Canadian Corrugated and Containerboard Association

Association canadienne du cartonnage ondulé et du carton-caisse

Research Study:

Microbiological Sanitary Status of Reusable Plastic Crates Sampled in British Columbia, Quebec and Ontario

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January 2017

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Version 2: Jan 2017

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Summary

Sampling trials of Reusable Plastic Crates (RPC) were performed at different locations within British Columbia, Ontario and Quebec. On each sampling visit a set of freshly delivered RPC's were inspected for visual residues (plant, soil and dust), labels and damage. Further assessment of RPC's was performed by taking ATP measurements along with enumerating microbial counts of fecal/spoilage indicators (total aerobic count, enterobacteriaceae, coliforms, *E. coli* and Yeast & Moulds). Samples were taken from visibly soiled crates (plant or soil residues excluding labels) with the majority being randomly selected. It was found that the sanitary status of RPC's was independent of the geographical location were sampling was performed or if visible soils were present. RPC's were defined as sanitary based on the following microbiological criteria ATP <1000 RLU; TAC <4 log cfu/crate, enterobacteriaceae <3 log cfu/crate; coliforms <3 log cfu/crate; Yeast and Moulds <3 log cfu/crate and absence of *E. coli*. Collectively, the study found 83% of RPC's samples had total aerobic counts and Yeast & Mould counts, exceeding the standard. Overall, 19% of RPC's failed on ATP readings with 7% failing on high levels of enterobacteriaceae. *E. coli* was recovered on one sampling trip and represented a carriage rate of 4% of the total number of crates tested (114 units). When the results of the trials were compared to that performed in 2014 it was evident that the numbers failing on total aerobic counts had increased in 2016 but the frequency of fecal indicators had decreased significantly. However, the reoccurring incidence of labels from previous uses and visibly soiled RPCs may suggest the low incidence of fecal indicators is more related to best handling practices rather than improved sanitation.

Background

Fresh produce remains the leading cause of foodborne illness outbreaks within North America (Francis et al. 2012; Olaimat and Holley 2012; Goodburn and Wallace 2013). To date the majority of focus on improving food safety has been at primary production and post-harvest levels (Warriner et al. 2009). In addition to the microbiological standards relating to soil and water there is additional focus on food contact surfaces that could potentially act as sites of cross-contamination for virulent pathogens. For example, it is established that slicers and knives along with hands of workers can be potential sites for cross contamination during produce handling (Keskinen et al. 2008a; b; Keskinen and Annous 2011; Bin et al. 2012; Brar and Danyluk 2013; Jensen et al. 2013; Sreedharan et al. 2014; Zilelidou et al. 2015). The role of crates in disseminating pathogens is less well defined despite the recognized risk. It has been reported that *Salmonella* introduced onto RPC's can be transferred and more significantly, develop biofilms that represents continuous sources of contamination (Shi et al. 2016).

Moreover, biofilms cannot be removed during common sanitizing cycles employed by industry with the consequence of circulating human pathogens through production, processing and retail (Shi et al. 2016).

In recent times the major retail chains have requested suppliers to replace board packing with reusable plastic crates (RPC's). The main perceived incentives for RPC's include sustainability, price stability and reduce waste handling at the retail level (Koskela et al. 2014). Yet, because crates are reused there is the potential to disseminate contamination from one grower to the next. Plant pathogens are one concern but of equal, if not greater, is the potential of virulent pathogens such as STEC, *Salmonella* and *Listeria* monocytogenes, in addition to enteric parasites to be disseminated (Shi et al., 2016). To address food safety concerns, RPC's are sanitized at centralized facilities after being returned by retail. A typical sanitizing cycle encompasses a water rinse followed by a caustic wash and finally passing through a peroxyacetic acid shower (IFCO, personnel communication). The RPC's are inspected for damage and visual organic residues then passed or returned for further cleaning. At the end of the process the RPC's are stacked, wrapped in plastic film then re-distributed to growers/packers. In principle, it may be expected that the aforementioned sanitation cycle and inspection would provide adequate assurance on the sanitary quality of RPC's. However, in recent studies performed both in Canada and the US has raised food safety concerns. Specifically, sampling trials performed in 2013 and 2014 identified issues relating to sanitary standards of RPC's delivered to grower/packers (Mullinder, 2014). From the data generated it was found that over 70% of the RPC's had total aerobic counts in excess of those expected from a clean surface (4 log cfu/crate. Enterobacteriaceae levels on 51% exceeded the 3 log cfu/create limit with 13% harboring *Escherichia coli*. From visual inspection, 10% of the crates were dusty or had visible debris with over 30% carrying labels from previous use. In a corresponding study performed by Dr Suslow of UCDavis using the same microbiological criteria but sampled both random creates, in addition to selected units carrying visible soils. It was found that 37.5% of randomly selected RPC's exceeded bacterial counts expected of a clean surface. Of the visibly soiled RPC's over 30% tested positive for high enterobacteriaceae counts along with over 50% having decaying plant matter residues.

