Submission response to Biosecurity Australia's Revised Draft Import Risk Assessment for the Importation of Decrowned Pineapple (*Ananas comusus* (L.) Merr.) fruit from Malaysia

December 2011

Growcom
68 Anderson Street Fortitude Valley
PO Box 202 Fortitude Valley QLD 4006
Tel: 07 3620 3844 | Fax: 07 3620 3880

Growcom's Response to Biosecurity Australia's Revised Draft Import Risk Assessment for the Importation of Fresh Decrowned Pineapple (*Ananas comusus* (L.) Merr.) fruit from Malaysia.

Introduction

Growcom is the peak industry body for the pineapple industry and as such is providing the industry response to the Draft Import Risk Assessment for Fresh Decrowned pineapples from Malaysia (hereafter referred to as the Draft IRA). The information presented in this response has been gathered from detailed consultations with pineapple growers, pineapple agronomists and scientific experts in pineapple genetics and plant pathology.

Biosecurity Australia released the Draft IRA on the 21st of October 2011and comments were requested by the 19th December 2011 to comply with the 60 day comment period.

Growcom notes that the Draft IRA proposes that the importation of fresh decrowned pineapple fruit from all areas of Malaysia be permitted, subject to a range of quarantine conditions. Growcom examined the risk in relation to the major pineapple pests found in Malaysia. Our primary concern relates to the likelihood and consequences of an incursion of Bacterial fruit and heart rot (caused by a species of *Dickeya,* formerly *Erwinia chrysanthemi).* The Draft IRA does not adequately deal with the risk presented by this pest and the huge range of unresolved scientific issues relating to its likelihood of spread. We also note that there are no quarantine measures that can be put in place to deal with this pest. We contend that until there is a greater level of certainty around the likelihood of this pest establishing the risk assessment should be reviewed and Malaysian pineapple imports should be restricted. The potential impact on the pineapple industry should this pest become established are too great to risk.

Growcom notes that quarantine conditions apply to *Dysmicoccus grassii, Dysmicoccus neobrevipes, Planococcus minor* and *Pseudococcus jackbeardsleyi.* Industry is satisfied with the proposed quarantine arrangements put forward for these pests. Industry is however, concerned about the use of the fungicide benomyl put forward as part of the quarantine arrangements (Draft IRA, pg 20) which is not registered in Australia due to human health concerns.

General information on the Australian pineapple industry

According to the Pineapple Industry Strategic Plan 2011, Australian pineapples are grown year round, with approximately 44,000 tonne of fresh fruit and 41,000 tonne of processed fruit marketed in 2009-10. It is currently estimated that there are 114 production businesses in Queensland. Key production districts include North Queensland, Yeppoon and Cawarral, Bundaberg, Maryborough and Hervey Bay, Mary Valley and Nambour, Glasshouse Mountains and Beerwah, Wamuran, and Elimbah.

Australia supplies 0.5 per cent of fresh pineapple world production and a negligible per cent of fresh pineapple world exports. More than 70 per cent of fresh pineapples are packed and marketed through four primary packhouses. There is one primary pineapple processor; Golden Circle, recently acquired by Heinz Australia. However there are at least two small scale processing operations dealing with fresh cuts. The industry is becoming an increasingly fresh-market based industry, due to new fresh-market varieties and competition with imported processed fruit.

Approximately 60 per cent of pineapple plantings are Smooth Cayenne and Queen (Rough leaf) varieties, and 40 per cent of plantings are of hybrid varieties. Increased plantings of hybrid varieties, which have higher sugar to acid ratios and greater consistency, are expected (Pineapple Industry Strategic Plan 2011). Current hybrid varieties include 73-50, MD2 (73-114), which are marketed under various proprietary brand names, and Aus-Jubilee and Aus-Carnival.

The gross value of production of Australian pineapples at the farm gate is currently estimated at $79 million ($13m processed, $66m fresh).

Bacterial fruit collapse of pineapple

The pineapple industry's major concern relates to the potential transmission of bacterial fruit collapse (a species of *Dickeya,* formerly *Erwinia chrysanthemi).* This pathogen is not present in Australia and poses an extreme biosecurity risk to the industry. The pathogen causes two major diseases, bacterial heart rot and bacterial fruit collapse. As presented in the draft IRA (pg 47), bacterial heart rot and fruit collapse are of major economic importance to pineapple producers (Rohrbach ,1983). The pest has been identified in the National Biosecurity Plan for pineapples as being high priority emergency plant pests by the pineapple industry.

The Draft IRA defines the overall unrestricted risk estimate for bacterial heart rot and fruit collapse as very low. Industry contends that this assessment is based on a number of assumptions that are either incorrect or unsubstantiated. We accept there is a paucity of information relating to critical aspects of how this disease might enter, spread and establish in Australia but argue that this lack of information should not be used to justify the importation of Malaysian pineapples but instead imports should be restricted until these outstanding scientific issues are resolved.

The lack of understanding of this disease is exemplified by the fact that its taxonomy is unresolved. A review of the current science (Appendix 1), put forward to Biosecurity Australia clearly outlines that bacterial heart rot and fruit collapse are not *Erwinia chrysanthemi* but are instead a species of *Dickeya.* Whilst the Draft IRA accepts that this pathogen is not present in Australia, confusion around its taxonomy sheds doubt on the level of scientific certainty that can be ascribed to other aspects of its ecology and epidemiology.

This submission will deal with each component of section 4.3 relating to bacterial fruit collapse separately. There will be significant crossover, however it is important to highlight how these assumptions potentially affect evaluation of all aspects of the infestation pathway. For ease of reading we have used the same sub-headings as presented in the draft IRA.

Probability of Entry

The Draft IRA states that the likelihood that pineapple heart rot and fruit collapse will arrive in Australia is low. Industry contends that this is based on some incorrect assumptions about the manifestation of the pathogen and that there is insufficient data to make this assessment. We contend that the actual likelihood of entry has the potential to be significantly higher but accept that the lack of data makes it impossible to make an accurate assessment at this stage.

The Draft IRA puts forward that there is only one pathway by which this pathogen can enter Australia and that is via latent infections of the fruit with the bacterial fruit collapse pathogen. Our review of the scientific literature and understanding of the morphology of pineapples leads us to the conclusion that there are three mechanisms by which the pathogen can enter Australia. That is: 1) latent infections in fruit, 2) as limited decay resulting from surface contamination of fruit and entry through growth cracks and 3) as latent infections in basal and scale leaves.

