Assessment of General RPC Cleanliness As Delivered for Use in Packing and Distribution of Fresh Produce

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Purpose: To assess the typical microbiological cleanliness and quantitative bacterial profile of pool RPC's at the time of delivery to a packing materials distribution facility for subsequent, secondary delivery to a grower harvest or packing operation.

Background: The design and implementation of a modern systems-based produce safety management program requires that operations understand and consider the potential sources and risk associated with all forms of contamination including physical, chemical, and biological hazards (FDA, 1998; FDA, 2009). The regulatory agency guidance documents (FDA, 2001) and multiple sources of industry guidance documents and Best Practice standards (e.g. UFPA, 2008; CCAB, 2013; LGMA, 2013; WGA, 2013; WGA, 2014) focus specifically on the need for a comprehensive assessment of microbiological risks for the fresh and fresh–cut (minimally-processed) produce supply including all aspects of inputs and points of contact or cross-contamination during distribution. Furthermore, this guidance also serves to elevate grower, shipper, receiver, and affiliated industry awareness of the need for science-based prevention programs and validation of any corrective actions.

Even before the release of the guiding principles of Good Agricultural Practices (GAPs), as published by the US FDA (1998), the potential for microbial hazards and their potential for cross-contamination associated with diverse fomites (any inanimate object, other than food or water, capable of serving as a vector or vehicle for transfer of pathogens) including harvest knives, gloves, totes, and bins was widely recognized. Internationally, the FAO technical document, *Management of reusable plastic crates in fresh produce supply chains* (Rapusas and Rolle, 2009) highlights the need for special attention to routine cleaning and sanitation to prevent decay, spoilage, and human foodborne illness.

Whether ultimately covered under the FDA's Food Safety Modernization Act (FSMA, 2014) Produce Rule or Preventive Controls Rule or any of the multiple Good Agricultural Practices (GAPs) and Good Handling Practices (GHPs) standards and audit schemes; growers, handlers, and shippers have the expectation that newly delivered reusable plastic containers (RPC) would not be a source of indicator organisms sufficient to exceed general cleanliness standards for audit compliance nor be a factor to consider as a plausible source of human pathogens. Recently, however, growers, handlers, and shippers have expressed a building concern, based on observations and experiences over several years, regarding the

microbiological cleanliness of RPC's. Inspection prior to use of RPC's by growers and packers has been a long-standing Best Practice for *ready-to-wash* and *ready-to process* fresh produce, whether direct marketed, shipped to foodservice providers, or sold at retail establishments. However, produce suppliers have acknowledged these inspections rarely, to date, have included any form of microbiological cleanliness verification of the RPC lot delivered. Given the nature of pool RPC management systems, this cleanliness standard is assumed and expected. Although there is no direct evidence, at this time, for the transfer of microbiological hazards from RPC surfaces to their product or any direct role in documented foodborne illness this confidence has been somewhat eroded by recent studies. A recent survey report from the University of Guelph (Warriner, 2013), on the cleanliness of RPC's in the Canadian pool RPC system delivered for produce packing, presented some outcomes that have stimulated a heightened interest to assess regional situations. An overview of the Univ. of Guelph outcomes in relation to broad national concerns for general cleanliness of RPC's is presented in an associated article by Sanders (2014). In response to the reactions to the Warriner study within the produce industry, we sought to determine whether these findings were reproducible in a geospatially distinct and major region of fresh produce production and shipping.

Aim: To determine whether the University of Guelph outcomes represent an outlier situation or are representative of the general limited efficacy of washing, cleaning, and sanitizing that occurs at RPC depots during each cycle of reuse. The aim is to develop for produce suppliers, which choose to or are required by customer specifications to pack produce into RPC's, a science-based and data-based informed view of the microbiological status of their multiple-use packing supplies. In addition, the anticipated outcomes were designed to create an opportunity, if warranted, to engage RPC suppliers in a dialogue for improved sanitization of their RPC's between shipments to their users in the pool loop system shared by diverse food providers both nationally and internationally.

