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FINAL REPORT

Evaluation of isopropyl alcohol for the sanitation of maple sap collection systems

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ABSTRACT

In 2009 and 2010, trials were conducted at six maple sugarbushes to assess the efficacy of 70% isopropyl alcohol (IPA) in conjunction with vacuum for the sanitation of maple sap collection systems, in comparison with sodium hypochlorite solution at 600 ppm (SH) pushed with air in the system. The results obtained by ATP bioluminescence demonstrated a notable reduction in internal surface contamination of the sap collection system when IPA was utilized, with a sustained effect observed and after a few months following the treatment, in accordance with the established procedure. A reduction in contamination was observed for all systems evaluated and all types of surfaces tested (mainlines, lateral lines, droplines and spouts). Nevertheless, the performance of the SH was only observed following a brief period (two days) after the treatment. Following an extended period (several months), the systems treated with bleach solutions exhibited contamination levels that were nearly equivalent to those observed prior to treatment. This contrasts with the findings for IPA. Consequently, sanitation with IPA represents an efficacious method for maintaining low contamination levels in the sap collection system. Further research will be conducted in order to ascertain whether this practice affects the quality of maple products and sap yield. As this IPA is flammable, it must be utilized and stored in accordance with the established safety standards.

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Figure 4 Results of microbial contamination (ATP bioluminescence) of the CE system for the mainlines and lateral lines, with and without sodium hypochlorite treatment at 600 ppm (SH) prior to sanitation and in fall. Means with different letters are significantly different ($p < 0.05$).

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CONTEXT

The impact of microbial contamination of maple sap on the quality and properties of maple syrup has been well documented in the literature (Edson, 1912; Morselli and Whalen, 1991; Fabian and Buskirk, 1935; Holgate, 1950; Lagacé et al, 2002). The metabolic processes of microorganisms can induce alterations in the composition and properties of the sap, which can have a detrimental impact on the flavor, color, and texture of the resulting syrup. More recently, research has also attributed a negative impact on sap flow performance to microorganisms developing in the sap collection system (Perkins, 2009; Perkins et al., 2010). This phenomenon may be explained by the potential return of contaminated sap residue from the system to the taphole when conditions conducive to this process are present. These conditions may include negative pressure in the taphole induced by a significant temperature difference between night and day, as well as significant contamination of the system.

In light of these considerations, it becomes imperative to maintain an acceptable level of cleanliness within the collection system. This is why the most recent editions of the good practice guides recommend the sanitation of the sap collection system using sanitizers that have been approved for this purpose (Allard and Belzile, 2004; Anonymous, 2004; Chapeskie et al., 2006). However, the proposed sanitation procedures are confronted with a number of challenges. One such challenge is the formation of biofilm within the collection system, which has been demonstrated to reduce the effectiveness of sanitizers and contribute to the maintenance of microbial viability within the system (Lagacé et al., 2006a, Lagacé et al., 2006b). A second potential issue is the possibility of residual sanitizers being present in the maple syrup. This possibility is attributed to the fact that rinsing the system after sanitation is a challenging process, and that a low residue content in the sap can potentially become a concern when it is processed into syrup since, for some sanitizers, the residues will concentrate (up to 40-50X) during evaporation. The sap collection network is typically sanitized using a 600 ppm sodium hypochlorite solution (SH), which is introduced into the system at the conclusion of the sugar season through a process that incorporates air to enhance the turbulence effect. A minimal quantity of the solution is expelled from each spout and the head of each main mainline, followed by a rinse with potable water or reverse osmosis filtrate. Recently, the Canadian Food Inspection Agency (CFIA) approved the use of a 70% isopropyl alcohol solution (IPA) for cleaning maple sap collection systems. This product has been utilized in experimental settings in select maple sugarbushes in previous years. The anticipated advantage of utilizing this product is that it provides an alternative to SH, which contains chlorine and has the potential to leave residues (sodium). For example, IPA does not contain chlorine and is unlikely to leave a residue in the syrup since it is volatile at low temperatures. Nevertheless, prior to recommending this product for use in the sanitation of the maple sap collection system, it was necessary to conduct tests to verify its effectiveness.