According to the *Dictionary of Biological Terms,* (Lawrence, 1997latent means lying dormant but capable of development under certain circumstances. In pineapple, the pathogen lies dormant (latent) in the base of the ovary until sugar levels rise and polyphenoloxidase levels decline in ripening fruit (Rorhbach and Schmitt 2003). At this stage there is no known way to measure at which point latency will break in any individual pineapple fruit.

Industry is concerned that the draft IRA only deals with latent infections of fruit collapse disease. This limited focus is predicated on the statement that the scope of the IRA is for fresh pineapples from which all crowns and all basal and scale leaves have been removed (Draft IRA pg 37). While we accept that this is the proposal put forward by Malaysia, we contend that the nature of pineapples means it is almost impossible to remove all basal and scale leaves without chopping into the actual fruit which would render it unsuitable for sale. This was demonstrated to Biosecurity Australia at the recent stakeholder meeting. Therefore, the risk of latent infection being carried in the basal and scale leaves must be considered. These infections would arise when basal and scale leaves were splashed by infested juice from nearby rotted fruit. This means the risk assessment must also cover off on the likelihood of entry of bacterial heart rot.

The second mechanism relates to the entry of partially infected fruit. According to Johnson 1957, disease can be induced by spraying uninjured fruit with the bacteria. In some cases, fruit collapse could not be induced, but infection did occur on part of the fruit which would not be evident until the fruit was cut open. This means a fruit could be infected via exudate from a freshly collapsed fruit in the field without having to enter via the flowers through an insect vector. This fruit would have a low level infection which could remain undetected until entry to Australia where the infected part of the fruit would be discarded by the consumer/processor. This cannot be referred to as a latent infection but rather a partially manifesting infection. This information is supported by recent comments from the breeder of the Josapine and N36 varietals who states that in mildly infected stages one cannot detect the malady from external examination (Chan pers comm., 2011 see appendix 2). There is no data on how many fruit have this limited ‘eye’ (fruitlet) rot and we contend there is definite potential for such infected fruit to enter Australia.

Because disease incidence is sporadic and unpredictable it is extremely difficult to predict levels of latent infection. Industry is concerned that the 2% figure put forward in the Draft IRA as being the upper limit of the percentage of fruit with latent infections that will remain undetected is not scientifically valid. For a number of reasons we see this figure as having no credible basis and there can be no accurate assessment can be made regarding the incidence of latent infection based on existing scientific information. The draft IRA cites Lim (1986) and Lim and Lowings (1979a) as putting forward the data which identifies the level of undetected latent infections as up to 2%. Review of these papers indicates that they are drawing this conclusion from statements made in Thompson (1937). We are aware that Biosecurity Australia did not review this paper themselves (Nathan Sibley, pers comm. 2011) and on review of this paper industry does not see that these conclusions can be substantiated. Thompson (1937) reported the disease but did not isolate the pathogen involved. The pathogen was not isolated until 1957 (Johnston, 1957). Johnston (1957) found a bacteria to be associated with fruit collapse which he concluded was a strain of *Erwinia carotovora.* As the pathogen was not known in 1937 it is possible that some of the 2% rejections in the 1937 paper were due to other diseases such as yeasty rot. It seems most inappropriate to incorporate such data in the risk analysis. In addition, Thompson (1937) states that these fruit (the 2%) were apparently sound when harvested 24 hours previously. According to Lim (1985), 5 to 6 days are required for the entire fruit to be soft rotted so one would assume that latency had already broken so this observation cannot be used to support any statements relating to post harvest latency. According to Rorhbach (2003), the pineapple is exemplary as a host for a floral infection (e.g bacterial fruit collapse) where an extended latent period occurs. The flower of pineapple is the portal for several major pathogens and the period of latency ranges from four to six months. Therefore it is highly conceivable that some fruit will be picked whilst carrying latent infection and that this infection is unlikely to be detected before entry into Australia. This is supported by Rorbach (1983) but that setting the figure at 2% is arbitrary to say the least. A laboratory within the University of Hawaii is currently developing screening assays to detect the bacteria in latent form from plant tissue (Taniguchi, University of Hawaii, pers comm., 2011 – see appendix 2). This will assist in providing more definitive data relating to levels of latency.

Another issue relating to latency that has not been addressed in the IRA is the possibility that resistant species could potentially be carriers of the bacteria. The Draft IRA acknowledges that both varieties earmarked for export are highly susceptible therefore this issue will be dealt with in relation to probability of distribution and establishment.

The Draft IRA also makes a number of points in this section that do not correspond to industry knowledge. According to Chan (pers comm., 2011 – see appendix 2), there are no detectable symptoms in ripe fruit when only one or two eyes (fruitlets) are infected. The statement on page 39 that sorting, grading and quarantine inspection at packing would reduce the low percentage of latently infected fruit is completely unsubstantiated as latent infections are by definition, unmanifested. It is also highly unlikely that partial infestation will be able to be detected (Chan pers comm., 2011 – see appendix 2) so this statement should be removed and not form part of the risk assessment.

There is no evidence that copper sulphate or NAA (Draft IRA pg 38) will have any effect on infestation levels of either latently or partially infected fruit.

The statement on page 39 of the Draft IRA that fruit will be stored for 4-5 weeks at 8-12 degrees is flawed as fruit do not store at that temperature for more than 3 weeks (Sanewski, DEEDI, pers comm., 2011). This will favour latency as the shorter time between harvest and sale means less opportunity for latency to break and be detected prior to sale.

In conclusion, industry cannot support the current risk assessment for importation as **LOW** (Draft IRA pg 40). A significant proportion of the information put forward in this section of the Draft IRA is inconclusive. The figure of 2% is completely meaningless even for latently infected fruit and does not even consider the issue of partially infected fruit. Based on the statement that there is a possibility that small (but undefined) amounts of (latently) infected fruits will be included in commercial shipments (Rohrbach 1983, Draft IRA 2011) and the potential for partially infected fruit to enter Australia undetected. Industry contends the risk assessment should be reviewed as the risk is potentially significantly higher. We do not see how this risk assessment can be accurate without additional scientific information. That said, we do not see even with the current information how the risk level can be rated as anything less than **MODERATE** which refers to an even probability that the event will occur.

Probability of Distribution

The probability of distribution has been identified in the Draft IRA as **LOW**. Industry contends that this assessment is based on some incorrect information about management of pineapples and pineapple waste in the supply chain as well as some unsubstantiated assumptions relating to the behaviour of the pathogen.