Scope: For expediency in addressing the immediate questions raised by growers and shippers, a limited duration baseline survey program was conducted in Santa Maria, CA on RPC's taken directly following discharge from a delivery vehicle at a packing materials distributor. The microbiological survey was designed only to test for indicator bacteria and did not include methods to detect or recover any pathogen or pathogen virulence-markers. The purpose, as described above, was to indirectly assess the general sufficiency of cleaning and sanitizing procedures used by RPC providers without raising unreasonable or unnecessary fears regarding the potential for unintended adulteration on produce packed and shipped interstate, an actionable compliance and reporting issue under existing FDA regulations, by users of the tested RPC lots.

Methods:

Design of sampling regime - With an unknown microbiological heterogeneity among a population of pool-system RPC's and high potential for an unbalanced quantitative distribution of bacteria at specific spatial points among individual RPC units, it was determined to be unrealistic to develop a statistically

derived sampling plan. In advance of having a baseline of data against which to derive the predicted standard deviations, it was necessary to assign practical assumptions that defined the survey sampling approach. A mock 'power analysis' was performed using a combination of past experience in similar microbiological studies of harvest totes and bins and the general outcomes reported by Warriner (2013). Using this as guidance, a standard replicate-unit and date sampling format was developed. A population of 34 RPC units was collected and swab-sampled on each of six dates at a single central distribution location over a 16 day period in 2014. There were two sub-populations selected and tested on each date from among a delivered lot of palletized and wrap-protected/stabilized RPC's. *Random Sample* units were taken from the top, middle, and lower pallet positions of incoming RPC's; two visually clean and dry RPC's were taken from each position and included RPC's from each of four intact pallets delivered (24 total per date). This survey was designed to maximize the detection of viable microbial populations on interior surfaces of delivered RPC units. One large area sponge-swab was collected from the bottom panel of the *Random Sample* RPC and a second large area sponge-swab was applied to the side-panels and hinge areas.

For Cause units (10 per date) were selected from among the pallets sampled by observation to be visibly 'soiled'. The criteria for anticipated *For Cause* triggers were determined from grower and handler input across several commodities and include, primarily, plant/produce residues or intact recognizable tissues, less distinct adhering organic matter or sediments, indistinct concentrated or diffuse residues, free water, and decaying plant matter. During *For Cause* sampling, a typical tipped-swab was applied, using standard technique, to the visibly-soiled or heavily wetted target area(s) that triggered selection. For this survey, 3M™ Swab-Sampler with Letheen Broth (3M Corp; US Food Safety Division) were used. Following this swabbing, one large area sponge-swab was collected from the bottom panel of the *For Cause* RPC and a second large area sponge-swab was applied to the side-panels and hinge areas.

Sampling and Site Preparation – Appropriate measures were taken to protect and ensure the objectivity of the sampling protocols and integrity of the resulting data outcomes. An on-site area to conduct the swabbing of interior surfaces of each unfolded RPC was established to minimize interference from on-going local operations, to facilitate proper aseptic swabbing, and to maximize aseptic swab sample handling. Palletized RPC's in a newly delivered lot were placed in a protected area, as practical, to prevent dust, debris, or other likely sources of external microbial introduction from affecting results during unstacking and sampling. RPC's were removed from the pallet by individuals wearing new sterile disposable gloves; gloves were exchanged between removing exterior stabilizing wrap and between de-palletizing to access sampling locations for each pallet. Reasonable care was taken to ensure handling of the RPC's from their folded edges to minimize possible transfer between unstacking and selection and during observations to separate *For Cause* units from others. The sampler was charged with making a decision as to whether glove exchange was necessary or prudent after handling individual *For Cause* units; typically this was done only when excess free water was abundant. Digital image documentation was conducted for each *For Cause* RPC target area with the swab identification label included and

sequentially coded for each date. Reasonable measures to separate swabbing activities from RPC de-palletizing and selection activities included setting up a separate sampling station to exclude significant environmental dust intrusion from surrounding operations. A small folding table was used as the platform for laying out each RPC for swabbing. Prior to start-up, the table surface was sprayed with a 20% sodium hypochlorite solution and 30 s contact time followed by a 70% isopropyl alcohol solution spray and wipe-down with a new, clean paper towel between each pallet-group being tested.

Swabbing Method – Standard swabbing procedures will be utilized. A visual SOP guide will be made available if requested but for this DRAFT we assume that all contracted parties are familiar and proficient with these standard methods. Rather than a typical 12”x12” area for large format sponge-swabbing, a full surface swab was utilized, as described above. *For Cause* targeted-area swabbing was at the point of visible soil/product residue rather than a typical 4”x 4” area.

