apple calyces not being detected by plating cells either directly on solid media, which is the method commonly used in all bacteriological laboratories, or on nylon membranes placed on solid culture media for detection by DNA hybridization (Clark *et al*, 1993; Hale *et al*, 1996). On the basis of their findings Ordax *et al* (2005; 2006a; 2006b) conclude "……**the occurrence of phytopathogenic bacterial cells in the VBNC state could have serious implications in plant pathology, since epidemiological studies are usually based on plate counts of culturable cells (Wilson and Lindow, 2000)" (Ordax *et al* (2005; 2006a; 2006b)**.

(d) Effectiveness of *E. amylovora* harvesting (recovery) methods: The implications of not using effective harvesting or recovery techniques were briefly mentioned in the preceding paragraphs. Ineffective harvesting has implications for detection. Inability to detect does not mean there is no infestation; the reliability of a detection result will be qualified by the accuracy and/or relevance of the harvesting technique. The technique commonly used for harvesting epiphytic bacteria is the one used by Crosse in the late1950s and in the 1960s (Crosse *et a,l* 1960; Crosse *et a,l* 1972). Most of the publications cited in the RDIRA-2005 document use a form of the Crosse isolation/harvesting method. This technique or modifications of it are capable of harvesting planktonic (unattached) bacteria but are unlikely to harvest the attached bacteria. Bacterial attachment prevents the removal of bacteria by washing. In experiments designed to assess epiphytic *Ea* on leaves Thompson and Gouk (1999) compared the indirect method of Crosse with the direct method of implanting the leaves on agar. They found the direct method was 4 times more sensitive than the indirect method. Similar results have been obtained by other workers too. Working with *Escherichia coli* on apples Sapers (2000) found that 90% of the bacteria were recoverable 30 minutes after inoculation, almost none after 24 hours, indicating that it takes some time for the bacteria to firmly attach to the inoculated surface.

(e) Biofilms/Aggregates, Multicellular behaviour, Sigma Factor and Quorum Sensing: These are areas of new science that are now being increasingly examined in order to understand certain events in the infection process which have hitherto baffled

plant pathologists. With respect to fire blight, scientists still cannot explain why epidemics suddenly flare up in orchards when similar conditions, suspected as likely, have existed in previous seasons but without causing any significant disease. This was the reason why van der Zwet *et al* (1988) commented as follows: "Fire blight is one of the most erratic and unpredictable diseases of pear and apple. Our perplexity is due mainly to our lack of fundamental knowledge of the bacterium and its mode of infection, especially just before and during bloom". Prior to that Schroth *et al* (1974) stated "Fire blight continues to be one of the most intensively studied bacterial diseases of plants. …. In spite of this effort, the disease is still not satisfactorily controlled; it continues to spread throughout continental Europe and remains a major concern in most countries where pome fruits are grown". Commenting on this statement Johnson and Stockwell (1998) stated " Twenty four-years later, this summation by Schroth *etal* (1974) of the status of fire blight is unchanged" (Johnson and Stockwell, 1998).

There is increasing evidence that *Ea* engages in **multicellular behaviour.** Bacterial multicellular behaviour begins when free living planktonic bacteria engage in **quorum sensing.** An outcome of multicellular behaviour in planktonic bacteria attachment to surfaces is often facilitated by flagella. Following attachment surface colonisation in the form of aggregation develops, facilitated by pilli and other adhesions leading to biofilm formation (Stoodley et al, 2002). More complex adhesive structures called pellicle may follow (Yap *et al.,* 2005).

Most recent publications confirm the reported ability of *Erwinias* to form biofilms; in particular *E. chrysanthemi* (Barak 2004) and *E. carotovora* (Marques *et al,* 2004). Although formation of biofilms by *Ea* has not been demonstrated as yet, it has a number of characteristics that are known to be required for the formation of biofilms.