Distribution of the imported commodity and waste generation

As discussed extensively in the previous section, the figure of 2% should not be used as the potential level of latent and partial infection could be higher. Distribution of pineapples generally occurs throughout the supply chain at 15° C (Simon Newett, DEEDI, pers comm., 2011) not ambient temperature as stated on page 40 of the Draft IRA. Lower temperatures delay the breaking of latency. Therefore the statement that latently infected pineapples will display symptoms during distribution is flawed and the lower temperature increases the likelihood of waste disposal occurring at the consumer level. This section also does not take into consideration partially infected fruit and the fact that some imported fruit may be directed toward processors whose waste management methodologies also need to be considered.

Transfer of the pest from waste to suitable host

Industry agrees with the statement that transfer of a pathogen from infected fruits in waste to a susceptible host plant is complex, variable and dependent on a number of critical factors (Draft IRA pg 40). We contend that again the scientific information available is inconclusive and on balance the Draft IRA significantly underestimates the risk in light of this complexity.

Industry sees there are three major false assumptions that underpin the argument put forward in this section of the Draft IRA. They relate to proximity of pineapple growing areas to purchasing locations, waste disposal particularly in light of processor practices and assumptions around host specificity, susceptibility and vectors.

The low risk ranking seems to be predicated on the assumption that imported pineapples will not come into contact with pineapple growing regions (Draft IRA pg 43). Industry cannot see how this assumption can be supported. Growing regions identified in the IRA are close to major population centres e.g. Brisbane, Rockhampton and Townsville. The Sunshine Coast (current population more than 300,000) is a rapidly expanding area in close proximity to 60% of production. More than 74 percent of consumers purchase their fruit and vegetables at supermarkets (Nielson Homescan data, 2011) and these are supplied by central distribution centres (as are a significant proportion of independent fruit shops via the Brisbane markets). There is no data supporting the statement that only 1% of the population lives in proximity to pineapple growing regions (Draft IRA pg 43) and there is no clear guidance as to what Biosecurity Australia defines as proximity for the purposes of this IRA

The claim that imported product is less likely to be consumed near pineapple growing regions is patently false and not supported by any data relating to existing imported product. If the imported product is cheaper (highly likely) or there are supply or quality issues, then supermarkets will supply it to all outlets regardless of the proximity to local production areas. The production areas in South East Queensland are characterised by a mosaic of use patterns and is considered rural-urban (http://www.abc.net.au/elections/qld/2009/guide/glas.htm). Should imported pineapples be sold in supermarkets in Caboolture, Caloundra, Maroochydoore or even Brisbane then it is highly likely that any infected pineapples could be dumped at a property near a pineapple farm.

The statements in this section relating to waste disposal do not consider the full spectrum of pineapple uses or waste disposal practices and in particular do not consider the waste disposal practices of processors which could potentially increase the potential for spread.

Heinz pty ltd is the major processor of pineapples in Australia. They are based at Northgate on the outskirts of Brisbane. According to Doug Jones (Grower Integration Manger, Heinz), the cannery cannot rule out using imported pineapples although their preference is for Australian product. The cannery has no system for removing sub-standard pineapple before the peeling procedure so any infected waste would go into the general waste system. This waste is then transported via open trucks to cattle farms throughout the South-East Queensland region (Jones pers comm.., 2011). Again these farms could potentially be adjacent to pineapple farms or at the very least the trucks would drive through pineapple growing areas.

There are a number of other smaller processors who deal with fresh cuts including Tropico based in Palmwoods. This processor transports their waste in open trucks to the Mary Valley, again potentially moving through pineapple growing areas or ending up adjacent to pineapple farms. Fresh cuts and pre-packaged product are becoming increasingly popular with consumers (Nielson Homescan data, 2011) and therefore this type of waste disposal will become more prevalent and will present an increasing risk.

It has been acknowledged in the Draft IRA on page 41 that wind does play a role in disease transmission so therefore contaminated droplets could conceivably be blown into pineapple fields. The high volume of fruit contained in such trucks could have a sufficiently high percentage of infected fruit to provide a high inoculum load.

As discussed above, there is potential for individual consumers to discard their pineapple waste on properties adjacent to pineapple farms and we cannot agree with the statement that this waste will end up in landfill. Many people, particularly those in peri-urban areas have compost heaps which could act as a source of infestation via insect vectors.

The issue of host specificity is contentious and the scientific research is moving quickly. Industry cannot support the contention that this bacterium is highly specific to pineapples (Draft IRA pg 42). Not only is there no evidence that there are no other hosts but the disease did not co-evolve in South America with pineapples, therefore it must have some other host in Malaysia to be consistent with the evolutionary pattern of such pathogens (Ken Pegg, DEEDI, pers comm., 2011). Reports indicate that the bacterial fruit and heart rot pathogen has only recently been introduced into South America (Marrero and Alvarez, 2011)

Recent evidence from Hawaii indicates that isolates of *Dickeya* with the same genome as the pineapple infecting pathogen have been recovered from maize, taro and bromeliads (Marrero and Alvarez, 2011). This indicates a wider host range than what has been previously thought and contradicts the statement on page 42 of the Draft IRA that there are no reports of other hosts present in Australia for this pathogen, as all of these plants are found here. This has huge significance in terms of the likelihood of spread as given the possibility of alternative hosts, the proximity of pineapple farms to imported fruit is less critical for an outbreak to become established. The proximity of potential alternative hosts is initially of greater importance.

Pathogenicity tests are currently in progress in Hawaiii on the infested species (Marrero and Alvarez, 2011). The issue of alternative hosts has huge significance in terms of the likelihood of spread, particularly as the low risk status accorded in the Draft IRA is largely predicated on the assumption that no infected plant materials will come into contact with pineapples farms in Australia. In terms of the implications, there are a number of scenarios whereby infected pineapple could come into contact with other host species and the bacteria could become established in Australia.

It is easy to envisage a scenario whereby an infected pineapple leaking exudate is removed from a pineapple display by a worker in the produce section of a supermarket or greengrocer (as per pg 40 of the Draft IRA). This worker could conceivably handle a susceptible ornamental plant before washing their hands and transmit the pathogen and the infected plant could contribute to the establishment of the pathogen in Australia . Industry is also concerned by reports of ornamental pineapple plants being sold at fruit shops around Brisbane and placed next to the fresh pineapple display.

Page 41of the Draft IRA states that the bacterium does not survive long in soils. According to recent information from the University of Hawaii (Taniguchi pers comm. , 2011 – see appendix 2), soil transported from contaminated pineapple fields subsequently grew corn which became infected with *Dickeya* with the same genome as the pineapple pathogen. This would indicate that the potential for survival in soil and water is greater than previously thought. The Draft IRA uses data from Lim (1974) however it should be pointed out that these experiments used laboratory techniques involving the artificial contamination of sterile soil in an attempt to understand the field behaviour of the pathogen. At the time of the experiments, modern molecular techniques were not available and there has been no attempt to verify these findings in field conditions. Industry contends that Lim (1974) did not fully ascertain the pathosystem of the bacteria as he did not have the technology available that is now widely used. It is well known that many of the *Dickeya* species do persist in soil and irrigation water (Toth et al 2011) and the recent discovery in Hawaii would support the same conclusion for this pathogen.