Swabs were placed in the pre-labeled sample bag or within the tip-swab capped tube. All individual sample bags containing swabs were uniquely labeled with a sequential coded label and placed in a master container for each RPC pallet location and for each pallet. Swabs were immediately transferred to an insulated cooler with sufficient frozen gel-ice packs to maintain at least 10°C, or lower, during transport to the analytical lab. The total time from sample collection to sample processing was less than 24h. If samples were held before processing, they were placed in a secure area within a refrigerator at approximately 4.0°C $\pm$ 1.5°C

Sample Processing – Standard microbiological methods were be applied for quantitative recovery by adding 25 ml of Butterfield’s Phosphate Buffered Saline directly to the large area sponge-swab bags which was sufficient to ensure the swab was fully covered. Release of bacteria from the swab was performed in a Stomacher at the Medium setting for 45 sec. For the 3M™ Swab-Sampler, the tube was vortexed in the pre-filled 5ml letheen broth prior to aseptic transfer to plating media.

Sample Enumeration - Standard microbiological methods appropriate for each media used were applied for quantitative recovery and enumeration. 3M™ Coliform Count PetriFilm™ (3M™ Microbiology Products Div. St Paul, MN) was used for thermotolerant (fecal) coliform enumeration following the AFNOR validated method 3M 01/2 -09/89C. Following inoculation, PetriFilm™ was incubated for 24h ± 2h at 44°C ± 1°C. 3M™ Enterobacteriaceae Count PetriFilm™ was used to enumerate total enteric bacteria, which overlaps and includes coliform bacteria but is also representative of a broader group of closely related environmental and food borne bacteria. From a sanitation perspective, the group Enterobacteriaceae is a preferred and more stringent indicator of compliance with GMP sanitary process controls due to a generally higher threshold for sub-lethal sanitizer injury and more robust post-exposure recovery compared to the coliform group. Plating, incubation and enumeration followed the AOAC® Official Methods^{SM} 2003.01; 24 hours ± 2 hours at 35°C ± 1°C. Spiked controls and Blank controls were used on each sampling date and for each lot of PetriFilm™ used in this study.

Data Analysis – Data reporting included Mean values, Standard Deviation within and among the 4 pallets tested, Median values, Minimum values, Maximum values, and value Range. Appropriate statistical analysis (e.g., ANOVA) was performed using appropriate SAS protocols to identify significant differences ($P < 0.05$) among RPC populations and SAS Tukey's multiple-comparison tests to compare bacterial count MEANS Statement among pallets, pallet position, *Random* and *For Cause* sample units, and sampling dates.

Results:

The RPC Cleanliness survey was conducted on six dates representing six different deliveries of RPC's from the regional distribution center of the provider. The clearly evident results confirm that there is a high degree of heterogeneity (variability) in numbers of viable bacteria on the interior, direct product-contact RPC surfaces across all evaluated pallets and among all dates. The mean, or average counts, per full surface swab are not particularly high but, from a produce packing and handling perspective, the median and range of values determined are the critical and most risk-relevant data sets to consider. These values are more informative of the overall elevated populations of recoverable indicators of 'cleanliness', due to the potential for individual RPC units to impact food safety, rather than only focus on the collective 'cleanliness' value. The quantitative microbial outcomes from this survey for thermotolerant (aka fecal) coliform (TTC) and Enterobacteriaceae (ENTB; Total Enteric Bacteria) are presented in Tables 1 to 4. Across the six date survey time-course, the Random Sample *per-pallet* range of TTC exceeded log 5 Colony-forming Units (CFU; approximately equivalent to cells)/swab nine of twenty-four times or 37.5% and exceed log 6 CFU/swab two times or 8.3%. These are equivalent to more than 100,000 cells per RPC unit and 1,000,000 cells per RPC unit, respectively.

In these same categories, the *For Cause per-pallet* range of TTC exceeded log 5 Colony-forming Units (CFU; approximately equivalent to cells)/swab seven of twenty-four times or 29.2% and exceed log 6 CFU/swab three times or 12.5%. On one sample date, June 11 (Table 3), the *For Cause* range of viable TTC exceed log 7 CFU/swab or more than 10,000,000 cells when one includes the forty RPC unit replicates for that sampling date.

