Antimicrobial Silver in Maple Sap Collection

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INTRODUCTION

The ability of some metals to inhibit the growth of or to kill certain microorganism is well established. In particular, the use of silver as a method to control microorganisms to maintain water quality has been known for centuries (Landau 2006). Silver coins were added to pots or barrels of water to preserve freshness during extended land and sea voyages when potable water sources were uncertain. Ions of silver (and other oligodynamic metals) work by interfering with the metabolism and reproduction of microbes, resulting in their death. Contact between the microbe and silver-containing material is required to affect surface exchange of ions. Silver does not readily dissolve in water, so there is little or no residual action of silver in solution.

The role of microorganisms in the premature cessation of maple sap flow (taphole drying) has been well recognized for some time (Naghski and Willits 1955). Bacteria, fungi, yeasts, molds and algae can colonize tapholes and sap collection systems, thereby reducing sap flow via a combination of vessel plugging by microbial biomass (Ching and Mericle 1960), or through the normal response of trees to close off the wound (taphole) to prevent microbial spread throughout the tree tissues (Shigo 1965, Walters and Shigo 1978). In both cases, the result is the same: sap flow is reduced and eventually stops.

During the late-1950s and early-1960s, research was conducted to investigate ways to reduce or eliminate microbial contamination and attendant taphole drying in maple sap collection systems (Sheneman et al. 1958, Costilow et al. 1962). A number of different compounds were tried at that time, including paraformaldehyde (PFA), oligodynamic silver, antibiotics, sorbic acid, mercuric iodine, and sodium hypochlorite (Sheneman et al. 1958). Of all the substances tried, ". . . only paraformaldehyde appeared promising for commercial use." (Shenaman et al. 1958). Subsequently, PFA in tablet form was introduced as a taphole disinfectant, and registered by the E.P.A. as an approved pesticide for maple use.

Using PFA in tapholes resulted in increased sap flow, especially in the latter part of the sap collection season, and could result in increases in sap yield up to 96% (Costilow et al. 1962). After a period of use in the maple industry, it was found that PFA interfered with the wound healing response of trees, resulting in greatly increased staining columns and decay, and higher levels of morbidity and mortality in trees in which PFA was used (Shigo and Laing 1970, Walters and Shigo 1978). Consequently, the use of PFA was banned in both the U.S. and Canada, and, as a result, the E.P.A. registration of PFA as an approved pesticide for maple use eventually lapsed.

A review of the role of microorganisms on premature drying of tapholes was

conducted during the Second Conference on Maple Products held at the U.S.D.A. Pennsylvania Laboratory (1953). At that time, a question was raised about the possible use of silver to control bacteria in maple spouts. The answer given was, "The bactericidal property of metals was first investigated some forty years ago and has been of considerable academic interest. However, the effect is of a slight magnitude and of little practical value." (Naghski 1953). Regardless of this conclusion, antimicrobial silver (along with other substances) was investigated by Shenaman et al. (1958). Cotton balls soaked in a solution of "O-Silver" were placed into tapholes and these tapholes were compared with untreated tapholes and tapholes into which other antimicrobial substances were added. O-Silver did not produce a substantial reduction in microbial contamination.

More recently, interest in antimicrobial silver has experienced a resurgence as bacterial antibiotic resistance has increased in medical applications and the desire to find "natural" alternatives to chemicals has increased. Current applications of antimicrobial silver utilize nano-scale technology (microscopic particles) to increase the ion exchange surface area. Minute particles of an inert, inorganic, crystalline (usually ceramic) carrier material is embedded with silver ions and incorporated into the material in which antimicrobial properties are desired. The positively-charged silver ions are attracted to negatively-charged surfaces, like bacterial membranes. In maple, most plastic spouts (typically food grade nylon) are made by an injection molding process, while

tubing is made by an extrusion process. For tubing incorporating nano-silver, there are frequently two layers: an outer layer without nano-silver, and an inner coextruded layer with nano-silver. The nano-silver mixture is simply mixed into the plastic prior to molding or extrusion. U.S. E.P.A. regulations allow a maximum of 2% silver by weight, and, in certain applications, the use of antimicrobial silver is U.S. E.P.A. and FDA approved. Because of the need for contact between the microbe and silver, only the very outmost layer of silver-containing material is effective in transferring silver ions to microbes. Silver embedded beneath the surface of the plastic or on the outer surface that does not come into contact with sap during normal collection procedures does not contribute to the antimicrobial effectiveness of the material.