If the pathogen persists in soil, irrigation water will be infested (the pathogen has been recovered from irrigation water in Hawaiii). If this water is not disinfested, it could be used in a nursery growing bromeliads or used to irrigate other susceptible hosts including pineapple plants.

The combined impact of a broader host range and increased potential for survival in soil and water significantly increases the likelihood of this pathogen to establish and spread in Australia. Contaminated soil from a compost heaps or cattle farm could easily come into contact with Bromeliads which are a very popular ornamentals throughout Australia. Run off water is often re-used for irrigation and could again be a source of contamination of Bromeliads, pineapples or other susceptible hosts. The susceptibility of other commercial crops to this pathogen also increases the overall consequence of this disease establishing as well as its capacity to spread.

The discussion on page 41 of the Draft IRA relating to transfer mechanisms fails to consider the behaviour of potential Australian vectors such as native bees (Sanewski 2007) and birds. Native bees pollinate broad acre crops and therefore have a reasonable foraging range which increases the distance across which the disease can be spread (at least 500 metres). Additionally, the potential for wind transmission cannot be discounted until the issue of percentage of latent or partial infection can be resolved.

According to the Draft IRA (pg 42), entry points for the pathogen are restricted to a small number of days throughout the year. This is based on the incorrect statement that flowering only occurs on a limited number of days throughout the year. According to 3rd generation pineapple growers, pineapple production occurs year round so flowering is induced throughout the year. Secondly, breakthrough flowering is a regular occurrence and cannot be effectively regulated.

A final area of concern relates to the statement that because 60% of pineapple plantings are smooth cayenne and these pineapple are resistant, the likelihood of initial transfer is significantly reduced Draft IRA pg 44). Firstly, hybrids such as the highly susceptible MD2 (Sanewski, pers comm., 2011) are becoming increasingly common as the industry moves from cannery fruit to fresh, so while the 60/40 split may be accurate at the time of writing, the trend is for increased plantings of hybrids. Secondly, there is no guarantee that resistant varieties are not carriers of the pathogen. According to the University of Hawaii, it has never been determined one way or the other as being true or false so it cannot be assumed that they are not carriers and that 60% of the plantings do not represent a risk (Taniguchi pers comm., 2011 – see appendix 2).

Industry cannot support the statement that the probability of distribution is **LOW** and again would like the risk assessment revised to reflect a **MODERATE** risk. Table one summarises the industry position on each of the factors supposedly mitigating against entry used in the Draft IRA to make this determination.

Table 1

IRA determination	Draft IRA rationale	Industry/scientific position
Probability of entry (importation x distribution) is **Very Low**.	Probability of importation is low with only 2% of fruit being latently infected on arrival in Australia.	**Cannot support** No valid scientific data to support 2% post harvest latency figure. No assessment of potential importation of partially infected fruit. No valid detection or treatment measures for latently infected fruit. Infected fruit will enter Australia.
	Imported pineapples will be consumed and discarded away from pineapple growing regions so there is limited likelihood of imported infected pine material coming into contact with flowering Australian pineapples.	**Cannot support** Statement based on flawed assumptions about: - supermarket distribution mechanisms and population distribution - processor waste disposal techniques - -frequency of flowering
	Pineapples are the only host in Australia for the Bacterial Heart and Fruit Collapse pathogen	**Cannot support.** No scientific evidence provided in the IRA that pineapples are the only host and strong new evidence that the pathogen has been found on Bromeliads, Taro and Maize in Hawaii. All of these hosts are found in Australia. Basic understanding of the evolution of such pathogens would indicate that an alternative host must exist but there is no information on what that is or its presence/absence in Australia.

Probability of establishment and spread

Industry agrees with the statements in the Draft IRA that the probabilities for establishment and spread are **High** (pp44 and 45). We also contend that the likely presence of additional hosts and the capacity for the pathogen to survive in soil and water increases the risk of establishment and host. Overseas experience indicates that once the pathogen establishes, it spreads quickly and cannot be controlled.

Consequence

Industry contends that the consequence of the pest should it become established in Australia is **High** rather than **Moderate**. This is based on the likelihood of alternative hosts (as discussed above) causing it to become established nationally rather than just regionally and the potential commercial impact on the nursery industry and taro and maize production.

Other Issues

Whilst industry's primary concern relates to the importation of Bacterial heart rot and fruit collapse, there are some other areas we would like to see addressed. Firstly, industry is concerned that *benomyl* is being put forward as an anti-fungal treatment for Malaysian pineapples (Draft IRA pg 20). This chemical is unregistered in Australia due to its link with birth defects and is banned in Europe and the United States. NAA is also not a registered treatment in Australia and industry is unsure of the purpose of its use in Malaysia.

Conclusions and recommendations

Industry does not see there is any valid scientific basis for the risk assessment profiles put forward in the Draft IRA for the importation of Malaysian pineapples and in most instances, on the balance of probabilities relating to understanding of such organisms, the risk estimations have been set too low. We think that it cannot be stated with any confidence that the overall risk is very low.

It is our primary contention that there is insufficient recent scientific information to validate the claims put forward in the Draft IRA and there are a number of false assumptions that are used to define the risk estimates.

The industry considers that more carefully focused research using modern techniques is requires to answer the many scientific questions that remain unanswered before the importation of pineapple from infested production areas can be allowed. The industry is so concerned about the pathogen it has elected to fund research in Hawaii (which has been curtailed due to lack of funding) so that isolates with the same genome as the pineapple pathogen which have been recovered from diseased corn, taro, bromeliads and irrigation water can be pathotyped.

As disease occurrence in Malaysia is sporadic and unpredictable but commercially significant and the pathogen remains latent in the fruit with no detectable symptoms, it is impossible to determine the level of "risk" in imported fruit. Industry recommends that the final determination on the importation of Malaysian pineapples be delayed until a more acccurate risk assessment can be undertaken that can be substantiated by modern scientific data. We do not think that the importation of this potentially devastating disease can be allowed based on such scant data and flawed assumptions. As outlined above, industry is prepared to fund further research to clarify some of the most important outstanding issues.