Risk mitigation measure (i): Areas free of disease symptoms: The stakeholder believes that the effect of this risk mitigation measure has been highly over rated by the IRAT. The reasons for this are as follows:

- It is not practically possible to see all the symptoms in an orchard/block, especially the presence of small cankers, during inspections. Therefore, the assumption that an orchard/block is free of symptoms is strictly not correct.
- There is evidence in the literature where both immature and mature apples collected from disease free orchards have been found to carry calyx infestations. This is despite the possible occurrence of the VBNC state and the inability of conventional harvesting methods to capture attached bacteria having not been considered with these detections.
- There is evidence in the literature of the occurrence of endophytic fruit infections from orchards with symptoms; as it is not possible to see all the symptoms during inspections an orchard having disease symptoms may be assumed to be free of symptoms. Such orchards may carry fruit with endophytic infections.
- There is evidence in the literature of fruit collected from orchards free of symptoms having *Ea* infestations in the calyx when these orchards have been in close proximity to trees with symptoms.

- Some of the export orchards/blocks (apparently free of symptoms) may have trees with systemic *Ea* infections carried over from previous years. These bacteria could move into the fruit. There is experimental evidence in the literature that this could occur.

Risk mitigation measure (ii): Disinfection treatment:

The stakeholder agrees with most of the points discussed in the RDIRA – 2005, with respect to chlorine treatment. However, it needs to be realised that chlorine will have no effect on *Ea* in the calyx, those deep down in the stem cavity and on endophytic infections. Although it will kill some bacteria on the surface (planktonic bacteria) it will not kill all the bacteria; those not killed will be the bacteria firmly attached to the surface.

Stakeholder's other concerns with respect to the RDIRA – 2005

- **Trash:** Although IRAT has identified the importation of trash as a potential pathway for the introduction of *E. amylovora* into the country it seems to be somewhat confident that thorough offshore inspections can prevent the trash from coming in with fruit consignments. It is widely accepted by fruit packers that avoiding trash in large consignments is an enormous task that is almost impossible to achieve. IRAT further states that trash from orchards inspected and found to be free of symptoms will not have contaminations over and above that of fruit. This is incorrect on two counts. Firstly, twigs with buds are known to carry relatively high levels of epiphytic bacteria (van der Zwet and Keil 1979; McManus and Jones 1995); furthermore, these could have very small but active cankers on them (Brooks, 1926; Ritchie and Klos, 1975). Secondly, the trash may not necessarily come from the export orchards; it could come other orchards and may be found in picker's bags, bins, in the packing house etc.
- **Resistance to streptomycin:** Quite apart from the risk of introducing fire blight into Australia there is another risk that is as important as introducing fire blight. This is the likelihood of importation of strains of *Ea,* with infested/infected apples, that are resistant to streptomycin. Streptomycin resistance in these bacteria is becoming more and more widespread in countries having fire blight where this antibiotic is routinely used for control. Streptomycin is widely used in New Zealand in the management of fire blight and resistance to this antibiotic has been found in that country. There are two types of resistance to streptomycin *viz*. chromosomal based resistance and plasmid based resistance. The resistant type occurring in New Zealand on *Ea* is reported to be of the chromosomal type which is generally transferred during cell division and does not cross the species barrier (Vanneste and Voyle, 2001). In the latter paper these authors report that *Ea* bacteria carrying the chromosomal type are resistant to **very high levels of streptomycin, exceeding 1000 μg/ml.** Thus**,** if fire blight is introduced into Australia with New Zealand apples, the control of the disease, let alone its eradication would become extremely difficult if the chromosomal type streptomycin resistant

strains of the bacterium flourish here. **Currently streptomycin is the only effective plant safe pesticide available for the control of fire blight**.