Across the six date survey time-course, the *For Cause per-pallet* range of ENTB exceeded log 3 Colony-forming Units (CFU; approximately equivalent to cells)/swab eight of twenty-four times or 33.3% and exceed log 4 CFU/swab six times or 25%. These are equivalent to more than 1,000 cells per RPC unit and 10,000 cells per RPC unit, respectively. A different threshold is used for ENTB than TTC as a reflection of the different media and protocols for enumerating this group of indicators. Therefore, although all coliforms belong to the Enterobacteriaceae family, differential traits detected by the method often result in lower numbers of ENTB than TTC in the same sample. High numbers of ENTB, which would

include pathogens such as *Salmonella*, *Shigella*, and *Yersinia*, are widely held to reflect a cleaning and sanitation procedure inadequate to ensure lethality to bacteria of concern for human health.

There were five key categories (Figure 1) defined as the observational basis for separating an RPC into the *For Cause* grouping including; Leafy Green residues (36%), Decay or Brown Leaf residues (16%), Other Plant residues/fragments (6%), Dry White residues (20%), White Mucoid residues (4%), Stickers and Labels (18%). If RPC units with abundant free water are included as a combined factor with other categories this would represent 25% of the *For Cause* selections (Figure 2). Examples of these *For Cause* selections are shown in Figure 3.

Statistically using Mean Separation and Tukey's multiple comparison test, Random sample outcomes for TTC were significantly less than *For Cause* samples four times and less than ENTB five times. *For Cause* units were lower in ENTB only one time. For all other comparisons across pallets and dates, there was no significant difference in Mean values between the two RPC groups or indicator type.

Discussion:

The fundamental conclusion from this survey of multiple-use RPC's in the common reuse pool for direct packing of Raw Agricultural Commodities intended for fresh consumption supports a similar survey conducted by Warriner (2013) in that a significant number of packing-units exceed a reasonable expectation for general cleanliness and frequently fail expected microbiological standards for surface sanitation. Although there are currently no regulatory critical limits or microbial standards for food contact surfaces (FCS) or established and widely recognized industry standards, studies of a similar nature cite values of 125 CFU/50 cm^2 Aerobic Plate Count (APC) as the upper limit for a clean and sanitized FCS (Cunningham et.al., 2011). Using a typical, conservative conversion of 1:100 (TTC:APC) and a value of 2,400cm^2 for the RPC surface, a generous comparable outcome in this survey would be 60 TTC/cm^2 or 144,000 TTC/RPC swab. This was the basis for describing a *high exceedance* at log 5 CFU/swab in comparing individual RPC's. The model Food Code 2013 released by the FDA provides no metric or guidance in assessing FCS microbial limits but common industry practices anticipated a much higher degree of consistency and homogeneity among cleaned and sanitized FCS than observed in this survey across six independent dates. Whether by ATP bioluminescence (not conducted in this study) or direct contact plating, the outcomes of this survey are similar to Cunningham et.al. (2011) and other studies cited therein which commonly find a general lack of agreement between visual (Food Code standard) and objective surrogate or quantitative assessments FCS cleanliness. The need for improved standards and methods to ensure adequate cleaning and sanitization of bins, totes, and other reusable containers is emphasized in Foong-Cunningham et al. (2012).

The primary concern for the RPC user is the potential for these random but often large numbers (> 100,000 CFU), at least nine times in among six dates and three instances on a single date, to represent a risk of direct contact transfer to fresh produce packed or re-packed into these units. The outcome that the majority of RPC units were not statistically different in bacterial indicator levels (indicators of an

effective sanitization process) between randomly selected-visually clean and dry RPC's and visually soiled and wet RPC's was somewhat surprising (Table 1-4). It is plausible, if the sanitization step was inconsistent or inadequate for some incoming RPC's, that a few heavily contaminated RPC's released bacterial loads from the surface, organic and soil residues, or biofilms to process water. The result would be to allow cross-contamination among many RPC's in a given timeframe during daily wash operations. The results from several dates among units sampled from a pallet that had very low to less than detectable levels of the selected indicators would tend to support this possibility. To some degree, it is likely that the experimental design which included a large area swab rather than a typical 50-100 cm^2 area negated some of these differences when evaluating the RPC as a unit rather than the target *For Cause* result.