References

Johnston A. (1957) Bacterial Heart Rot of the Pineapple. *Malaysian Agricultural Journal.,* **40** : 2-8

Johnston A. (1957) Pineapple Fruit Collapse. *Malaysian Agricultural Journal.,* **40** : 253-263

Lawrence, 1997, Dictionary of Biological Terms, 11th Edition, Longman Singapore Publishers)

Lim WH (1974a) The etiology of fruit collapse and bacterial heart rot of pineapple. *MARDI Research Bulletin* **2,** 11-16.

Lim, WH. (1985). Diseases and Disorders of Pineapples in Peninsular Malaysia. MARDI Report No. 97. 53 pages.

Lim WH, Lowings PH (1979a) Pineapple fruit collapse in peninsular Malaysia: symptoms and varietal susceptibility. *Plant Disease Reporter* **63,** 170-174.

Marrero G and Alvarez AM (2011) Hawaiian Strains of *Erwinia chrysanthemi* (*Dickeya* sp.) Associated With Pineapple Heart Rot Disease *Pineapple News* **18**: 57- 59

Rohrbach KG (1983) Pineapple diseases and pests and their potential for spread. In: Singh KG (ed.) *Exotic Plant Quarantine Pests and Procedures for Introduction of Plant Materials.* ASEAN Plant Quarantine Centre and Training Institute, Serdang, Selangor, Malaysia. pp. 145-171.

Rorhbach KG, Schmitt D (2003) Disease of Pineapple *In* Ploetz RC (*Ed)*Diseases of Tropical Fruit Crops. CAB International, Wallingford

Sanewski, G (2007). The role of insects in the pollination of pineapple in Australia. Pineapple News. **14**, 7-9.

Thompson, A. (1937) Pineapple fruit rots in Malaya. A preliminary report on fruit rots of the Singapore Canning Pineapple. Malaysian Agricultural Journal **25**: 407 - 420.

Toth, IK, van der Wolf, JM, Saddler, G, Lojkowska, E, Helias, V, Pirhonen, M, Tsror, L and Elphinstone, JG. (2011). Dickeya species: an emerging problem for potato production in Europe. Plant pathology. 60 (3), 385-399.

Appendix 1

Submission of scientific evidence to Biosecurity Australia in regards to the risk to the Australian pineapple industry posed by Malaysian pineapple-infecting strains of *Dickeya* sp.

Prepared by: Anthony Young and Ken Pegg

Plant Pathology, Agri-Sciences,
Department of Employment, Economic Development and Innovation (DEEDI),
Ecosciences Precinct, GPO Box 46, Brisbane 4001, Queensland, Australia.

anthony.young@deedi.qld.gov.au; ken.pegg@deedi.qld.gov.au

Executive summary

We present evidence that Malaysian pineapples are subject to infection by a poorly characterised bacterium belonging to the genus *Dickeya*. This bacterium is distinct from described strains and is not present in Australia. We believe there is a high likelihood of these bacteria entering Australia in semi-processed fruit, and that there exist adequate pathways for their introduction into the Australian pineapple industry. In the advent of their introduction, there will be significant deleterious impacts on Australian pineapple production, and we consequently recommend that any import determinations are based on a sound understanding of the risks involved, which we believe have hitherto been understated.

Introduction

Pineapples are an iconic crop for Queensland and Australia. There are one hundred and seventy pineapple-growing businesses throughout seven production areas in Queensland. These directly employ some two thousand people, in addition to associated industries supplying farm machinery, fertilizer, chemicals and packaging, with the positive flow-on effects for tourism and other regional service industries. Between the Sunshine Coast and Wide Bay/Burnett region, pineapples are grown for both processing and fresh market, and high value fresh fruit hybrids are grown as far north as the Atherton Tableland. The fresh fruit hybrids, which are high in vitamin C, are much sought after by the food service industry in key tourist destinations.

There are very real threats to our ability to produce enough food to feed a growing world population. One of the key concerns to plant pathologists is preventing the establishment of pathogenic micro-organisms and other plant pests that will further erode production. This is especially relevant to the pineapple industry in Queensland. The pineapple plant is most productive under xerophytic conditions, where low rainfall is supplemented by irrigation in well-drained soils. Queensland production areas are subject to periodic cyclones, tropical storms and low pressure systems when even the best drained soils become temporarily saturated and productivity depends largely on the successful management of pests and diseases. The introduction of any new pineapple disease would severely impact the Australian industry. In this respect, we have justifiable concerns regarding the import conditions of semi-processed pineapple from Malaysia, which appear to offer an avenue for the incursion and establishment of a serious new disease into the Australian pineapple industry.

There are three key questions that need to be addressed when considering the risk of importing semi-processed fruit from Malaysia:

1. Are the Malaysian pineapple-affecting strains of *Dickeya* the same as those in Australia?

2. Is there a chance that semi-processed pineapple can carry viable *Dickeya* strains?

3. Are there any possible pathways for the transfer of *Dickeya* strains from Malaysian semi-processed pineapple into the Australian pineapple industry?

We provide evidence that the Malaysian pineapple strains are distinct from endemic strains, that they can be transported in a viable state in semi-processed pineapple, and that there exist avenues by which they can gain access to the Australian pineapple industry.

1. Are Malaysian pineapple-affecting strains of *Dickeya* the same as Australian strains?

In previous correspondence, the bacterium in question has been known as any of the following: *Erwinia chrysanthemi, Pectobacterium chrysanthemi, Dickeya chrysanthemi, D. zeae* and *Dickeya* sp. Throughout this report the term *Dickeya* sp. is used in relation to the Malaysian pineapple-affecting strains, reflecting the currently unstable taxonomic arrangement. While the taxonomy of the soft-rotting bacteria has recently received considerable treatment, the position of the pineapple-affecting strains has not been satisfactorily resolved. There have been no targeted taxonomic treatments for the Malaysian pineapple-infecting strains of *Dickeya*, but it is fortuitous that several isolates have been included in the general systematic studies of the broader group of bacteria. From these there is conclusive evidence that the Malaysian pineapple-affecting *Dickeya* strains are distinct from Australian strains.