PRIMARY AND SPECIFIC OBJECTIVES

Primary objective

To assess the efficacy of 70% isopropyl alcohol (IPA) as a sanitizing agent for the maple sap collection system.

Specific objectives

- To evaluate the effectiveness of IPA for sanitizing the maple sap collection system in multiple sugarbushes by comparing it with a control system that has not undergone sanitization.
- To compare the effectiveness of IPA with that of a 600 ppm sodium hypochlorite solution (SH).

MATERIAL AND METHODS

Description of Maple Sap Collection Systems

To assess the efficacy of sanitation treatments, six sugarbushes were selected for study. The control group consisted of unsanitized samples, while the treatment group underwent sanitization with either IPA or SH. Table 1 presents a summary of the primary characteristics of these systems. It should be noted that, for the Centre ACER system, no control was employed, as the two available systems were utilized to evaluate the efficacy of IPA and SH.

Table 1 – Description of the systems employed for the assessment of the effectiveness of sanitation

Producer	Line / Treatment	Diameter x Manifold Length (ft)	Number of taps	Age (years)	Previous sanitation method
#1 BF	Control	1" x 1332'	319	5 +	IPA
	IPA-Sanitized	1" x 1295'	330	5 +	IPA
#2 GTL	Control	1" x 1115'	363	5 +	IPA
	IPA-Sanitized	1" x 1499'	196	5 +	IPA
#3 MS	Control	1" x 790'	148	5 +	IPA
	IPA-Sanitized	1" x 862'	265	5 +	IPA
#4 RL	Control	1" x 1564'	469	5 +	IPA
	IPA-Sanitized	1" x 1551'	205	5 +	IPA
#5 ACER	SH-Sanitized	3/4" x 800'	130	4	SH
	IPA-Sanitized	3/4" x 1000'	164	4	SH
#6 CE	Control	1" x 1138'	362	5 +	SH
	SH-Sanitized	1" x 1138'	197	5 +	SH

Sanitizing with 70% Isopropyl Alcohol

The product utilized in these experiments was isopropyl alcohol (IPA) from SANI MARC (Victoriaville, Quebec) and supplied in 20-liter containers. The product is a 70% pre-diluted solution authorized for use by the Canadian Food Inspection Agency (CFIA). It was utilized in conjunction with sponges for the sanitation of mainlines. These sponges are available in various diameters from suppliers of maple syrup equipment. For each sugar bush, a treatment system was sanitized with IPA, while an equivalent control system was not sanitized (with the exception of Centre ACER systems, where SH was used instead of a control).

Mainlines and lateral lines (including droplines and spouts)

The procedure for sanitizing the treatment systems with IPA was conducted in two stages. The first stage involved the sanitation of the lateral lines, droplines, and spouts, while the second stage focused on the sanitation of the mainlines. In the initial stage of the procedure, the operator injected 15ml of IPA into each spout while maintaining the vacuum of the system. To achieve this, the worker utilized a backpack tank equipped with a graduated dose injector, thereby facilitating the operation. Subsequently, the spout was sealed with a cap, thus concluding the procedure. The procedure was conducted in a systematic manner, beginning with the final spout of the lateral line and proceeding in a proximal direction towards the initial spout near the mainline. Once all spouts had been injected, the mainlines could be sanitized. During this phase, the operator introduced a sponge soaked in IPA into the valve situated at the head of the main line, which was under vacuum. Subsequently, the same valve drew in 2 L of IPA, followed by a second sponge soaked in IPA. Then, the vacuum pump was deactivated and the valve situated at the extractor was closed. Finally, 2 L of IPA were introduced through the valve located at the head of the main line, which was then sealed. The IPA then remains in the system until the next season.

Sanitizing using a 600 ppm sodium hypochlorite solution (SH)

To provide a point of comparison with IPA, two sugarbushes (ACER and CE) were utilized to assess the efficacy of sanitation procedures in accordance with the stipulated SH guidelines. The systems were sanitized using SH, in accordance with the methodology delineated in the "Cahier de Transfert Technologique en Acériculture" (Allard and Belzile, 2004), without rinsing.