- **Movement of *Ea* around the world:** According to the latest statistics on the worldwide distribution of fire blight, the disease is now known to occur in 47 countries, with the first introduction being to Canada in 1840, and last introduction being to Liechtenstein in 2004; apart from the USA, which is regarded as the centre of origin of the disease, the exact means by which the disease has been introduced and subsequently established is known with certainty for only one country, namely Egypt (Dr T. van der Zwet 2005, personal communications). The speculative means of introduction range from means like air currents in 29 countries (65%), nursery stock in 9 countries (20%), migratory birds in 6 countries (13%) and contaminated fruit boxes in one country. From the above it is apparent that the means by which fire blight has been introduced to over 95% of the countries where it is currently known to occur is based on **pure speculation**. Apple and pear fruit may have been exported to numerous pome fruit growing countries from the time the disease was first reported in the USA in 1983. **However, it is a mystery as to why among these suspected means of introductions fruit has not been to date implicated, especially when it is known that fruit could be both infected (without exhibiting external symptoms) and infested (calyx).**

Assessment of Unrestricted and Restricted Risks

Although IRAT has done the assessments quantitatively the stakeholder prefers to do it qualitatively using basic principles of plant pathology for this purpose. However, the stakeholder will refer to Table 12 in the RDIRA – 2005 document for comparison with assessments done by the IRAT. In stakeholder's opinion quantitative assessments are well suited to "exact sciences" that could be defined and described using mathematical concepts and formulae. However, as plant pathology, based on biological sciences, is not strictly an "exact science" the stakeholder does not believe that conclusions based on quantitative assessments are as accurate as they are often claimed. As an example, if the are **3 successive biological events, each with a likelihood of "Moderate"** leading to the establishment of disease, qualitatively (plant pathological point of view) the outcome would still be **"Moderate".** However, quantitatively it may be rated as Moderate (0.5) x Moderate (0.5) x Moderate (0.5) = **"Very Low"** (0.125), which is not realistic from a plant pathological point of view.

Unrestricted Risk:

Imp 1: The stakeholder agrees with the assessment of the IRAT on this Imp as **"Certain"**.

However, the stakeholder has some concerns about the effect that a couple of recommended orchard management practices in New Zealand (listed in the RDIRA on page 50) would have on the assessments. If these are carried out routinely in source orchards they would lead to erroneous results on Restricted Risk. The practices in question are: (1) pruning out infected shoots; this would lead to wrong conclusions by inspectors in regard to the disease status of the orchard. If the inspectors are not advised about pruning out of diseased material from the orchard concerned they are likely to consider the orchard as disease free. (2) frequent

inspections of the orchard (*and pruning and burning infected material*). If these are done under assessment of unrestricted risk then it should not be considered again under assessment of restricted risk; it amounts to double counting.
Imp 2: In the light of what has been stated above under epiphytic *Ea* (a), endophytic *Ea* (b), VBNC (c), effectiveness of harvesting techniques (d) and areas of new science (e) the stakeholder considers the assessment of this Imp by the IRAT as 0.03 (Very Low) is far too low. The stakeholder would assess this Imp as **"Moderate"**.

Imp 3: The IRAT has assigned a most likely value 0.02. Considering (a) the relatively high levels of epiphytic *Ea* present in orchards (unrestricted risk category), (b) the likelihood of infested/infected trash being accidentally picked up by pickers, and (c) infested/infected trash already present their bags and in bins which are all likely to contaminate apparently clean fruit the stakeholder would assess the likelihood for this Imp as **"Moderate"**; assessment by the IRAT as Very Low (0.02) is considered too low.

Imp 4: Considering the fact that there are no effective measures in the packing house that would reduce the levels of *Ea* already on the apples, and the possibility of further contamination occurring in the dump tank (5-50 ppm chlorine is grossly inadequate especially if there is no monitoring of chlorine levels and replenishment) the stakeholder would assess Imp 4 as **High.** The assessment by the IRAT of 0.65 (most likely value), which is just below High, is considered somewhat low. However, the stakeholder agrees with the material presented in the RDIRA – 2005 document to support the IRAT's assessment.

Imp 5: The IRAT has assigned a value of 0.025 for this Imp. The procedures in the packing house for this Imp are no different from the procedures and conditions that applied to Imp 4. The value assigned by the IRAT (0.025 = Very Low) is far too low and the stakeholder suspects that the basis for this very low value given by the IRAT would have been the perception that the water in the dump tank would kill some of the bacteria getting into the tank**. It is true that some species of bacteria would not survive in <u>distilled water</u> for too long**. However, the dump tank contains dirty water with an enormous amount of organic matter dumped into the tank along with the apples. This will enable the *Ea* to multiply and progressively increase the inoculum levels in the dump water. Furthermore, as stated above under Imp 4, the levels of chlorine in the tank are far too low to effect any elimination of bacteria. The stakeholder would assess Imp 5 as **"High"**.