In 2015, the Reusable Packaging Associated (RPA) issued guidelines on handling and sanitation of RPC's. Within the guidance document, the importance of best practices to be implemented when using RPC's although fell short of suggesting microbiological criteria to define a satisfactory sanitation process for disinfecting crates. Regardless of this omission, the following study was directed towards determining if the guidelines, along with other initiatives, within the RPC sector had improved the sanitary quality of crates used within the fresh produce sector.

Objectives

- To determine the sanitary status of RPC's freshly delivered to grower/packers within Ontario, Quebec and British Columbia.

- To establish if visual soils on RPC's and/or ATP measurements can be used as a predictor for reporting the sanitary status of RPC's.

Methods

Five fresh produce packing operations located in British Columbia, South Western Ontario and the South Shore area of Quebec were visited in the course of the study between July and November 2016. On each sampling visit up to 36 RPC units were sampled that included a maximum of 12 visibly soiled units (For Cause) with the remainder being randomly selected. Up to three visits per location were performed with a separation of a minimum of 5 weeks to ensure new batches of RPC's were sampled. In total, 144 RPC units were sampled over the course of the study.

A visual inspection was performed to note visible soil or organic debris and labels attached from previous users. After visual inspection samples were collected to determine ATP and microbial counts. ATP swabs were taken from approximately half the area of the base and a further swab used to sample half an interior panel (Outline in SOP Annex 1). The sponge samples were returned to the laboratory and submerged in 30 ml saline then stomached for 30s. A dilution series was prepared in saline then plated on Total Aerobic Count, *E. coli*/Coliform and enterobacteriaceae petri films and Yeast & Mould Petri Films. The TAC was incubated at 34°C for 48h with the other petri films being incubated at 37°C for 24h. Yeast & Mould petri films were incubated at 25°C for 5 days.

There is no specific criteria set for the microbiological standards for RPCs and as a consequence those based on food contact surfaces were used to designate pass or fails. Specifically, for ATP testing a fail was designated at >3 log RLU, for TAC the limit was 4 log cfu/crate, enterobacteriaceae or coliforms >3 log cfu/crate and presence of *E coli*. The limit for Yeast & Mould counts limit was set at 3 log cfu/crate.

Results

Visual Assessment

The participating grower/packers were requested by the University of Guelph to segregate visually soiled RPC's during the course of their activities. Although, not quantitative the grower/packers reported sporadic occurrence of visibly soiled RPC's with produce residues being most frequently encountered along with dust. When one packer was asked to make an estimate they consider less than 2% of RPC's delivered to their facility would be considered unclean as depicted in Annex 2.

In the course of random sampling of RPC's it was notable that labels from previous users of the RPC's remained attached (Annex 2). Again, although not quantitative, it was estimated that more than 10% of the RPC's tested had intact labels or the remnants of previously applied labels. In the course of sampling no damaged RPC's were encountered.

ATP and microbiological sanitary assessment of RPC's

RPC's were sampled at different locations within Canada with the same protocols being applied at each site. It should be noted that not all sites had access to an ATP meter and on occasion no For Cause RPC's were set aside by the participating grower/packer.

Table 1: ATP (RLU) readings taken from unused Reusable Plastic Crates sampled at different produce packing stations located in Southern Ontario, Quebec or British Columbia.

		ATP (log RLU/100cm^2)				
Location #	**Total RPC Tested**	**Min**	**Max**	**Median**	**Pass**	**Fail (%)**
Ontario 1	24 selected	0.91	2.61	1.61	24 (100%)	0
	52 random	0.90	4.38	2.16	36 (69%)	16 (32%)
Ontario 2	8 random	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested
Quebec	10 random	1.57	3.39	2.31	8 (80%)	2 (20%)
BC	24 selected	1.52	3.28	2.34	19 (79%)	5 (21%)
	44 random	1.48	3.21	2.26	42 (95%)	2 (5%)

RPC returning an RLU reading > 3 log. Ten RPCs were sampled on each visit and taken from a different lot of crates on each occasion. Selected are For Cause with Random being RPC's randomly selected from pallets of delivered crates.

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Table 2: Microbial counts recovered from unused Reusable Plastic-Crates sampled at different produce packing stations.