Microbial Contamination Sampling and Analysis

Prior to sanitation, microbial samples were obtained for each of the control and treatment groups. These samples were taken in early May and fall (between mid-September and late October 2009). In the case of the Centre ACER systems, an additional microbial sample was obtained two days following the SH treatment. The samples were collected using the System Sure II bioluminometer and Ultrasnaps swabs in accordance with the manufacturer's instructions (Hygiena). A total of 10 samples were collected from the mainlines at 10 distinct connections with the lateral lines, extending from the head to the extractor. The tee located at the junction between the mainline and the lateral line was removed, allowing for the swabbing of approximately 4 cm² of the internal surface of the mainline, and replaced with a new one. Subsequently, 10 new connections were sampled in the same manner for the fall period. Ten samples were obtained from the lateral lines prior to sanitation, with each sample taken from a different line and distributed across the mainline. The lateral lines were severed between the spout and the initial dropline. Subsequently, the inner surface was swabbed for approximately 2 cm², and the tubes were reconnected using a union. For the autumn sampling, the same lateral lines were again cut at approximately 30 cm towards the mainline, from the point of the initial sampling. The evaluation of the droplines and spouts' inner surfaces was conducted the following season, in 2010. The aforementioned sanitation procedure was then applied in an identical manner to an RL system. In this instance, 15 randomly selected droplines and 15 spouts were measured with a bioluminometer both before the sanitation procedure and in the autumn of 2010. The droplines were evaluated in a manner analogous to that employed for the lateral lines in the preceding year (2009). Prior to the sanitation procedure, the internal surface of the spouts was swabbed in order to obtain a measurement. This was conducted on the section of the spout that is situated within the tree during the harvesting process. Subsequently, a measurement was taken following the sanitation procedure in the autumn. This measurement was conducted on the same spouts, but this time on the portion that is inserted into the dropline.

Statistical analysis

The efficacy of the sanitation treatments was evaluated using the ANOVA (analysis of variance) and Tukey multiple comparison tests ($\alpha = 0.05$), which were applied to the mean microbial contamination results (log RLU/unit area) per system.

RESULTS AND DISCUSSION

Mainlines and lateral lines

In order to assess the efficacy of the IPA sanitation process in reducing microbial contamination in the maple sap collection system, two lines (treated and control) were evaluated in four sugarbushes in 2009. Figure 1 illustrates the findings of this assessment, conducted just prior to the implementation of sanitation procedures in the spring and after a period of several months in the fall of the same year. As illustrated in Figure 1, the microbial contamination results indicate that the control system under examination appears to exhibit a relatively consistent level of contamination between the spring and fall samples. This suggests that microbial contamination of these systems (mainlines and lateral lines) remains largely unchanged during this period in the absence of sanitation. As a result, microbial contamination will have the opportunity to persist in an unsanitized system throughout the off-season, despite the temperature and dehydration conditions that prevail during this period. This increases the likelihood of sap contamination at the outset of the next season. However, the only exception was observed in the control lateral lines of the RL, where microbial contamination was relatively lower in the spring (before). Thus far, no conditions have been identified that could explain this result, except that this system exhibited a slightly steeper slope than that of the other systems. However, it is noteworthy that the contamination of the lateral lines in the spring continued to develop during the off-season, reaching a level comparable to that of other systems in the fall.

The IPA-sanitized systems demonstrated a notable reduction ($p < 0.05$) in microbial contamination of the lateral lines and mainlines between the spring (pre-sanitation) and fall (post-sanitation) measurements for the four sugarbushes evaluated in Figure 1. An exception was observed once again with the lateral lines prior to the sanitation of the RL system, which exhibited lower contamination on average than the other systems. Consequently, no discernible difference was evident between the pre-sanitation and post-sanitation results for these lateral lines. The results of this evaluation indicate that the IPA sanitation method, as practiced in this study, is effective in significantly reducing microbial contamination. It is important to note, however, that this method of sanitation does not result in the complete elimination of microbial contamination. In all cases, residual microbial contamination persists, but at a significantly lower level than before sanitation and than for unsanitized control systems. This may have a beneficial effect on the maintenance of lower microbial contamination in the collection system in general and potentially on the microbial quality of the sap at the outset of the subsequent season, although this has yet to be evaluated.