Imp 6: The stakeholder agrees with the **"High"** assessment given by the IRAT

Imp 7: The stakeholder agrees with the **"Negligible"** assessment given by the IRAT

Imp 8: The stakeholder agrees with the **"Certain"** assessment given by the IRAT

Conclusion – Probability of Importation:

On the basis of qualitative likelihoods assigned to the above 8 importation steps the probability of importation was assessed (qualitatively) as **"Moderate".** This was despite the fact that 3 of the likelihoods were "High", one was "Certain", 2 were "Moderate" and one "Negligible"; thus, it is a conservative assessment.

Probability of entry, establishment and spread

The stakeholder agrees with the IRAT on most of the material presented under probability of entry, establishment and spread. Specifically, the stakeholder disagrees with the discussions on "transfer mechanism" and "potential movement of pest with commodities or conveyances". From a qualitative point of view certain probabilities needed to be increased over and above that allocated by the IRAT. **Consequently, the Unrestricted probability of entry, establishment and spread was (qualitatively) worked out as "Moderate".**

Assessment of Consequences

The stakeholder agrees with the impact scores given in the RDIRA – 2005, for most of the criteria except for that given for "Control and eradication"; the costs given in the RDIRA – 2005 for the control of fire blight in the USA are too low. The reason for this may be because the figures have been based on costs worked out in 1997 by Oliver *et al* (1997). The stakeholder obtained costs for control of fire blight on pears in the Sacramento Valley, California from Dr Broc G. Zoller, Pear Doctor Inc, Kelseyville, California in October 2005. On the basis of those figures the cost of control in Australia would work out to Australian $ 2377 per hectare per season; this would include the costs of chemicals, application of chemicals and the cost of removing cankers and pruning out strikes. This is almost double the value ($1275) given in the RDIRA – 2005. The average size of an apple or pear orchard in Victoria is about 15.4 ha while the average size for the whole of Australia is around15.5 ha. On this basis the cost of fire blight control on an average size orchard in Australia would be approximately $36,843 per year.

Conclusion – Consequences

The stakeholder agrees with IRAT on all the impact scores assigned except for the one on Control or Eradication. Stakeholder believes that in the event of a fire blight breakout the industry and governments will spend enormous amounts of money in trying to eradicate. However, eradication will be impossible to achieve as by the time the disease is detected, based on symptoms, it would be too late to eradicate. Also, the cost of control would be nearly double the amount stated in the RDIRA – 2005. Therefore, the stakeholder would assign a score of "F". Hence, the overall Consequence is assessed as **"Certain"**.

Unrestricted risk

Stakeholder assessed the Probability of importation as "Moderate", Probability of entry, establishment and spread (PEES) as"Moderate", and the Consequences of entry, establishment and spread as "Certain". Thus, taking purely a qualitative approach and using the Risk estimation matrix.table in the RDIRA – 2005 document (Table. 11) the **Unrestricted annual risk** for *E. amylovora* was estimated as **"High".**

Risk management for fire blight

Areas free from disease symptoms as a Risk Mitigatiom Measure

RDIRA – 2005 states that areas free from disease symptoms could be established and maintained following the guidelines described in ISPM 4 and ISPM 22. However, there are several logistical problems that MAFNZ is likely to encounter in trying to apply ISPM 22 to establish areas free from fire blight (ALPP). The principal problem centres around the question of whether low pest (*Ea*) prevalance is going to be considered by MAFNZ as the same as low disease prevalence. The latter position is not strictly correct. The only alternative then is to have ALPP determined based on actual *Ea* levels. If the latter is the one to be adopted then the follow-up question will be what level of *Ea* is going to be the cut-off point.