A		**Log cfu/Crate**				
Ontario 1 (selected)	**Total RPCs Tested**	**Min**	**Max**	**Median**	**Pass**	**Fail (%)**
TAC	24	4.84	8.32	6.58	0	24 (100%)
Enterobacteriaceae	24	2.62	7.47	5.05	1 (13%)	23 (88%)
Coliforms	24	0	6.13	3.07	14 (58%)	10 (42%)
E. coli	24	0	0	0	24 (100%)	0
Yeast and Moulds	24	2.48	6.58	4.53	0	24 (100%)

A		**Log cfu/Crate**				
Ontario 1 (Random)	**Total RPCs Tested**	**Min**	**Max**	**Median**	**Pass**	**Fail (%)**
TAC	52	3.32	10.00	5.19	7(13.5%)	45(86.5%)
Enterobacteriaceae	52	1.95	9.47	4.38	8(15.4%)	44(84.6%)
Coliforms	52	0	3.66	2.08	50(96.2%)	2(3.8%)
E. coli	52	0	2.15	2.09	47(90.4%)	5 (9.6%)
Yeast and Moulds	52	0	9.77	4.94	3(5.8%)	49(94.2%)

B		**Log cfu/Crate**				
Ontario 2 (random)	**Total RPC Tested**	**Min**	**Max**	**Median**	**Pass**	**Fail (%)**
TAC	8	2.78	5.10	4.50	1 (14%)	7(86%)
Enterobacteriaceae	8	0	4.48	4.0	6 (67%)	2 (33%)
Coliforms	8	0	3.26	2.76	7 (86%)	1 (14%)
E. coli	8	0	0	0	8 (100%)	0
Yeast and Moulds	8	0	4.64	3.03	4 (50%)	4 (50%)

C		Log cfu/Crate				
Quebec (random)	**Total RPC Tested**	**Min**	**Max**	**Median**	**Pass**	**Fail (%)**
TAC	10	5.71	9.8	7.25	0	10 (100%)
Enterobacteriaceae	10	0	2.14	2.07	10 (100%)	0
Coliforms	10	0	4.33	2.07	9 (90%)	1 (10%)
E. coli	10	0	0	0	10 (100%)	0
Yeast and Moulds	10	1.48	4.59	3.95	2 (20%)	8 (80%)

D		Log cfu/Crate				
BC (selected)	**Total RPCs Tested**	**Min**	**Max**	**Median**	**Pass**	**Fail (%)**
TAC	24	4.73	7.8	5.63	0	24 (100%)
Enterobacteriaceae	No	No	No	No	No	No
Coliforms	24	0	3.37	3.35	22 (92%)	2 (8%)
E. coli	24	0	0	0	24 (100%)	0
Yeast and Moulds	24	3.74	6.8	4.71	0	24 (100%)

D		Log cfu/Crate				
BC (Random)	**Total RPCs Tested**	**Min**	**Max**	**Median**	**Pass**	**Fail (%)**
TAC	44	4.0	6.37	5.04	0	44 (100%)
Enterobacteriaceae	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested
Coliforms	44	0	2	0	44(100%)	0
E. coli	44	0	0	0	44 (100%)	0
Yeast and Moulds	44	3.3	4.9	4.02	0	44(100%)

Table 3: Pooled data for ATP readings and microbial counts recovered from RPCs sampled at different produce packers located within Ontario, Quebec and British Columbia.

Test		**RLU/100 cm^2 or Log cfu/Crate**				
	Total RPCs Tested	**Min**	**Max**	**Median**	**Pass**	**Fail (%)**
ATP	48	0.9	3.28	1.95	43 (90%)	5(10%)
TAC	48	4.73	8.32	6.15	0	48(100%)
Enterobacteriaceae	24	2.62	7.47	6.24	1 (4%)	23 (96%)
Coliforms	48	0	6.14	5.19	36 (75%)	12 (25%)
E. coli	48	0	0	0	48 (100%)	0
Yeast and Moulds	48	2.48	6.8	5.12	0	48 (100%)

Random sample

Test		**RLU/100 cm^2 or Log cfu/Crate**				
	Total RPCs Tested	**Min**	**Max**	**Median**	**Pass**	**Fail (%)**
ATP	106	0.90	4.38	2.23	86 (81%)	20 (19%)
TAC	114	2.78	10.01	5.12	8 (7%)	106 (83%)
Enterobacteriaceae	70	1.77	9.47	3.94	22 (93%)	48 (7%)
Coliforms	114	0	4.33	2.08	110 (97%)	4 (3%)
E. coli	114	0	2.15	2.10	109 (96%)	5 (4%)
Yeast and Moulds	114	0	9.77	4.15	9 (8%)	105 (82%)

Sanitary status of RPC's sampled in different regions

The indicators applied in the study can be sub-divided into general microbial loading (ATP, TAC), potential source of fecal contamination (enterobacteriaceae, coliforms, *E. coli*) and potential spoilage microbes (yeast and moulds). There is no microbial criteria for RPC's and hence those expected of a food contact surface were selected.

RPC's randomly sampled within Ontario 1 location recorded 82% failure on TAC with 27 % ATP. There was also a high failure rate for enterobacteriaceae and significantly, a proportion (5%) tested positive for *E. coli* (Figure 1). The RPC's recorded >90% failure rate on Yeasts & Moulds. Those RPC's sampled in Ontario 2 location exhibited a similar profile to that of Ontario 2 although the % fails on fecal indicators were lower with no *E. coli* being recovered. The failure rate for Yeasts & Moulds was also lower but still unacceptable high at >50%. The microbial levels found in RPC's recovered from RPC's tested in Quebec and British Columbia exhibited intermediate profiles between Ontario 1 and Ontario 2. Specifically, the failures based on TAC and Yeast & Moulds were high with lower proportion failing on fecal indicators.