The concern and potential for RPC-to-product cross-contamination may be substantiated by the observed free water and evidence of multiple instances of decaying plant matter (Fig 3) and sporulation of mold on adherent plant debris (Fig. 4). A similar concern prompted the risk assessment for multiple use corrugated fiberboard cartons used in fresh tomato re-packing (Danyluk and Schneider, 2012). In this study with inoculated *Salmonella*, dry surface to dry surface transfer coefficients were very low but became greater in both directions (carton to fruit and fruit to carton) if moisture or free water was present. Although this survey did not include detection for specific human pathogen indicators, the frequent presence of high numbers of Enterobacteriaceae and thermotolerant coliforms is indicative of an apparent inadequate effectiveness and control of RPC washing and sanitizing which, by extension, would fail to remove or inactivate enteric pathogens. Other studies with similar objectives, but of well recognized hazards, also identified concerns regarding the sufficiency of disinfection process for multiple use plastic containers (Neely et al., 2003). The purpose of this study was not to exaggerate or escalate the concerns shared with us by produce suppliers electing or required to use pool RPC's, but simply to provide the objective data as a baseline for dialogue to implement changes in RPC handling and a basis for informed decisions about multiple-use packing materials.

Recommendations:

The simple and direct outcome from this survey study is that growers, shippers, and re-packers that choose or are required by their customer specifications to pack into RPC's should develop and implement a Standard Operating Procedure for receiving and inspecting incoming loads of RPC or any multiple-use packing container. The results from this study and the other parallel studies conducted by the University of Guelph should be the springboard for discussions with receivers requiring RPC use and with the RPC providers about system-wide improvements in the cleaning and sanitizing process. What is particularly surprising was the frequency of plant residues among the palletized RPC's across the dates of the survey. This may suggest a lapse in implementation of internal standards and procedures for inspection of RPC's passing through the multiple wash phases at the processing facility. The potential for microbial growth, including human bacterial pathogens, on the water saturated and senescing or decaying nutrient source presents a risk of contamination. This is an area of RPC management that clearly needs attention.

The following recommendations appear warranted based on the cumulative evidence for an unreasonable frequency and quantitatively excessive abundance of indicator bacteria on RPC's delivered for use in packing fresh fruits and vegetables that have direct contact with the interior surfaces;

- RPC providers should re-assess the current validation of sanitization process under a range of controlled and standardized surface bio-burden challenge studies.
- Growers, contract harvesters, or handlers should implement a regular inspection SOP for acceptance or rejection of palletized or otherwise delivered RPC's.
- RPC users that elect to wipe down RPC's on-site due to residual water and/or soil and debris so carefully consider evaluating the potential for this procedure to spread microbial contamination from unit to unit, potentially increasing risk.
- Harvest crews and packing operations staff should be trained to recognize and inform supervisors if RPC's, or any multiple-use packing container, do not meet company standards for 'cleanliness'.
- Growers and Handlers should establish a Master Schedule for routine ATP bioluminescence testing or rapid Total Bacterial Load swabbing of RPC's prior to packing into un-lined units.

Citations:

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Figure 1.