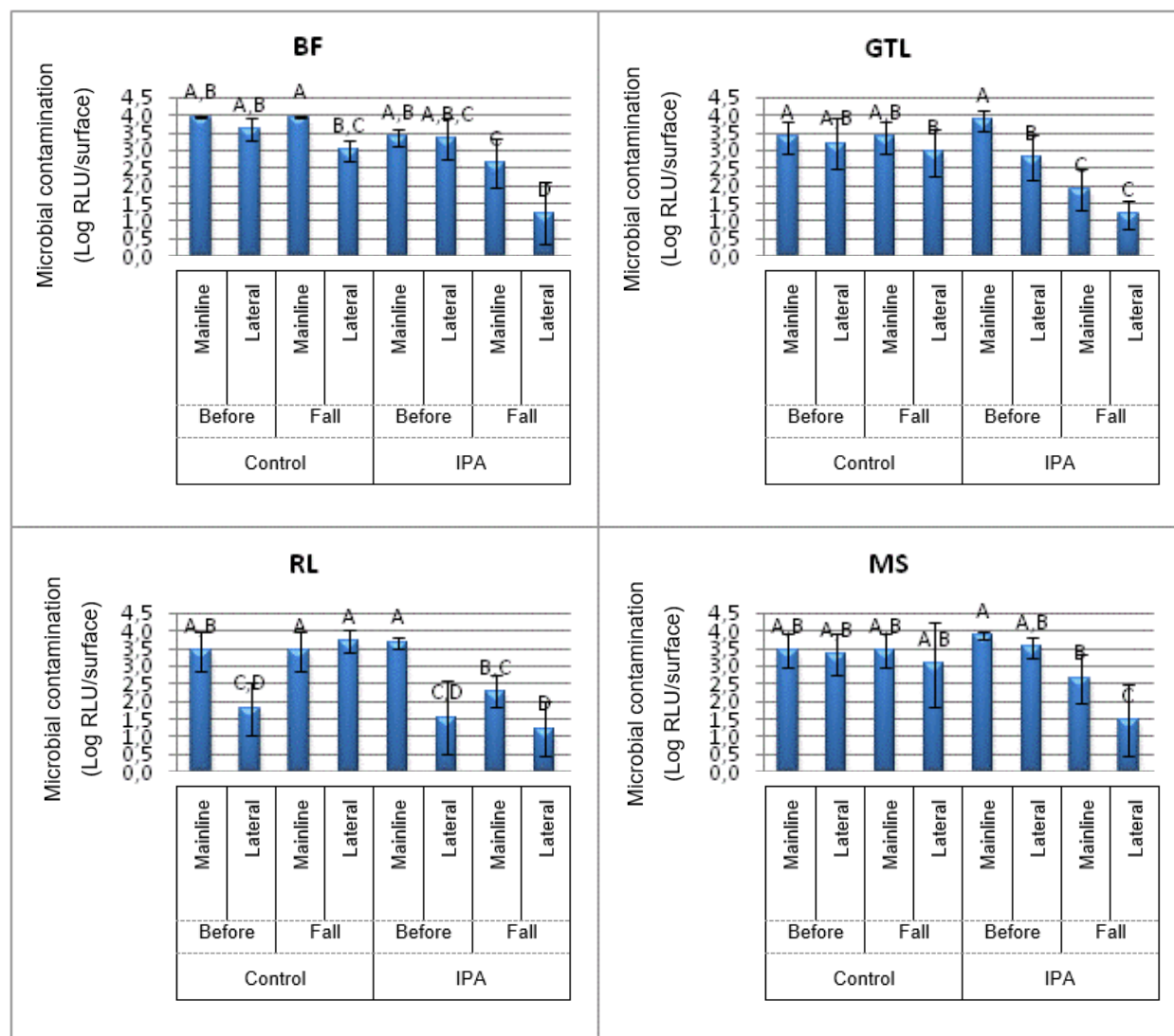


Figure 2 – Results of microbial contamination (ATP bioluminescence) of the maple sap collection system of the GTL, BF, MS and RL producers for the control and isopropyl alcohol (IPA) treated mainlines and lateral lines before and after sanitation (fall). Significant differences ($p < 0.05$) are indicated by means with different letters