As mentioned before implementation of some of the measures recommended in the Integrated Fruit Production Program Manual (Fact Sheet 7) will interfere with the work that orchard inspectors would be doing in export orchards. Also, a single inspection of the orchard/block is not considered adequate.

On the basis of above and on the basis of what was stated earlier under "Areas free from disease symptoms" the risk estimate with this risk mitigation measure was worked out as "Moderate".

Stakeholder's Unrestricted likelihoods for Imps 2, 3, 5 PEES and Consequences in Unrestricted risk category were:
Moderate, Moderate, High, Moderate and Certain respectively

The application of this risk mitigation measure (as spelled out in the RDIRA – 2005) would lower the likelihoods of all 3 Imps to the next category below. Thus, following application of this risk mitigation measure the Restricted likelihoods will be as follows:
Low, Low, Moderate, Moderate and Certain respectively for Imps 2, 3, 5 PEES and Consequences.

As a result of above the Restricted risk estimate using "areas free from disease symptoms" as a risk mitigation measure would be **"Moderate**".

Disinfection treatment as a Risk Mitigation Measure

In the light of what has been stated earlier under chlorine treatment, and also because of the fact that about 37% of the packing houses already use chlorine (unrestricted risk, even though at rates and conditions that are below optimal, some allowance has been made for this in assessing chlorine as a risk mitigation measure.

Under Unrestricted Risk Imps 3, 4, 5, PEES and Consequences were rated as :
Moderate, High, High, Moderate and Certain respectively.

Thus, the Unrestricted Risk estimate would be "High".

Under Restricted Risk, with chlorine being used and **maintained at 100 ppm right through ,under optimal temperature and pH,** the stakeholder's assessment of the likelihoods would be: Moderate, Moderate, Moderate, Moderate and Certain for. Imps 3, 4, 5, PEES and Consequences respectively.

As a result of above the Restricted risk estimate using chlorine as a risk mitigation measure would be **"Moderate**".

Storage as a Risk Mitigation Measure

The stakeholder agrees with the IRAT that a conservative view should be taken with regard to cold storage. It is an established norm in basic bacteriology that low temperatures in the range of 0-4^{0}C do not kill bacteria (Salle, 1967). These temperatures affect certain physical properties within the bacterial cell, which in turn decrease the rate of metabolic reactions leading to increased longevity of the cells. Rapid cooling of apples, carrying surface and calyx infestations.would cause internalization of fire blight bacteria that would result in having the opposite of the desired effect (Seeman, 2002; Seeman *et a,l* 2002). As stated in the RDIRA document a decline in bacterial numbers is likely but a two-fold reduction would be too optimistic. Several researchers have found that *Ea* on mature apple and pear to be unaffected following cold storage (Anderson, 1952; Dueck, 1974; Nachtigall, 1985). Other workers, studying the survival of *Ea* on pears in cold storage were able to reisolate the bacteria from calyces of pears even after 101 days of cold storage (Ceroni *et al*, 2004).

RDIRA states that the analysis of the effect of storage was based on application of the storage measure at the pre-export and transport step (Imp 6). However, the stakeholder maintains that the effect of cold storage would be almost zero regardless of the stage in the export chain at which the cold storage measure is applied. Any small reductions in numbers observed following cold storage would be **predominantly** due to normal declines that may occur with time with storage at **room temperature**; even this reduction would be arrested or impeded if the apples are stored at temperatures of 0-4^0 C.

Under Unrestricted Risk the likelihoods for Imp 6, PEES and Consequences were: High, Moderate and Certain respectively.

Under Restricted Risk the likelihoods for Imp 6, PEES and Consequences would be: Moderate, Moderate and Certain respectively.

Thus, the Risk estimate with Storage as a risk mitigation measure would be **"Moderate".**

Systems Approach

Areas free from disease symptoms and Chlorine treatment

Under Unrestricted Risk the likelihoods for Imps 2, 3, 4, 5, PEES and Consequences were: Moderate, Moderate, High, High, Moderate and Certain respectively.

Stakeholder's comments made earlier with respect to Areas free from disease symptoms and Chlorine treatment separately apply here too.