Because of this new approach to using nano-silver, the fact that PFA has been banned, and the desire to control microorganisms in maple sap collection systems, the University of Vermont Proctor Maple Research Center investigated the use of spouts and tubing containing antimicrobial nano-silver for suitability for increasing maple sap yield.

2008 EXPERIMENTS

Sap Yield Methods. During the spring of 2008, we tested fittings and droplines containing antimicrobial silver. Both 5/16" antimicrobial fittings and 5/16" (ID) FlexeleneTM FXAG silver antimicrobial tubing (Figure 1) were purchased from Eldon James, Corp. (Loveland, Colorado). The 5/16" fitting was a standard straight-through tubing coupler fitting that we used as a spout. We did not observe any abnormal leakage around the fitting when used in this way. Henceforth, silver spouts will be referred to as AG spouts and silver-containing dropline will be referred to as AG dropline.

The 2008 treatments were: a) a new Leader spout and 1-year old, used 30P dropline that served as a control, b) a new AG spout (fitting) with 1-yr old, used 30P dropline, and c) a new AG spout with new AG dropline and d) a new Check-valve spout with 1-yr old, used 30P dropline (this treatment was discussed in a prior publication and will not be addressed further in this paper). All used dropline was cleaned prior to use. Each tree received four tapholes, one for each treatment, with a total of 10 trees used.

Droplines were connected vertically to vacuum chambers. All droplines were 36" in length. Chambers were connected to a mainline which was under approximately 22" Hg vacuum supplied through a 5/16" lateral line at the top of the chamber. Trees were all tapped on the same day using the same 5/16" drill bit and tapholes were placed such that the orientation of tapholes for the treatments were rotated from tree to tree. The sap volume in each chamber was measured following each sap flow period, after which the chambers were drained. Sap yield for each period was totaled for the 2008 season.

Microbial Estimates. At two dates near the end of the 2008 sap flow season, sap samples were collected from each chamber for microbial contamination testing using a Charm Sciences, Inc. (Lawrence, Massachusetts)

Figure 1. 5/16" straight-through silver antimicrobial tubing coupler used as a spout and FlexeleneTM FXAG (silver antimicrobial) tubing used as a dropline. Fittings and tubing were sourced from Eldon James, Corp. (Loveland, Colorado).

FireFly® ATP Luminometer and Watergiene® swabs. This system provides a rapid estimate of the microbial population of liquid samples. In the lab, Watergiene swabs were immersed into the samples for 30 seconds, then immediately inserted into the luminometer and a reading taken.

Silver Concentration in Sap. Sap collected in the study described above was also analyzed for silver concentration by ICPAES at the UVM Plant Testing Laboratory.

Tree Wounding. An AG spout and a control (food-grade nylon) spout were placed into opposite sides of three small (2.5-3.0" diameter) understory trees as a preliminary study of possible incompatibility of silver with tree healing processes. Taps were removed in late-spring after the sap flow season had ended. Trees were cut during the summer of 2009 to examine the size of the internal wound.

2008 RESULTS

Sap Yield. Over the course of the 2008 sap-flow season, the control spout produced 14.3 gal sap/tap, while the AG spout with a used 30P dropline produced 16.6 gal sap/tap, for a total increase of 15.8% more sap than a standard spout at the same vacuum level. The AG spout with new AG dropline pro-

duced 18.2 gal sap/tap, for a 26.6% increase in sap yield. The majority of the increase in sap yield was found in the last week of the season.

