Maple Sirup. VI. The Sterilizing Effect of Sunlight on Maple Sap Collected in a Transparent Plastic Bag

J. NAGHSKI AND C. O. WILLITS
Eastern Regional Research Laboratory,[a] Philadelphia 18, Pennsylvania

The sterilizing power of sunlight in reducing the bacterial content of maple sap when exposed in plastic bags (transparent in the ultraviolet region) is described. Evidence was obtained that under the conditions of this experiment maple sap became practically sterile upon exposure to bright sunlight during cool weather.

As part of the program to isolate spoilage bacteria from maple sap, plastic maple sap bags were used for collecting the sap. Contrary to expectations the sap so collected was practically sterile. Although the sap contained traces of extraneous material including insects and bits of bark yet a plate count showed less than one bacterium per milliliter, indicating that the plastic bags were producing a sterilizing effect. This was of special interest since the maple industry has been much concerned with developing means of preventing contamination of the sap with microorganisms and reducing spoilage.

Edson, Jones and Carpenter in 1912 *(3)* in an extensive study of the bacteriology of maple sap showed that sap became contaminated by adventitious bacteria common in nature and that growth of these organisms in sap resulted in a poor quality, dark sirup—often with off-flavors. These results were confirmed by Hayward and Pederson *(5)*. However, no means was developed for controlling the contamination of the sap in the sugar bush or for preventing growth in storage tanks. Willits *(12)* and Holgate *(6)* evaluated the efficacy of many disinfectants for controlling microorganisms in maple sap but found objections to all. Either they constituted health hazards or impaired the quality of the sirup. Sirup prepared from sap collected in plastic bags was of exceptionally high quality and flavor, even though it had remained in the bag for as long as 2 weeks and at temperatures of 32° to 70°F. (0° to 21° C.). There were two possible explanations for the sterilizing power of the plastic bags. Either it was due to the presence of a bactericidal chemical in the plastic or to the sterilizing effect of ultraviolet rays in sunlight. Experiments were performed to test these hypotheses and the results indicate that the disinfecting action is due to sunlight.

EXPERIMENTAL

The bags used in this study were of Vinylite plastic, presumed to contain plasticizer and stabilizer, and are commercially available for the collection of maple sap. These bags were first rinsed with two liter-portions of boiling sterile water to remove the greatest number of extraneous bacteria. Into each bag was placed one gallon of sterile sap collected asceptically according to the technique of Holgate *(6)*. The bags were then inoculated with a bacterial culture isolated from maple sap which had spoiled when stored in a refrigerator room at 34° to 37° F. (1.2° to 2.8° C.). The bags were then exposed to outdoor sunlight by hanging onto a tree trunk. As controls, similarly filled bags were placed in covered metal cans also hung on a tree. A record of the sap temperature was made using a recording thermometer. Hourly readings were observed from the recordings and the average daily temperature calculated. Values for percent of sunshine were obtained through the courtesy of the U. S. Weather Bureau, Philadelphia, Pennsylvania.

Bacterial numbers in the original sap and in that held in the bags were determined by plating on nutrient agar after making appropriate dilutions to give 30 to 250 bacterial colonies per plate. The plates were incubated at room temperature for 2 days before counting. Although the culture was isolated from sap stored at 34 to 37° F., it was able to grow more rapidly at room temperature, making it possible to count the plates in 2 days.

RESULTS

In the initial experiment, sterile fresh maple sap was inoculated with approximately 3,000 bacteria per ml. and the bags were fastened to a tree in a southerly exposure. One day later a similar experiment, but in which the sap was inoculated with approximately 250,000 bacteria per ml., was set up to determine whether the sterilizing effect would be operative in the presence of large numbers of bacteria. The results presented in Table 1

TABLE 1

The bacterial content of maple sap inoculated with approximately 3,000 and 250,000 bacteria per ml. and exposed to sunlight in plastic bags