Under Restricted Risk the likelihoods for Imps 2, 3 4, 5, PEES and Consequences would be: Low, Low,Moderate, Moderate, Moderate and Certain respectively.

Thus, the Risk estimate with the Systems combination of Areas free from disease symptoms and Chlorine treatment would be **"Moderate".**

Areas free from disease symptoms and Storage

Stakeholder's comments made earlier with respect to Areas free from disease symptoms and Storage separately apply here too.

Stakeholder's Unrestricted likelihoods for Imps 2, 3, 5, 6, PEES and Consequences in Unrestricted risk category were:
Moderate, Moderate, High, High, Moderate and Certain respectively

The Restricted likelihoods will be as follows:
Low, Low, Moderate, Moderate, Moderate and Certain respectively for Imps 2, 3, 5, 6, PEES and Consequences.

Thus, the Risk estimate with the Systems combination of Areas free from disease symptoms and Storage would be **"Moderate".**

Areas free from disease symptoms, Chlorine treatment and Storage

Stakeholder's comments made earlier with respect to Areas free from disease symptoms, Chlorine treatment and Storage separately apply here too.

Stakeholder's Unrestricted likelihoods for Imps 2, 3, 4, 5, 6, PEES and Consequences in Unrestricted risk category were:
Moderate, Moderate, High, High, High, Moderate and Certain respectively

The Restricted likelihoods will be as follows:
Low, Low, Moderate, Low, Moderate, Moderate and Certain respectively for Imps 2, 3, 4, 5, 6, PEES and Consequences.

Thus, the Risk estimate with the Systems approach combination of Areas free from disease symptoms, Chlorine treatment and Storage would be between "Low" and "Moderate". The latter will be equivalent to a probability of 0.25 according to Table 12 of the RDIRA – 2005 document.

The Unrestricted Annual Risk was assessed by the stakeholder as "High". This is equivalent to 0.8 according to Table 12 of the RDIRA document. **Therefore, the reduction had been only 3.2 fold.**

Summary

In assessing the unrestricted risk associated with the importation of apples from countries having fire blight BA has to consider epiphytic and endophytic *Ea* bacteria found on or in apparently healthy fruit. The epiphytic bacteria could be found (a) on the surface of the fruit, (b) in the calyx sinus, and (c) in the stem end cavity.of the fruit and on the fruit stem (pedicel) itself.. Endophytic bacteria would be found in the core and the pulp tissue of the fruit. According to the literature there is evidence for the occurrence of these epiphytic and endophytic bacteria in fruit. These have been.determined using conventional methods of harvesting (epiphytic) bacteria and, conventional methods of plating which do not capture those bacteria that enter the viable but non-culturable (VBNC) state under conditions of stress. There is evidence in the more recent literature that demonstrates conventional harvesting methods based on washing, which are indirect methods, detect only around 25% of the actual epiphytic populations. The detectable bacteria comprise only the planktonic fraction. Similarly the occurrence of VBNC with *Ea* may indicate the total absence of the pathogen in or on fruit when it is actually present but surviving under stress; copper ions and nutrient stress are known to precipitate the VBNC state. Copper residues from routine copper based fungicides used on apples for control of black spot (*V. inaequalis)*, and nutrient stress that may occur in the calyx sinus are likely to induce the VBNC state in *Ea*. Thus, indirect harvesting techniques and VBNC would lead to underestimation of *Ea* numbers in or on apple tissue.

At present there are no known methods that could eliminate the epiphytic bacteria in the calyx or the endophytic bacteria within the fruit. As such, the "Moderate" unrestricted risk that IRAT has come up with cannot be lowered to Australia's ALOP. Lowering unrestricted risk from "Moderate" to "Very Low"involves a 19 fold reduction at the midpoint level, 300 fold reduction at the lowpoint level and a 70 fold reduction at the highpoint level. The very best that could be achieved with the risk mitigation measures proposed (systems approach) in the RDIRA – 2005 would only be about 4 fold. It may be possible to increase the latter to around 5 fold but that will entail an increased number of orchard inspections, use of a higher chlorine concentration and storage over a longer period of time. However, even this would not lower the risk down to less than "Low".