The distinctive feature of IPA is its volatility. The hypothesis is that when IPA is left in a closed system for an extended period, it undergoes a process of volatilization and condensation on the internal walls of the system, thereby affecting the viability of the microorganisms present. It thus appears that a sufficient holding period is necessary in order to obtain the desired results. The product is commonly utilized as a sanitizer in the food industry and has been approved for use in maple syrup production by the CFIA, including both standard and organic production. The advantage of this method is that the solution is pre-diluted, ready to use, and much more straightforward to apply than the SH method. Given its volatility, it is unlikely that any IPA residue

will be present in maple syrup following the passage of the sap through the evaporator. It is nevertheless recommended that the initial run be discarded, as the new procedure does not employ sufficient quantities of sanitizer to facilitate a comprehensive rinse and the removal of biofilm residues and other impurities from the system.

In order to facilitate a comparative analysis of the IPA and SH sanitation procedures, a series of tests were conducted at the Centre ACER and CE sugarbushes in 2009. As illustrated in Figures 2 and 3, the microbial contamination levels of the systems in the spring prior to sanitation were comparable to those of the systems in Figure 1 during the same period. Furthermore, the results of Figure 2 (ACER) for IPA demonstrate the efficacy of this treatment in markedly reducing microbial contamination of the mainlines and lateral lines. Conversely, the findings for SH sanitation, as illustrated in Figures 2 and 3, indicate that this treatment has a negligible or insignificant effect on the microbial contamination observed in the systems during the fall period. The fall microbial contamination results were comparable to those obtained prior to sanitation and to those of the mainlines and lateral lines controls (CE). No notable discrepancies were identified in the microbial contamination of the systems during the fall season in comparison to the pre-SH sanitation results, with the exception of the ACER lateral lines, which exhibited a reduction in contamination levels during the fall period in comparison to the pre-sanitation data. Nevertheless, the efficacy of SH sanitation was discernible at the ACER systems (Figure 2) on assessments conducted two days following the treatment. In this instance, a notable reduction in microbial contamination was observed in both the mainlines and lateral lines. Nevertheless, a certain degree of residual contamination persisted following the sanitation procedure. Given the bactericidal action of this product is limited in time, it is probable that this residual contamination multiplied significantly, reaching a level in the autumn that was almost comparable to that before sanitation.