Microbial Contamination. ATP luminescence showed that microbial contamination was moderate to high in all sap samples collected near the end of the season, but was significantly lower in sap collected from chambers with AG spouts both with or without antimicrobial droplines when compared to control spouts on both collection dates. In both cases, microbial loads in sap from AG spouts or AG spouts with AG dropline were about 55% that of control spouts.

Silver Concentration in Sap. All sap samples fell below quantification limits of 0.02 mg/l and there was no evidence suggesting higher silver levels in sap from antimicrobial silver containing spouts or droplines. Due to the concentration effect, further testing is necessary to determine whether this is also true of syrup.

Tree Wounding. The internal staining in trees using AG spouts appeared to be approximately the same size as that of a normal spout (Figure 2). In both cases, the size of the wound was quite small, probably due to the slow growth of these suppressed understory trees. Further work with a larger sample size of faster growing dominant or co-dominant maple trees is necessary to validate these results.

2009 EXPERIMENTS

Two field studies involving AG droplines were conducted over the course of the 2009 sap flow season. The basic design of both studies was described in

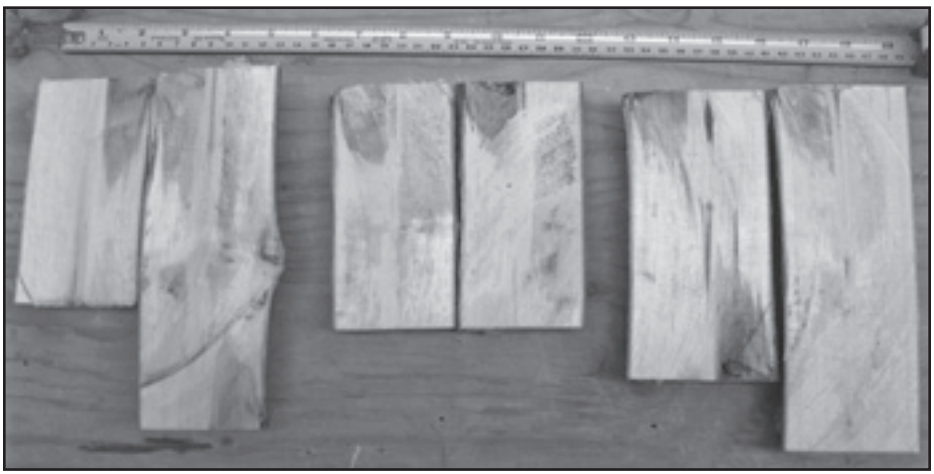

Figure 2. Internal staining in young maple stems tapped with regular (food-grade nylon) spouts and antimicrobial silver spouts. For each pair of stem segments, the regular spout is on the left and the antimicrobial silver spout is on the right. Note the very small stain pattern, probably the result of the slow growth and suppressed stature of these saplings.

a previous paper (Perkins 2009), and will only be briefly described except where further detail is required.

Study 1 Methods. Vacuum chambers were set up on 24 trees at the UVM Proctor Maple Research Center. All trees were connected to a common vacuum system operating at an average of 22.5" Hg throughout the 2009 season. Eight trees had 30P droplines that had been used for one year and then cleaned along with new Leader stub spouts and regular spout adapters. Another eight trees also had used 30P dropline and stub spouts, but had new Leader stubs and Check-valve adapters. A third set of eight trees had AG droplines that had been used for one year and then cleaned, along with new Leader stubs and normal spout adapters. Sap volume was measured after each flow period and the chambers emptied. Sap yield for each period was totaled for the 2009 season. Trees averaged 10.4" in diameter at breast heigh (dbh).