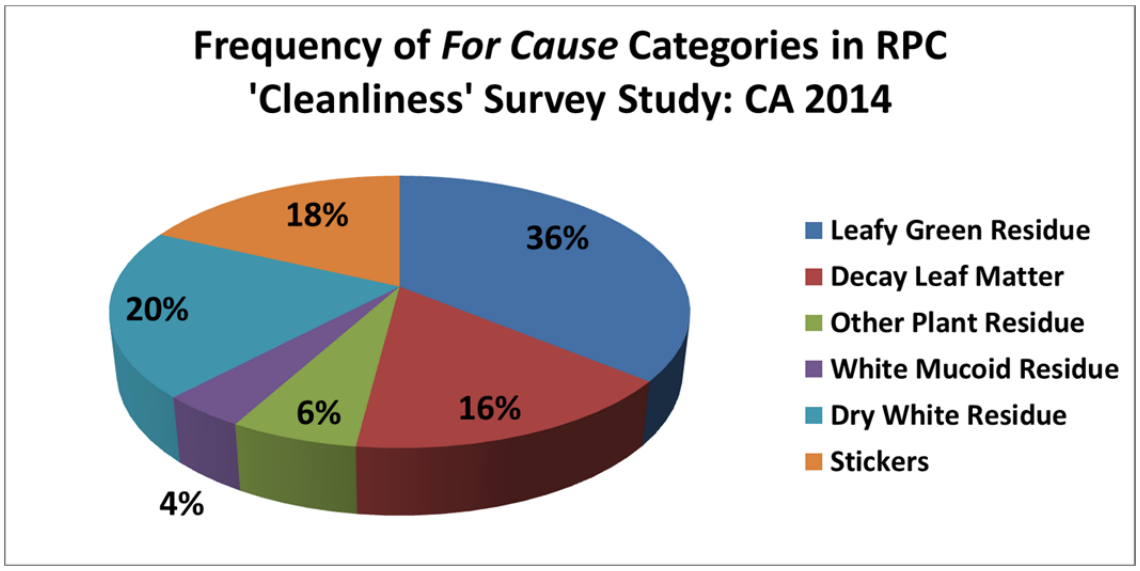

Figure 2.

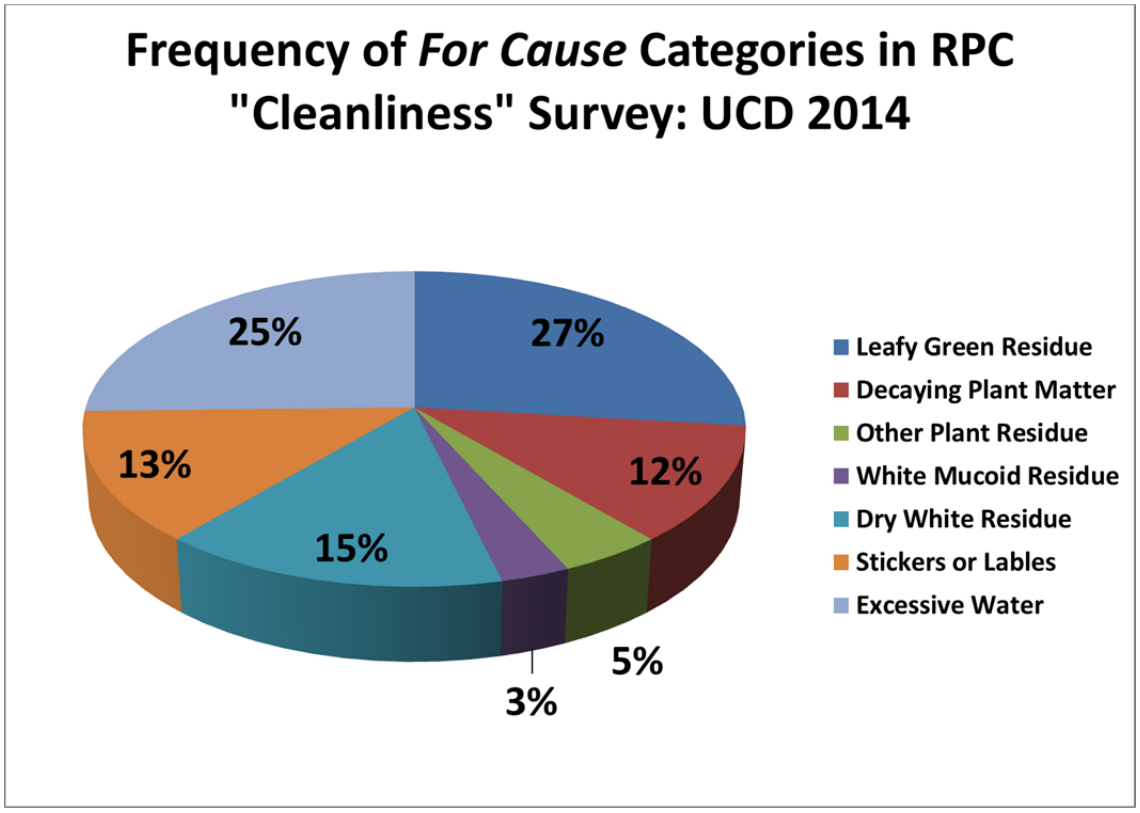

Figure 3. Examples of *For Cause* category across survey dates

Figure 4. Example of decay and fungal sporulation in an RPC unit