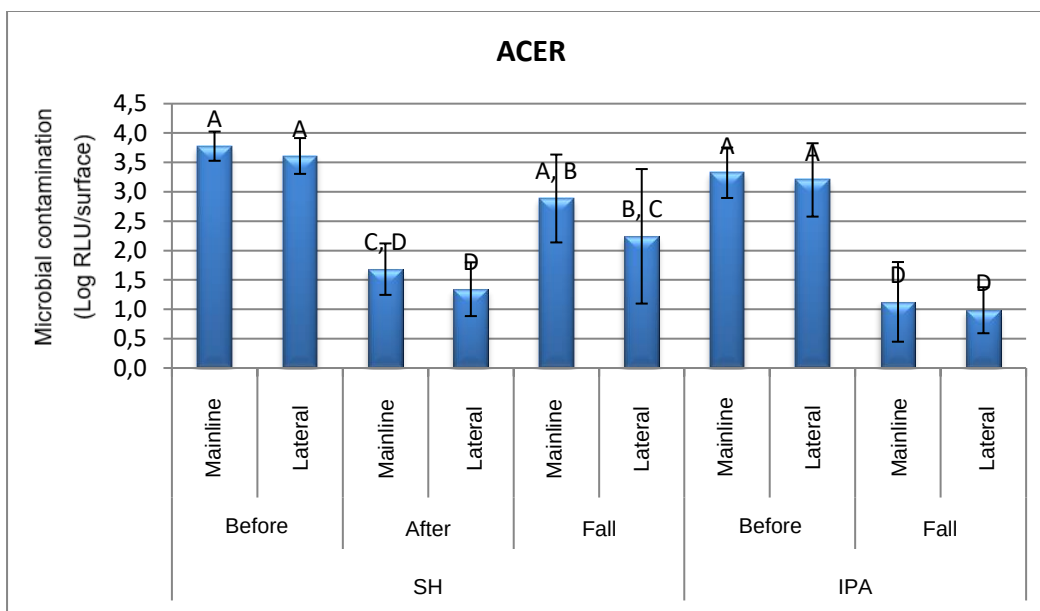


Figure 3 – Microbial contamination results (ATP bioluminescence) of the Centre ACER system for the mainlines and lateral lines treated with sodium hypochlorite at 600 ppm (SH) or IPA. The results are presented for the periods preceding and following sanitation (2 days after, only for SH treatment) and for the fall season. Significant differences ($p < 0.05$) are indicated by means with different letters.

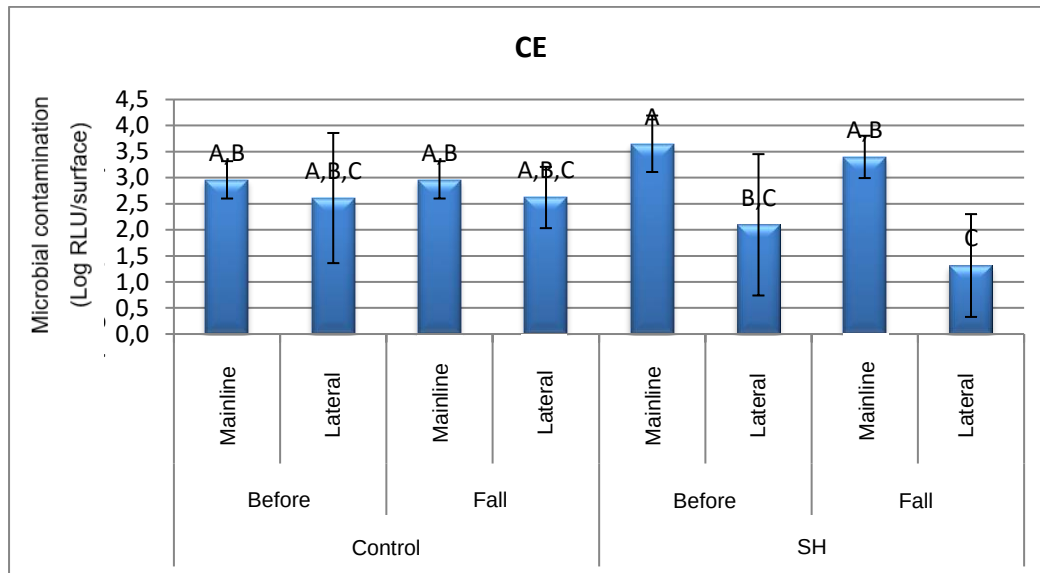


Figure – 4 Results of microbial contamination (ATP bioluminescence) of the CE system for the mainlines and lateral lines, with and without sodium hypochlorite treatment at 600 ppm (SH) prior to sanitation and in fall. Means with different letters are significantly different ($p < 0.05$).

Droplines and spouts

Additionally, the IPA was evaluated on droplines and the inner surface of the spouts. The sanitation procedure originally implemented in 2009 was subsequently repeated in 2010 for RL systems. The outcomes obtained prior to sanitation and in the fall are illustrated in Figure 4 for droplines and the spouts. As illustrated in Figure 4, the microbial contamination results obtained in the fall are markedly lower than those observed prior to sanitation. The results demonstrate that the performance of this sanitation procedure therefore seems to extend also to the droplines and spouts and is comparable to what was obtained for the mainlines and lateral lines in 2009.