The most comprehensive studies of the pineapple-associated *Dickeya* strains comes from the epidemiological work of Lim in the 1970's (Lim 1974, Lim and Lowings 1978, Lim and Lowings 1979). The salient finding was that the bacterium (then referred to as *E. chrysanthemi*) is highly-adapted to the pineapple host, to the extent that it can exist in a symptomless latent state for long periods of time before activating the plant collapse that so readily transmits it to new hosts. It is also unusual for a soft-rotting bacterium in that it can be readily vectored by flower-visiting insects. Throughout Lim's extensive characterisations, it was revealed that the Malaysian pineapple isolates were distinct pathovars from twenty five other *E. chrysanthemi* isolates from other hosts and regions (W. H. Lim, presentation to Australian Plant Pathology Society, Brisbane, 1976). Furthermore, Lim considered the pineapple fruit collapse or ghost rot pathogen to be, then, unique to western Malaysia. Subsequently, the disease has been reported from Costa Rica, Brazil, the Philippines and Hawaii, where it has been linked to the emergence of non-endemic strains of *Dickeya* associated with imported planting material (Melo *et al.* 1974, Chinchilla *et al.* 1979, Rohrbach 1983, Kaneshiro *et al.* 2008). A different bacterial heart rot (caused by *P. carotovorum* subspecies) has been recorded in Queensland, where, following foliar applications of urea, bacteria in stored surface water produce urease which breaks urea down to ammonium hydroxide, causing burns that provide entry points for the bacteria to enter. In Queensland, strains previously identified as *E. chrysanthemi* have been isolated from sugarcane and banana, but have not from pineapple. This is identical to the situation in Hawaii, where, until recently, bacteria nominally identified as '*E. chrysanthemi*' have been found to infect ornamental and other crops, but only since the propagation of imported planting stocks has the pineapple fruit collapse problem emerged (Kaneshiro *et al.* 2008).

The genus *Dickeya* was erected to accommodate bacterial species previously assigned to *Erwinia chrysanthemi* and *Pectobacterium chrysanthemi* (Samson *et al.* 2005). It had long been established that there were several distinct pathogenic groups of bacteria within the former 'species' (Dickey 1979), but it has only been through the application of molecular markers that it has been possible to accommodate these strains into a suitable taxonomic framework. The revised taxonomy proposed six new species, *D. zeae*, *D. dadantii*, *D. chrysanthemi*, *D. dieffenbachiae*, *D. dianthicola* and *D. paradisiaca*, but failed to accommodate the Malaysian pineapple-infecting group, which were different still. In their work they included two pineapple isolates: one from Martinique and one from Malaysia (O-serogroup 10 and 7 respectively), and placed the former in the novel species *D. zeae*, but they did not ascribe the Malaysian isolate to any species. This demonstrates what is already known: bacteria formerly known as *E. chrysanthemi* (like many other pectolytic bacteria) are capable of infecting pineapple, but there exists a related strain from Malaysian pineapple that is sufficiently distinct to escape taxonomic placement in a comprehensive study.

To avoid any future confusion, it is worthwhile pointing out two other pertinent features of Samson *et al.* 2005. First, there is an inconsistency in that pineapples are included as hosts for two (*i.e. D. zeae* and *D. dadantii*) of their new species, but this is incongruent with their presented results. The Martinique isolate has an O-serotype that makes it eligible for placement within *D. zeae*, while the Malaysian strain could not be accommodated in any of the new species and was consequently omitted from the new species descriptions (and is yet to be formally described). The new species *D. dadantii*, which was claimed to encompass pineapple within its host range, only has strains with O-serogroups 1 and 6, neither of which match the O-serotypes generated by the two pineapple isolates tested. What is critical is that while they found *D. zeae* (a widespread bacterium found in Australia) could infect pineapple, the Malaysian pineapple strain was distinct and could not be ascribed to any of the new species.

The second point is that Samson *et al.* (2005) found that the O-serotype 7 was found in an Australian isolate from sugarcane. This is not evidence that the Malaysian strains exist in Australia. The Australian sugarcane isolate and the Malaysian pineapple isolates are distinct, as borne out in the presented DNA:DNA hybridisation data, which, in having different levels of relatedness to the type species of *D. zeae* and *D. dadantii*, are patently not the same strain. Likewise, the phenotypic dendrogram, places the Malaysian pineapple infecting *Dickeya* sp. (CFBP 1278) and the Australian sugarcane *Dickeya* sp. (CFBP 1537) in a paraphyletic condition if they were to be considered the same species. Finally, the two strains in question differ across five of the seven differential phenotypic patterns for the delimitation of strains belonging to *D. zeae*, *D. dadantii* and these two undescribed *Dickeya* species. The authors justifiably consider both CFBP 1278 and CFBP 1537 as two genomically distinct strains.

More recent studies by Parkinson *et al.* (2009) involved a phylogenetic analysis of described and not described species of *Dickeya* based on the recombinase A (*recA*) gene sequence. Like the work of Samson *et al*. (2005), these investigators were not primarily concerned with Malaysian *Dickeya* strains affecting pineapple, but they included three in their study (NCPPB 1120, NCPPB 1121 (=CFBP 1278 of Samson *et al.* (2005)) and NCPPB 1125). Analysis of the *recA* sequences retrieved from GenBank (respective accession numbers FJ217110, FJ217111 and FJ217112) provide further evidence that the Malaysian pineapple-infecting strains are distinct from Australian strains. Strain NCPPB 1120 was unique, but had only two base differences from NCPPB 1121 and NCPPB 1125, which were identical for the 481 bases of sequence obtained. This may reflect evidence for genetic diversity of this pathogen in Malaysia (Sahilah *et al.* 2008), consistent with the views of Lim on the origin of the strains. At this locus, strains NCPPB 1121 and NCPPB 1125 were identical to several maize strains from India, one from wheat in France, and another Malaysian strain, this time from brassica, but none from Australia.

While Parkinson *et al.* (2009) attribute the Malaysian pineapple-infecting strains to the species *D. zeae*, it is clear that they are not the same as strains present in Australia. For example, of the seven Australian potato-infecting strains identified as *D. zeae* included in the study, the similarity at the functionally constrained (and therefore evolutionarily conserved) *recA* locus ranged between 96.5% (465/481) and 98.7% (475/481). The Australian sugarcane isolate, which has been previously proffered as evidence of the existence of the same strains in Australia and Malaysia, had a similarity, at this locus, of only 88.7% (422/476). There can be no further conjecture as to whether or not these constitute the same strain: they are indisputably distinct.

The results of the studies of Samson *et al.* (2005) and Parkinson *et al.* (2009) support previous work by Nassar *et al.* (1994) and Avrova *et al.* (2002). Studies by Avrova *et al.* (2002) found that a Malaysian pineapple infecting strain had a different amplified fragment length polymorphism fingerprint than other '*E. chrysanthemi*' strains, and Nassar *et al.* (1994) found that, based on rRNA pattern, the Malaysian pineapple strains formed a discrete group (cluster 6) to all other isolates tested. They also noticed that these isolates were related to other isolates (cluster 5 and 7), but were clearly not the same.