Study 1. Results. Sap yield from control trees (new spouts with used droplines) averaged 33.3 gal/tap (Figure 3). Sap yield from treatment trees (new spouts with used AG droplines) averaged 32.6 gal/tap. Patterns of sap

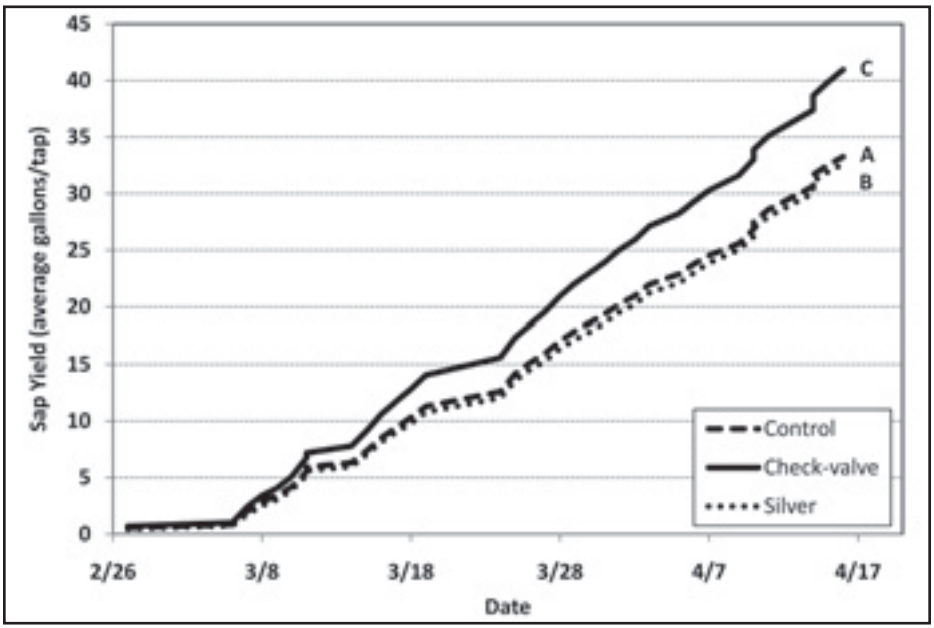

Figure 3. Seasonal progression of average sap yield (gal/tap) from trees on vacuum chambers during the 2009 season with:

A. 2-part spout stub/adapter with 1-yr old used 30P dropline (Control),

B. 2-part spout stub/adapter with 1-yr old used antimicrobial silver dropline, and

C. Leader Check-valve adapters with 1-yr old 30P dropline.

All dropline tubing was used for one season and then rinsed clean prior to use in this experiment. All chambers were connected to a common vacuum pump.

flow over the season were nearly identical. For comparison purposes, sap yield from new Check-valve spouts with used droplines was 41.0 gal/tap. The difference between the Check-valve spout treatment and the Control or AG dropline treatments grew steadily throughout the course of the season.

Study 2 Methods. Sections of the production woods at UVM Proctor Maple Research Center that had been completely re-tubed in 2004 were utilized. Sap yield from each section was measured each year from 2005-2008, and were roughly comparable each season (Perkins, Stowe and Isselhardt unpublished), with average yield above 0.5 gal syrup/tap each year. For the 2009 season, one section had all droplines and spouts replaced. Drops in that area were new semi-rigid tubing and new spouts were 5/16" health spouts. A second section had all droplines replaced with new antimicrobial silver FlexeleneTM FXAG tubing (Eldon James Corp.). Spouts were new Leader stubs with new Check-Valve adapters (Perkins 2009).

All sections were connected to individual releasers through individual mainlines. Releasers were calibrated and equipped with counters to record the number of dumps. Total volume was calculated each day and totaled for the 2009 season. The entire system was serviced by an Airblo Flood vacuum pump pulling a seasonal average of 22" Hg at the pump. The pump was turned on when the air temperature approached 32°F, and turned off automatically if the releaser did not dump within a four hour time span.