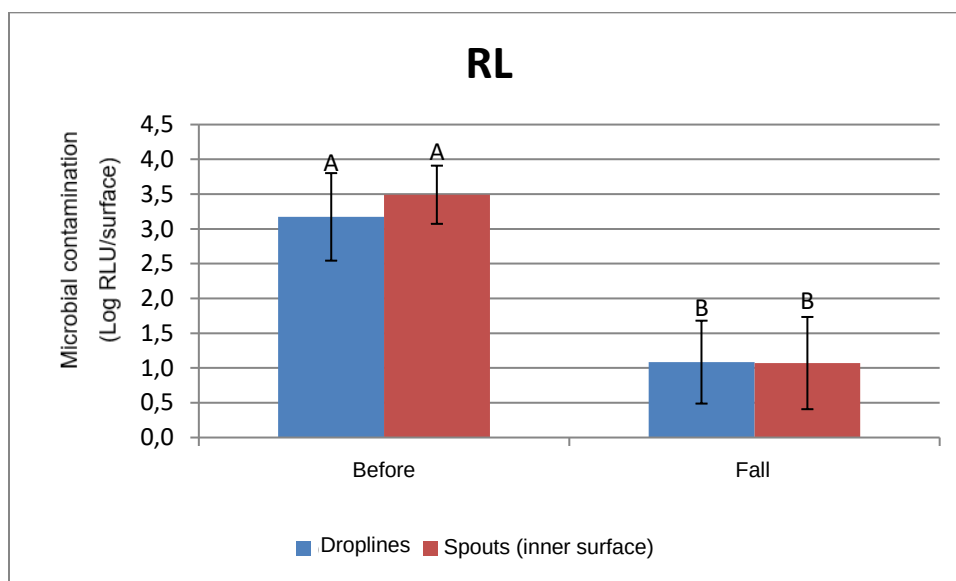


Figure 5 – Results of microbial contamination (ATP bioluminescence) of the maple sap collection system of the producer RL for control and IPA-treated droplines and spouts, presented before and after sanitation (fall). Significant differences ($p < 0.05$) are indicated by means with different letters.

Handling and storage of IPA

It is imperative that the handling and storage of IPA be conducted in accordance with the product's inherent volatility and flammability. To this end, sources of heat and sparks (such as engines, static electricity, and cigarettes) must be avoided. It is recommended that the MSDS be consulted for detailed information regarding the precautionary measures to be taken. It is recommended that products listed by the CFIA that are 70% pre-diluted be used in order to avoid unnecessary handling and reduce the risk of accidents (<http://active.inspection.gc.ca>). It is recommended that this product be stored in a cool, dry place with grounding of containers. It is inadvisable to store this product with other corrosive substances, such as sodium hypochlorite (bleach). It is

imperative that the original product label be consulted and that the rules set forth in the Workplace Hazardous Materials Information System (WHMIS) be followed with regard to the identification and storage of hazardous products. It is recommended that the requisite quantity for each season be purchased in order to avoid the unnecessary storage of the product. It is recommended that the transfer of the product be conducted in a well-ventilated area or, when feasible, in an outdoor setting with shade, provided that the temperature is suitable.

CONCLUSION

The findings of this study indicate that the sanitization of the maple sap collection system with 70% isopropyl alcohol (IPA) is an effective method for reducing the microbial load present in the maple sap collection system at the conclusion of the season. In addition to its microbiological efficacy, this procedure is described by growers who have used it as more practical and simpler to operate than the traditional method involving a sodium hypochlorite solution at 600 ppm (SH). This novel sanitation method is distinguished by its requirement for the IPA to remain in the system for an extended period, thereby ensuring its efficacy. This attribute confers a distinct advantage over SH, whose effectiveness is known to diminish over time. This observation was also made during the course of this study, wherein the efficacy of SH was noted only after a period of two days following treatment. Thereafter, a resumption of growth of the residual flora was observed. Consequently, the use of IPA for sanitation represents an effective method for controlling microbial contamination of the collection system, thereby contributing to the overall quality of sap and maple products. Nevertheless, the quality of the sap and other maple products following the sanitation procedure has yet to be evaluated in this study. It is essential to ensure that the collection system is flushed effectively by eliminating the initial run in order to achieve the desired sanitation outcome. Subsequently, further research will be conducted to ascertain whether this sanitation method confers advantages in terms of maple syrup product quality and productivity, as well as to confirm whether this practice affects tubing composition.

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