Regrettably, they did not include clusters 5, 6 and 7 in their pathogenicity analyses. For this it is necessary to consult the pathogenicity trials of Lim (Lim 1974, Lim and Lowings 1978, Lim and Lowings 1979), who found that the Malaysian pineapple strains were highly adapted to the pineapple host.

Although these raw data clearly demonstrate that the Malaysian pineapple strains are different from Australian strains, it is important to briefly address some of the limitations of DNA sequence-based phylogenetic reconstructions. Firstly, Parkinson *et al.* (2009) only used the gene sequence at one locus for their phylogeny. A single locus can provide an apparently clear result, but can only suggest an evolutionary history of that gene, and not of the broader genetic and physiological relations of bacterial species and strains. Horizontal gene transfer, strong stabilising selection, convergent evolution and higher rates of divergence at other genes (particularly those involved in host interactions) can all influence the veracity of a phylogeny inferred from a single gene. A cogent example of this is when Parkinson *et al*. (2009) failed to satisfactorily resolve the *D. dadantii* clade based on the *recA* sequence, they employed three other loci (*pfkA*, *rpoB* and *acnB*), which all yielded different phylogenetic relationships among the seven *D. dadantii* species tested. Furthermore, genomic plasticity, that is, the rearrangement of different genes and gene groups among strains of bacteria, can confer significant adaptive potential that is invisible from the nucleotide sequence for a given gene. Although there may be a broad host-range for any given strain, the genus *Dickeya* can be highly-adapted to their plant hosts (Dickey 1979, Ma *et al.* 2007). The surest conclusion to be made from any molecular data is that any observable difference translates to an evolutionarily significant divergence, and even if that does not amount, in the eyes of a molecular taxonomist focussing on a single gene, the delineation of a different species, it certainly warrants further consideration before saying that that organism is the same as another.

Based on the available, albeit limited, evidence, there can be no possible reason to suppose that the Malaysian pineapple infecting strains of *Dickeya* are the same as any Australian strains. To do so would be to say that both countries have the bacterial wilt pathogen *Ralstonia solanacearum*, so there is nothing to worry about (and in doing so ignore the devastating consequences of strain differences that are manifest in banana moko disease). There is clear genetic, physiological and epidemiological evidence that demonstrate that Malaysia and Australia have distinct strains of *Dickeya*. As such, there is a need to assess the incursion risk of Malaysian pineapple affecting strains of *Dickeya*.

2. Is there a chance that semi-processed pineapple can carry viable *Dickeya* strains?

Given the definitive evidence that the Malaysian pineapple-affecting strains of *Dickeya* are distinct from any Australian strains, it is important to be able to assess if it is likely that semi-processed pineapple fruit can be infected, and if so, whether or not the bacteria can survive transportation.

It is well established that bacteria have finely-tuned interactions with their host and other environments. Particularly significant in this discussion are the processes that govern latency. In basic terms, if an infiltrating bacterium immediately activates its cellulolytic enzymes (and others that break down host tissue), then the plant defence response counters it by isolating and chemically destroying the would-be pathogen. Bacteria generally avoid this by multiplying saprophytically until sufficient numbers exist to launch an effective, host-destroying attack. The bacteria can sense other bacteria in their environment by diffusible quorum-sensing molecular hormones. While there are many different kinds, Gram negative cells employ various *N*-acyl homoserine lactones. Three separate *N*-acyl homoserine lactones have been identified in at least one member of the genus (presumably *D. dadantii*) (Castang *et al.* 1998), and as they have been found to be important to pathogenic processes throughout a broad assemblage of other proteobacteria, it must be assumed they exist in other *Dickeya* species. This is supported by Lim (1974) who found conclusive evidence of latent infections in Malaysian pineapple.

While it is beyond the scope of this submission to discuss all of the statistical permutations for the likelihood of imported semi-processed pineapple being infected with *Dickeya* sp., if a tolerable field incidence of 1% is to be accepted as a basis, then there is a strong possibility that some of the imported product will contain undetectable levels of the bacterium. The figure of 1% can only necessarily indicate the incidence of symptomatic fruit within a field, and cannot take into account new or latent infections.

However, even at a 1% acceptable infection level there are ample opportunities for the bacteria to enter the packaging machinery and consequently the product. As all product is to be skinned and cored, a single latently-infected pineapple entering the system would result in the immediate contamination of multiple subsequently semi-processed fruit. This method of contamination is well documented in other fruits and crops. As these fruit would be stored in conditions unfavourable to the proliferation of the contaminating *Dickeya* sp., there could be little visible evidence of soft-rotting in the package until the product is removed from cool storage. That latency can play a key role in the dissemination of *Dickeya* induced soft rots (in this case potato), is shown by Tsror *et al.* (2010).

Therefore, given the potential for in field incidences higher than acceptable levels, the known latency of the bacterium, and the assured contamination of previously uninfected produce should a latently-infected fruit enter the processing plant, we believe there is a high likelihood that semi-processed pineapple fruit can harbour viable destructive strains of *Dickeya* sp..

3. Are there any possible pathways for the transfer of *Dickeya* strains from Malaysian semi-processed pineapple into the Australian pineapple industry?

It has been demonstrated that the Malaysian pineapple affecting strains of *Dickeya* sp. are distinct from Australian *Dickeya* species and strains, and that they can enter Australia in semi-processed pineapple. In order for these isolates to enter the Australian pineapple industry, there needs to be a potential pathway. We believe there is great potential for the *Dickeya* sp. to move from imported semi-processed fruit into the Australian pineapple industry.

It should be immediately acknowledged that what little we know of the taxonomy of Malaysian pineapple affecting strains of *Dickeya* sp., we know even less of their ability to infect other host plants. While it is tempting to adduce, based on sequence similarity, that the same strains can infect corn in India, wheat in Martinique, and brassica in Malaysia, this assumption would be unjustified for the aforementioned reasons. Nonetheless, based on the general properties of the genus *Dickeya*, as well as the related soft-rotting bacteria *Erwinia* and *Pectobacterium*, there is cause to suppose that the Malaysian pineapple affecting *Dickeya* sp. could infect alternate hosts and be transported in soil, water and farming equipment.

Members of the three genera, *Dickeya*, *Erwinia* and *Pectobacterium*, are facultatively anaerobic. This capacity allows them to survive in water-logged soils at reduced oxygen contents. By the nature of their attacks on plants, where they literally dissolve the plant tissues, it is necessary for these bacteria to be able to survive in the environment for as long as it takes to locate a new host. For this, each cell is furnished with several peritrichous flagellae that enable efficient movement in water and interstitial spaces. As several general pathogenicity trials have demonstrated (Dickey 1979, Dickey 1981, references therein and subsequent findings), strains within each of the soft-rotting bacterial species may have some preference to certain hosts, but can still be capable of infecting other hosts. Therefore, although there does not appear to be a sufficient body of research specifically directed towards the Malaysian pineapple affecting *Dickeya* species, it can be confidently expected that, like their close relatives, these bacteria can survive in water, the soil, anaerobic conditions and on currently unidentified plant species. Certainly, the potential of them being able to survive externally to pineapples should be factored into risk assessments until proven otherwise.