Study 2 Results. These overall results were discussed in a previous publication (Perkins 2009), but the results of the AG drop combined with the Leader Check-valve adapters are discussed in further detail here. The tubing section with new AG droplines and Leader Check-valve adapters produced 58.0 – 91.2% more sap than the other sections (Figure 4). The bulk of the increase was observed in the latter part of the season. The section of woods which had new drops and spouts showed an slight increase (10-20%) in yield, also during the late season, consistent with results of research on replacing drops and spouts under vacuum (Perkins 2009, Perkins, Wilmot and Stowe unpublished).

New AG dropline appeared to have little effect on the yield of sap. Sap yield in Study 1 (using one year-old 30P droplines with new Check-valve adapters) from 10.4" dbh trees averaged 41.0 gal/tap, whereas sap yield in Study 2 (new AG dropline with new Check-valve adapters) from trees averaging over 20" dbh produced 44.6 gal/tap. The small difference in production between the two studies is far more likely to be a result of the size of the tree than the use of antimicrobial tubing. Even in the highly unlikely case that all the difference in sap yield between the two studies was due to AG tubing, the total effect amounts to less than an 8% increase in yield.

Study 3. Methods. To examine the efficacy of the antimicrobial silver containing materials on bacterial kill, several 2 cm long pieces of tubing were cut from new, 1-yr old used, and 2-yr old used antimicrobial FXAG FlexeleneTM tubing, as well as new and 1-yr old used 30P tubing. Tubing pieces were

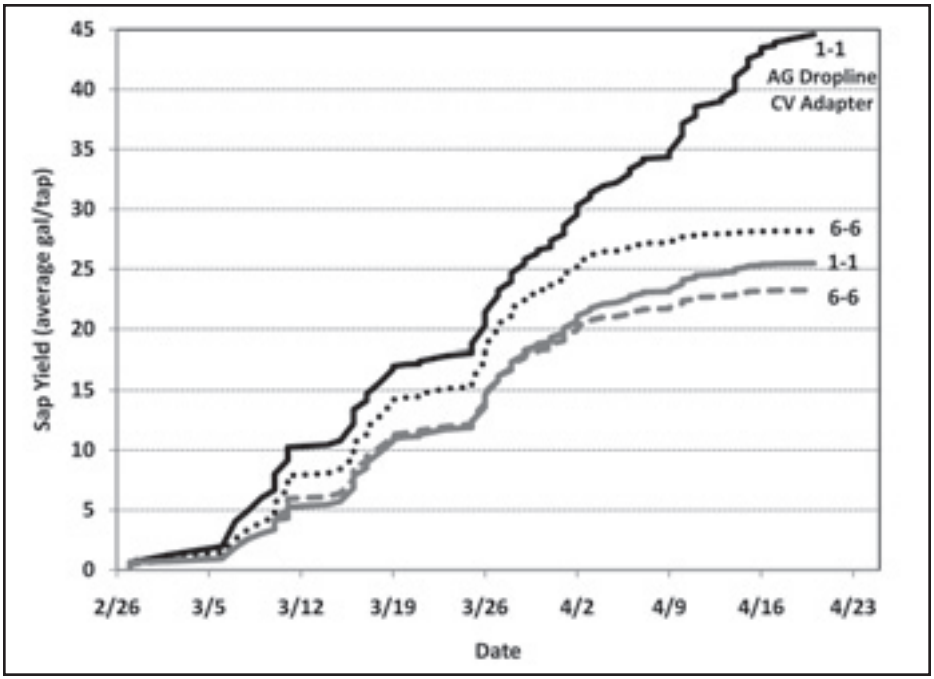

Figure 4. Seasonal progression of sap yield (gal/tap) from similar tubing systems at UVM PMRC during the 2009 sap flow season. All sections were retubed in 2004 and were connected to a common vacuum pump. Numbers refer to age of spouts and dropline (1 = new spout or tubing in 2009). Topmost line used silver antimicrobial dropline and Check-valve spout adapters.

placed into small vials with 25 ml of late-season (contaminated) sap and allowed to sit for for a period of time after which microbial contamination was assessed using the Charm luminometer and WaterGenie® swabs as described above. Three trials were conducted: a 1 hr period, a second separate 1 hr period, and a 4 hr time period. Vials of sap without tubing served as controls.