Conclusions

Because of the endemic nature of fire blight in New Zealand there are no orchards that are free of infections though they may be free of **apparent symptoms;** symptoms like small active cankers, 3-5 mm in diameter, high up on trees would be hardly visible from ground level. Thus, these orchards may carry fruit with infestations/infections at various sites. It was evident from discussions in the preceding sections that *Ea* bacteria in calyx infestations/infections and endophytic infections cannot be eliminated with any of the presently known risk mitigation measures; also difficult to remove are bacteria deep in the stem end cavity and bacteria firmly attached to the fruit surface (non-planktonic). The reason for the difficulty in removing bacteria in the calyx and deep in the stem-end is because of inaccessibility of these sites due to the formation of air pockets when the fruit in wash tanks. Endophytic bacteria in the fruit are embedded within tissues and are, therefore,

inaccessible. Primarily, this is the very reason why it is so difficult to reduce the risk level of the imported fruit more than 3 to 4 fold with the 3 risk mitigation measures proposed in the RDIRA document. In the exercise above done by the stakeholder the reduction in risk from "High" to a level between "Low" and "Moderate" was only 3.2 fold. Theoretically, the reduction from "High" to "Low" is 4.6 fold; from "Moderate" to "Low" is 2.9 fold, from "Moderate" to "Very Low" (which is IRAT's conclusion) is 19.2 fold. The best that could be expected with any combination of risk mitigation measures (**including several measures not mentioned in the RDIRA document),** using a Systems Approach, would be 5 fold reduction. This would reduce the risk level from "Moderate" to a level that is at least between "Very Low" and "Low". **The only other alternative which the stakeholder ventures to suggest needs to be done only in consultation with the industry; <u>this would be to increase Australia's ALOP to "Low" with respect ONLY to apple imports</u>.**

The protocol proposed below by the stakeholder may approach a nearly 5 fold reduction, **but still is unlikely to bring it down to "Very Low" which is Australia's ALOP.**

Protocol proposed by the Stakeholder

1. Designated export orchards/blocks must have been free of fire blight symptoms in the immediately preceding 3 years.
2. No fire blight symptom bearing hosts should be in the vicinity (within 250-500 meters) of designated export orchards/blocks.
3. Pruning or otherwise removal of any fire blight symptoms from designated orchards/blocks should not be permitted.
4. Designated export orchards/blocks must be inspected at times mentioned below:

    (i) First inspection is to be carried out at bud break. The purpose of this inspection is to exclude from the export program those orchards having any obvious overwintering cankers on the trees.

    (ii) Second inspection is to be carried out at full flowering. The purpose of this inspection is to exclude those orchards with any primary blossom blight symptoms and also any overwintering cankers that may have escaped attention in the first inspection.

    (iii) Third inspection is to be carried out just before harvest. The purpose of this inspection is to exclude those orchards with any secondary blossom blight symptoms, shoot blight symptoms on suckers or water shoots, and any cankers that may have escaped attention during the first and second inspections.

    (iv) An extra inspection may be necessary if hailstorm damage is experienced after the third inspection.

5 Coupled with orchard inspections statistically representative samples of mature fruit at the time of harvest should be tested for *Ea* using a highly sensitive technique to ensure, at least to some degree, that the

orchard is free from detectable infection. Appropriate tests are described in detail in the EPPO publication titled “EPPO Standards, PM 7/20” (2004).

6 The chlorine level of the packinghouse dump tank should be at least 200 ppm. This level must not be allowed to fall by constant monitoring. The pH and temperature should be maintained at optimal levels at all times. The exposure time must be at least one minute.

7 All packinghouse equipment, bins bags etc must be steam cleaned on a regular basis.

8 Every effort and care must be taken during packing to prevent any kind of trash getting into the boxes or bins to be exported.

9 Storage (not necessarily cold storage) prior to export should be for the maximum period practicable exceeding 6 weeks.

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