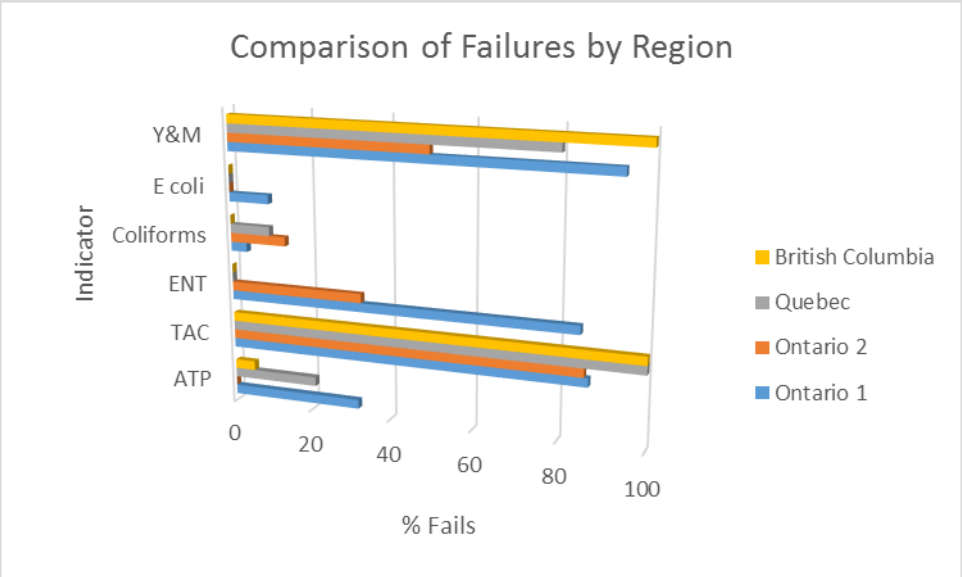

Figure 1: % of randomly selected RPC's that exceeded the ATP or indicator counts sampled in different geographical regions.

Selective vs Random sampling of RPC's

When available, visibly soiled RPC's were sampled and counts compared to those sampled randomly.

The microbial counts "For Cause" RPC's (i.e. visibly soiled excluding labels) were compared with those obtained from randomly selected, clean, units (Figure 2). It was found that the For Cause RPC's had lower variation in ATP measurements and antimicrobial counts compared to randomly sampled units. However, overall there was a poor correlation between the visual appearance of RPC's and the microbial loading. Nevertheless, the residues encountered on For Cause RPC's may represent a food safety hazard due to promoting biofilm formation that could include pathogens.