Study 3 Results. New antimicrobial silver tubing clearly reduced microbes (Table 1), however the efficacy of microbial reduction was greatly diminished with use. After 1-2 seasons, microbial reduction in used tubing was less than one quarter that of new silver tubing and was similar to that of used 30P tubing. Interestingly, new 30P tubing produced about a 20% reduction in microbial count.

DISCUSSION

In the 2008 study, new AG spouts in combination with a one-year-old used (but cleaned) droplines produced a 15.8% improvement in sap yield. Given that a new spout or spout adapter alone can yield an improvement in sap yield of 10-15% (Perkins 2009, Perkins, Wilmot and Stowe unpublished), the added

TABLE 1. *Efficacy of various types and ages of dropline material on microbial reduction. Values are percent reduction in microbial population as a result of exposure to different tubing materials and were estimated with a Charm Luminometer and WaterGenie® swabs in a controlled laboratory setting for 1 or 4 hours.*

	Reduction in Microbial Count (%)			
Treatment	**1 hr.**	**1 hr.**	**4 hr.**	**Average**
Control (sap only)	0.0	0.0	0.0	0.0
New 30P Tubing	16.0	29.9	10.6	18.8
1 Yr. Used 30P Tubing	4.5	8.9	6.4	6.6
New Antimicrobial Silver Tubing	40.7	59.1	37.3	45.7
1 Yr. Old Antimicrobial Silver Tubing	17.1	26.4	-12.4	10.4
2 Yr. Old Antimicrobial Silver Tubing	15.3	4.3	2.3	7.3

effect of the antimicrobial silver in spouts appears to be marginal at best. Similarly, a new AG dropline and new AG spout used in combination yielded a 26.6% increase in sap compared to a new conventional spout and one-year-old dropline, yet use of a new dropline and new spout (both non-antimicrobial) will produce a 15-20% increase in sap yield compared to materials that are four-years-old (Perkins, Wilmot and Stowe unpublished), again demonstrating that the antimicrobial silver effect, even in new silver containing spouts and dropline, is very small.

The lack of increase in sap yield over the control system in the 2009 study on vacuum chambers clearly showed that after one season of use the AG dropline was no longer effective in significantly controlling microorganisms that impact sap yield in the sap collection system. This is further verified by the lack of reduction in microbial populations in the controlled lab studies.

Thus it appears that the antimicrobial effect of AG droplines, and presumably also that of AG spouts made in the same way, is greatly diminished or exhausted after one season of use. This does not appear to be related to cleaning, as all used droplines were rinsed clean prior to use, but is more likely due to the depletion of the silver from exchange sites on the surface of the spout or tubing material. Although antimicrobial silver is reported to be effective in water systems for a long period of time, the substantially higher microbial loads in maple sap most likely overwhelm or exhaust the beneficial effect within a single production season when used in sap collection systems. This is not unprecedented: ozone works well as a sanitizer in water treatment systems, but is ineffective in killing microorganisms in maple sap (Labbe 2001).

Due to the low-moderate increases in sap yield and diminished-absent antimicrobial capacity after just a single season of use, and the very high cost of the antimicrobial spouts and dropline (approximately $2 per ft), it appears that antimicrobial silver usage in maple sap collection systems is likely to be economically prohibitive for the amount of sap gained.

In addition, it should be noted that antimicrobial silver is not on the list of

approved substances for organic maple production. Organic maple producers should be cautioned against utilizing antimicrobial silver in their operations, otherwise they will lose their certification and any premium that is associated with it. Finally, continued research is necessary to firmly establish that silver is not being released and accumulated in syrup, and that silver-containing materials do not impede the healing process in trees.

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