Once imported semi-processed fruit enter Australia, there can be no restriction on where they are distributed. If, as previously indicated, there is a high possibility of pathogenic *Dickeya* species being transported with the semi-processed fruit, and that these bacteria can be expected to be able to survive outside of the pineapple host, then the avenues for their infiltration into the Australian pineapple industry are too multitudinous to enumerate. Some basic examples are the movement of soil or compost, machinery and flood inundations. Therefore we suggest that the current proposed import conditions place the Australian pineapple industry at considerable risk of an exotic disease.

Conclusion

While the scientific evidence is incomplete on many fronts, and certainly warranting further research effort, we believe that we have provided a clear and detailed explanation of the reasons why we consider the current conditions for the import of semi-processed pineapple from Malaysia unsafe for the Australian pineapple industry. We have demonstrated, using literature available to all interested parties, that the Malaysian pineapple affecting *Dickeya* species is a unique and exotic pathogen, that it can be transported in semi-processed product, and has the potential to enter the Australian pineapple industry. However, having little direct experience with this pathogen (not having it in Australia), we believe that any concerned institutes or persons could contact the relevant authorities at the University of Hawaii, who are currently dealing with an incursion of this devastating pathogen.

There is currently no high level Australian research and development aimed at investigating the epidemiology, detection and control of pineapple affecting *Dickeya* species. It would seem that, in order to better safeguard Australia's biosecurity, and prevent the further globalisation of plant pathogens, it is imperative to refine our understanding of the risk posed by exotic pathogens, prior to their establishment.

References

Avrova AO, Hyman LJ, Toth RL, Toth I (2002) Application of amplified fragment length polymorphism fingerprinting for taxonomy and identification of the soft rot bacteria *Erwinia carotovora* and *Erwinia chrysanthemi*. *Applied and Environmental Microbiology* **68,** 1499-1508.

Castang S, Reverchon S, Gouet P, Nasser W (2006) Direct evidence for the modulation of the activity of the *Erwinia chrysanthemi* quorum-sensing reulator ExpR by acylhomoserine lactone pheromone. *Journal of Biological Chemistry* **40,** 29971-29987.

Chinchilla CM, Gonzales LC, Morales F (1979) Bacterial heart rot (*Erwinia chrysanthemi*) of pineapple in Costa Rica. *Agronomia Cost* **3,** 183-185.

Dickey RS (1979) *Erwinia chrysanthemi*: a comparative study of phenotypic properties of strains from several hosts and other *Erwinia* species. *Phytopathology* **69,** 324–329.

Dickey RS (1981) *Erwinia chrysanthemi*: reaction of eight plants to strains from several hosts and to strains of other *Erwinia* species. *Phytopathology* **71,** 23–29.

Kaneshiro WS, Burger M, Vine BG, de Silva AS, Alvarez AM (2008) Characterization of *Erwinia chrysanthemi* from a bacterial heart rot of pineapple outbreak in Hawaii. *Plant Disease* **92,** 1444-1450.

Lim WH (1974) The etiology of fruit collapse and bacterial heart rot of pineapple. *MARDI Research Bulletin* **2,**11-16.

Lim WH, Lowings PH (1978) Infection sites of pineapple fruit collapse and latency of the pathogen, *Erwinia chrysanthemi*, within the fruit. In: *Proceedings of the 4th International Conference on Plant Pathogenic Bacteria.* Angers, France, pp. 567-575.

Lim WH, Lowings PH (1979) Pineapple fruit collapse in peninsular Malaysia: symptoms and varietal susceptibility. *Plant Disease Reporter* **63,** 170-174.

Ma B, Hibbing ME, Kim HS, Reedy RM, Yedidia I, Breuer J, Breuer J, Glasner JD, Perna NT, Kelman A, Charkowski AO (2007) Host range and molecular phylogenies of the soft rot enterobacterial genera *Pectobacterium* and *Dickeya. Phytopathology* **97,** 1150-1163.

Melo SAP, Araujo E, Suassauna J (1974) Preliminary note of the occurence of *Erwinia* sp. causing a dry rot of pineapple fruits in Paraiba. *Review of Agriculture* **49,** 124.

Nassar A, Bertheau Y, Dervin C, Narcy J, Lemattre M (1994) Ribotyping of *Erwinia chrysanthemi* strains in relation to their pathogenic and geographic distribution. *Applied and Environmental Microbiology* **60,** 3781-3789.

Rohrbach KG (1983) Pineapple diseases and pests and their potential for spread. In: Singh KG (ed.) *Exotic Plant Quarantine Pests and Procedures for Introduction of Plant Materials.* ASEAN Plant Quarantine Centre and Training Institute, Serdang, Selangor, Malaysia. pp. 145-171.

Sahilah AM, Rozeita L, Umi Kalsum MS, Son R (2008) Typing of *Erwinia chrysanthemi* isolated from Josapine pineapple in Malaysia using antimicrobial susceptibility, plasmid profiles, ERIC-PCR and RFLP Analysis. *International Food Research Journal* **15,** 273-280.

Samson R, Legendre JB, Christen R, Fischer-Le Saux M, Achouak W, Gardan L (2005) Transfer of *Pectobacterium chrysanthemi* (Burkholder *et al*. 1953) Brenner *et al*. 1973 and *Brenneria paradisiaca* to the genus *Dickeya* gen. nov. as *Dickeya chrysanthemi* comb. nov. and *Dickeya paradisiaca* comb. nov. and delineation of four novel species, *Dickeya dadantii* sp. nov., *Dickeya dianthicola* sp. nov., *Dickeya dieffenbachiae* sp. nov. and *Dickeya zeae* sp. nov. *International Journal of Systematic and Evolutionary Microbiology* **55,** 1415–1427.

Tsror L, Lebiush S, Erlich O, Ben-Daniel B, van der Wolf J (2010) First report of latent infection of *Cyperus rotundus* caused by a biovar 3 *Dickeya* sp.(Syn. *Erwinia chrysanthemi*) in Israel. *New Disease Reports* **22**, 14.

Appendix 2.

The contents of this appendix were marked confidential by Growcom and are not reproduced here.