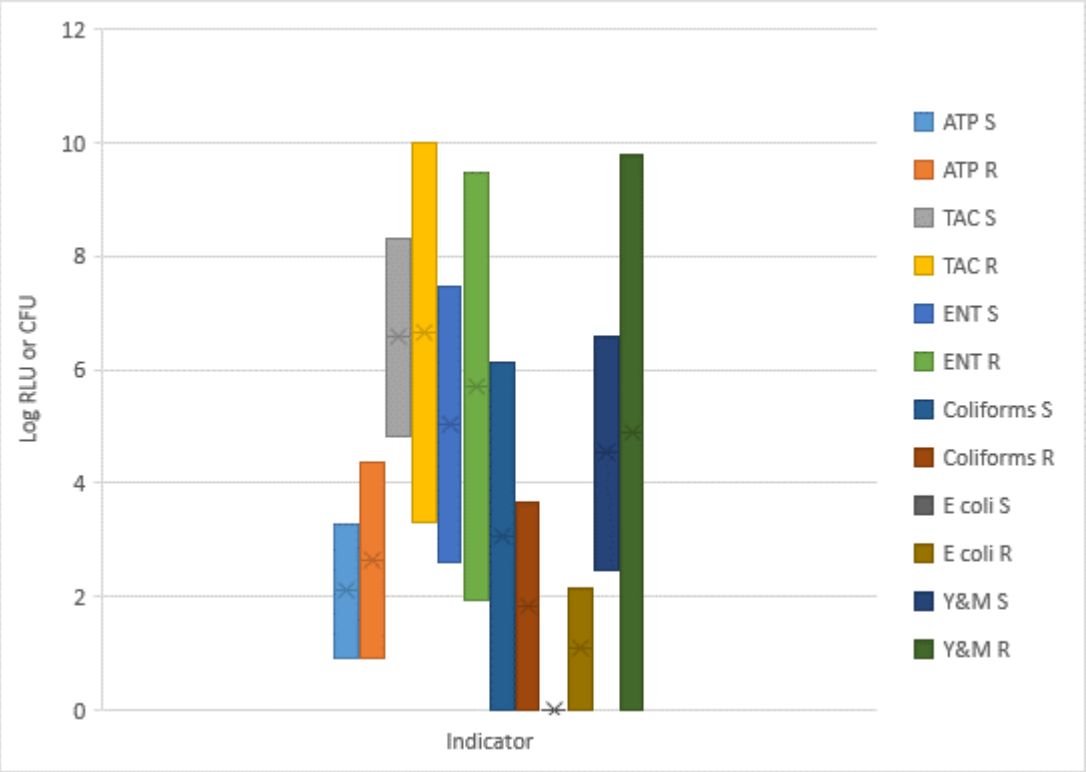

Figure 2: Range of ATP and microbial counts recovered from For Cause RPC's (S) or randomly selected crates (R). The trials were performed within Ontario and British Columbia with 12 visibly soiled being sampled along with 24 randomly selected clean RPC's.

ATP: ATP RLU; TAC, Total Aerobic Count; ENT, enterobacteriaceae; E. coli, *Escherichia coli*; Y&M, Yeast and Moulds.

Correlation between ATP readings and total aerobic counts

ATP readings provides a quick and low cost alternative to culture based methods when assessing the sanitary quality of RPC's. The pooled data of randomly selected RPC's from Ontario and British Columbia was used to determine if a correlation between ATP readings and total aerobic counts. No significant correlation was found between ATP measurements and microbial counts indicating that the former method for assessing sanitary quality of RPC's would not be reliable (Figure 3).

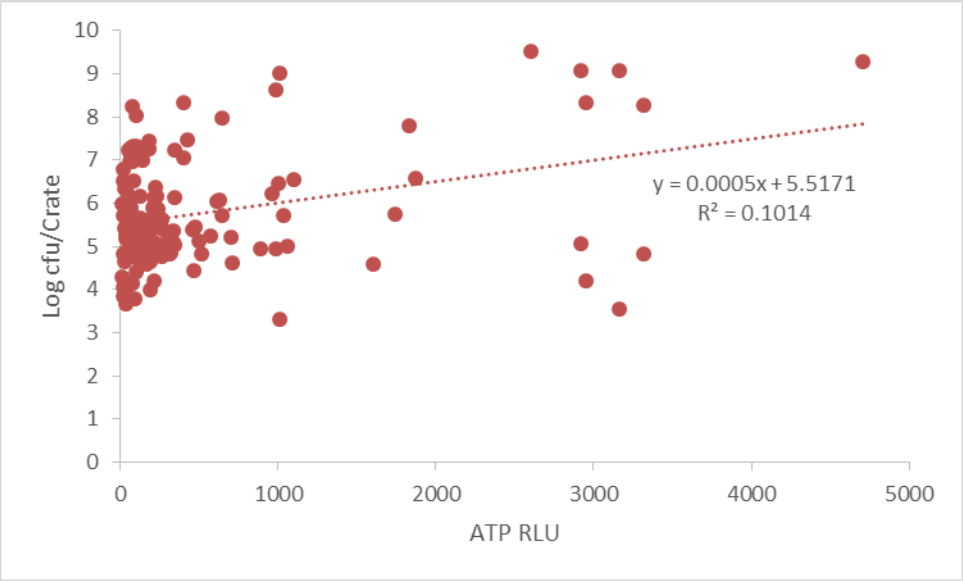

Figure 3: ATP (RLU) counts vs Total Aerobic Count (TAC) of RPC's sampled within Ontario and British Columbia.

Comparison of sanitary status of RPC's sampled in 2016 vs 2014

When the data on the sanitary status of RPC's was compared with previous studies it was found that there was generally on overall improvement with respect to fecal indicators although the fails associated with bacterial counts had increased (Figure 4). The greatest improvements related to the % RPC's failing due to levels of coliforms and enterobacteriaceae. It is unclear if

this could be attributed to improved sanitation or reflect the lack of contact with contamination sources.

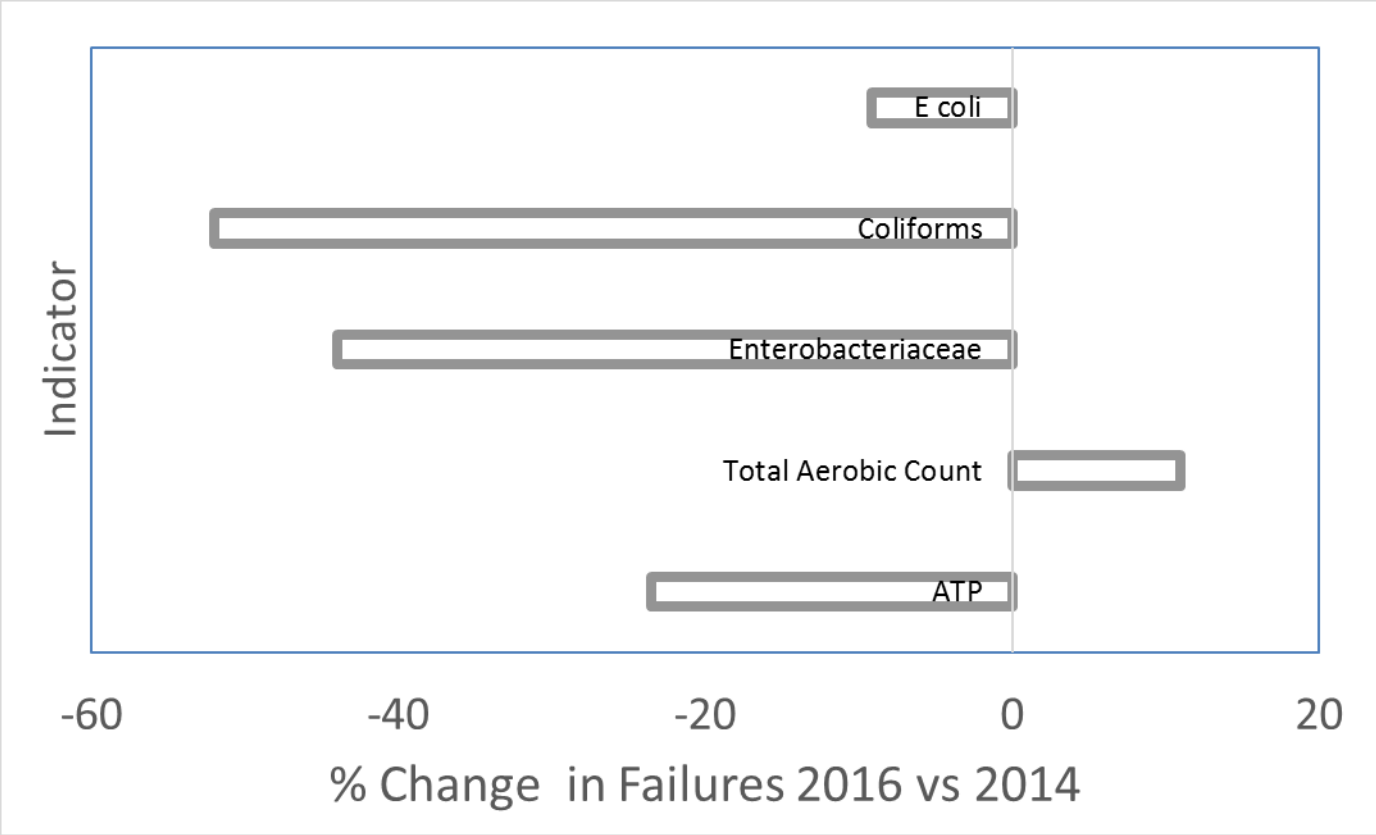

Figure 4: Comparison of % failures of RPC's sampled in 2016 compared to the 2014 study.

Discussion

Collectively the data collected would suggest that there has been improvements in the sanitary status of RPC's compared to sampling studies performed in 2014. The most significant change was the absence of damaged crates that were more frequently identified in previous studies. In addition, the low failure rate due to the presence of fecal indicators would also suggest improvements have been made. Such an improvement could have been to better handling of crates at different points in the produce chain or more effective sanitizing procedures. The latter is unlikely given the high failure rate of RPC's due to high levels of total aerobic counts and Yeast & Moulds that would suggest the sanitation procedures for crate disinfection were lacking. This is further supported by encountering visibly soiled crates along with the presence of labels from previous users.

Overall there were no differences in the sanitary status of RPC's sampled at the different locations. This would confirm that the effectiveness of sanitation of crates is dependent on

geographical location. Therefore, it can be assumed that although the current study was restricted to four sites within Canada the results are reflective across the country and probably North America.

There was a low recovery of *E. coli* from RPC's and the fecal indicator was only encountered on a single sampling trial. Although low, the finding again raises food safety concerns given that pathogens such as Shiga producing *Escherichia coli* can cause illness at levels of 10 cells in a susceptible host. In this regard, the food safety issues related to RPC's continue to persist.

Food safety management systems are moving from HACCP-based systems to more Prevention & Control. Given that contaminated RPC's represent a hazard likely to be encountered there is a need to establish prevention or control steps. With regards to the former, one preventative approach is to inspect or test RPC's prior to use. Microbial testing using culture based methods no feasible due to time and need for containment facilities therefore monitoring would be restricted to ATP measurements or visual inspection. However, the findings in the current study underlined that neither visual inspection or ATP measurements are suitable to report on the sanitary status of RPC's. Consequently, more effective control steps in the form of washing and sanitizing RPC's are required.

Conclusions

- The majority of RPC's sampled in the current study failed on the basis of total aerobic counts and Yeast & Moulds. The failure rate for fecal indicators was also high but an improvement based on previous years.
- The sanitary status of RPC's was independent on the geographical regions sampled suggesting that the findings of the study are reflective of sanitary status of reusable crates across Canada.
- Visual assessment and ATP readings are unsuitable approaches to assess the sanitary status of RPC's.
- The failure to sanitize RPC's effectively along with the recovery of *E. coli* from a proportion of crates would confirm that food safety concerns still persist with respect to reusable plastic crates within the produce sector.

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Date: December 2016

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ANNEX 1

Reusable Plastic Container Sampling and Testing Protocol (final)

May 3, 2016

This protocol provides information and methodologies for the follow-up microbial assessment / cleanliness evaluation of reusable plastic containers (RPCs) used for the storage and transport of fresh produce. RPC sampling will occur at multiple field sites across the United States with laboratory analyses being performed at Primus Laboratories Santa Maria, CA location. The protocol was developed by Haley & Aldrich and Primus Laboratories with input from Trevor Suslow and the Fibre Box Association.

1. Sampling sites:

   RPCs will be sampled at three unique grower/shipper locations in three different geographic regions: Florida, California and the Pacific Northwest.

2. Sampling date:

   Sampling will occur on a single day per site.

3. The container selection and sampling process will be conducted following Good Laboratory Practices (GLP). The RPCs chosen for sampling as well as actual swab samples will be handled according to standard GLP chain-of-custody technique to ensure sample integrity and identity.

4. Container selection:

   a. Containers will be selected from a four different RPC pallets, wrapped in green plastic directly as received from the RPC cleaning depot.[1] Any pallet wrappings will be inspected for evidence of substantial soil/dust deposits or other foreign materials. If deposits are observed alternative pallets will be selected. If all pallets have external deposits on pallet wrappings, a dry-brush procedure will be used to exclude as much as practical before removing the wrapping. Regardless of final condition, wrappings will be removed by technical staff wearing sterile disposable gloves and pulled outward and down from the top rather than lifting over the palletized stack.

      After the pallet is unwrapped, individual RPCs will be removed from the pallet for microbial sampling, by technical staff wearing new sterile gloves, across the bottom, middle and top of the pallet. Chosen RPCs will be handled by an exterior surface during unstacking and selection. Gloves will be changed as necessary to mitigate cross-contamination between RPCs. Generally, this will be required in cases of excessive gross visible contamination or if free water is abundant.

   b. Thirty six RPCs will be sampled at each site.

      i. Twenty four *at random* containers will be sampled per site.

         Note: *At random* RPCs are defined as containers that are visibly free of any gross sources of contamination (residual organic materials, old labels, or residual water).

[1] IFCO has indicated that the RPCs sent to grower shippers on pallets with "green wrap" have gone through their cleaning and sanitization process and are identified as clean and ready for use.

- Four pallets: Two pallets from two different lots per site (where available). If two lots are not available samples will be taken from four different pallets within the same lot."

- Six containers will be sampled per pallet.

- Two RPC's from the top, middle and bottom from each pallet.

ii. Twelve for cause RPCs will be sampled per site.

Note: *For cause* RPC are defined as containers that are visibly contaminated with organic materials, old labels, or residual water.

- Four pallets: Two pallets from two lots (where available) per site. If two lots are not available samples will be taken from four different pallets within the same lot.

- Three containers from each of the four different pallets.

- Digital documentation (image) shall be taken of each for cause RPC.

5. On-site sampling area:

a. An on-site area to conduct the swabbing of each erected RPC will be established with effective separation from on-going local operations, de-palletizing and selection activities, and any other potential sources of contamination or sampling interference. The on-site area will be prepared to facilitate proper aseptic technique in sampling/sample handling:

b. An on-site work bench or table, small folding table, or similar platform will be used for sampling activities

c. Prior to sampling, the table surface will be sprayed with a hard-surface sanitizing antimicrobial (bleach and/or 70% alcohol), and/or covered with a new sheet of protective paper. This activity will be performed between each pallet being tested.

6. Container Identification:

Each RPC selected for sampling will be labeled with a unique identifier and include:

a. Pallet specific prefix to include IFCO and pallet-specific numeric digits.

b. RPC specific number to include:

i. Position on the pallet: T (top), M (middle) or B (bottom).

ii. Type of Sample: R (at random), C (for cause).

iii. Pallet identifier: A, B, C or D

iv. Container number: 1-6 for at random containers and 1-3 for cause containers.

Note: The Lab may establish a different number system so long as the information noted in 6b is captured and consistently used to identify individual containers.

7. Microbial Sampling:

    a. Sampling of the RPCs will be performed using aseptic techniques, and in accordance with PrimusLabs SOP 14-20 “Environmental (Sponge) Sampling”.

    b. Two microbial samples will be taken from at random containers:

        i. One sponge will be used to wipe the entire interior bottom surface of the RPC.

        ii. One sponge will be used to wipe the interior side and corner surfaces of the RPC.

    c. Three microbial samples will be taken from for cause containers:

        i. One swab will be taken of the visible contamination on the container.

        Note: A digital image of the contaminated area of for cause RPCs will be documented with a digital image.

        ii. One sponge will be used to wipe interior bottom surface of the RPC (other than the visibly contaminated area – if applicable).

        iii. One sponge will be used to wipe the interior sides and corner surfaces of the RPC (other than the visibly contaminated area – if applicable).

    d. Interior dimensions or standard IFCO model number of the RPCs will also be recorded.

8. Sample Transportation:
    a. All individual sample bags containing swabs/sponges will be uniquely labeled with permanent ink or bar-code label and placed in a master container per individual corrugated box, pallet location, pallet and delivery.
    b. A “Sample Log Sheet” will be generated for each sampling event, reflecting transit time and receipt at the laboratory. This Sample Log Sheet will be signed by the Sampler and Laboratory Personnel to verify it’s accuracy.
    c. All samples will be placed in a cooler with blue ice, with the temperature of the cooler and three individual sample bags recorded upon receipt at the laboratory.
    d. If samples are not processed immediately upon receipt at the laboratory, they will be placed in a secure area in a walk-in cooler or refrigerator at 2.0 to 4.0°C. Total time from sampling to processing will not exceed 24 hours.

9. Microbial Sample Identification:

    a. Each microbial sample will be labeled with the RPC container identifier specified in 6b with a further notation regarding what part of the RPC was sampled:

        i. Interior bottom – B.

ii. Interior side/corners – S.

iii. Visibly unclean/free water interior surface – V.

Note: The Lab may use different identifiers so long as the information noted in 6b and 8a are captured and consistently used to identify individual samples.

10. Standard Microbial Methods:

    a. All microbial swabs/sponges will be processed in triplicate using standard quantitative microbiological methods for the *Enterobacteriaceae* and Coliforms. Swabs/sponges will be processed in accordance with PrimusLabs SOPs 14-05 (Coliforms) and 14-116 (*Enterobacteriaceae*), respectively.

    b. The number of colony forming units (CFU) for each of the triplicate samples will be recorded.

    c. The average CFU per swab and per surface area swabbed will be generated and recorded.

11. ATP swab sampling/analysis:

    a. ATP testing will be performed prior to sampling for Standard Microbial tests.

    b. ATP swab samples will be taken of the same surfaces noted in 7b and c. The surface area swabbed shall be recorded and be consistent with that recommended by the ATP system manufacturer (sampling will be performed in accordance with PrimusLabs SOP 14-118 “ATP Testing”).

    c. ATP samples will be labelled identically to the standard microbial samples (see 6b and 8a above), with the exception that ATP will be added as a final suffix.

    d. The laboratory will perform RLU measurements on-site within one hour of ATP sample collection.

    e. The laboratory will record the RLU reading associated with each swab sample.

12. Laboratory data reporting:

    a. Results of standard microbial analyses including individual sample and the sample averages (per swab and per surface area) will be determined.

    b. Results will be compiled and submitted as raw tabular data by the laboratory.

    c. Results of the ATP analyses will be recorded and submitted as raw tabular data by the laboratory.

    d. Digital images of RPCs with gross contamination will be submitted as individual files, labeled with the unique container/swab identifier.

ANNEX 2

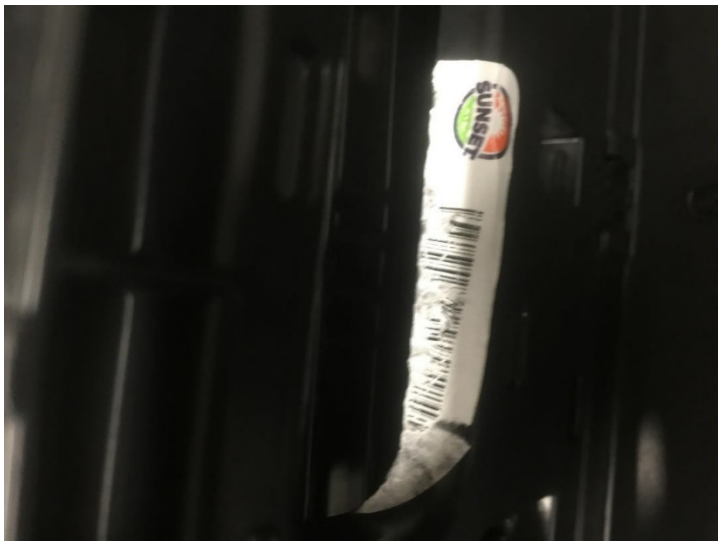
Examples of For Cause (Selected) RPCs

For Cause RPCs

Randomly Selected RPCs

Examples of RPCs with Labels

RPCs with Labels



RPCs with Labels

RPCs with Labels