Date	Amt. of sunshine	Inoculum, Approx. 3,000/ml.		Inoculum, Approx. 250,000/ml.	
		Exposed	Not exposed	Exposed	Not exposed
1952	*% of day*	*Bacteria/ml.*	*Bacteria/ml.*	*Bacteria/ml.*	*Bacteria/ml.*
2/25	....	3,300[b]	2,900[b]		
2/25	65	3,100[c]	3,300[c]		
2/26	....			208,000[b]	261,000[b]
2/26	81	2,400	3,400	212,000[c]	337,000[c]
2/27	73	2	8,500	142,000	343,000
2/28	92	1	44,000	230	2,200,000
2/29	63	0	66,000	27	2,100,000
3/1	32	....[d]	....	....	....
3/2	70	0	4,240,000	0	18,500,000
3/3	0	....	....	....	....
3/4	0	0	7,200,000	9	20,000,000

[b] Immediately after inoculation.
[c] Exposed only half day.
[d] .... indicates bacterial count not determined.

show that in both experiments the bacterial count was reduced to zero in the bags exposed to the sun but had increased to several million in the bags protected from sunlight by inclosure in the metal cans. These experiments demonstrate that the sun's rays transmitted by the plastic bags were not only bacteriostatic but also bactericidal.

To determine whether this sterilizing effect was limited to the action of direct sunlight, a third experiment was performed in which bags were hung about a tree exposing them to the 4 cardinal points of the compass. The results, presented in Table 2, show that all exposures (at the time of these experiments, February and March) were effective in reducing bacterial numbers but the sunny exposures (southerly and westerly) were more effective than the locations in which the bags were in the shadow of the tree most of the time (northerly and easterly). During the first few days of these experiments the weather was rather cloudy and warm with rain, with the result that the bacterial count showed a steady increase. Even so, the increase in bacterial count of the sap in the exposed bags was less rapid than in the bag which was not exposed to sunlight. This initial period was followed by 5 days of sunny and colder weather and

[a] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

the bacterial counts of the sap in the exposed bags dropped sharply, the sap becoming almost sterile in the one hung on the sunny side of the tree. This weather condition was followed by 3 warm, rainy days with no sunshine and the bacterial count again increased. This would indicate that during such exceptional periods at the end of the sap season when the weather is cloudy and warm for long periods, the sap cannot be allowed to stand in the bags if a product low in bacteria is to be obtained. However, even after this increase, the bacterial count of the sap in the exposed bags was only 1 to 10% of that in the bag not exposed to sunlight.

Since all of these experiments were conducted on artificially inoculated sap, it was desirable to determine whether sap originating from a naturally contaminated sap hole could be sterilized by collecting in a plastic bag. The sap flowing from such a contaminated hole was divided; one-half was allowed to flow into a plastic bag and the other half into a covered metal sap bucket. The sap flowing from the spile had 860 bacteria per ml. After 7 days (March 25 to March 31) the sap was collected and the bacterial counts determined. The sap in the plastic bag was clear and had only 3,100 bacteria per ml. while that in the sap bucket was milky and had 144,000,000 bacteria per ml. Samples of sap from the exposed bag and from the bucket were transferred to glass jars and photographed against a dark background (Figure 1) to show the milkiness developed in the sap collected in the sap bucket.

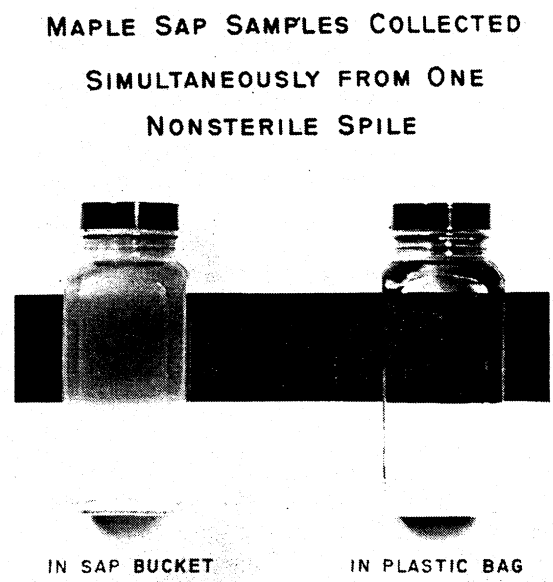

Figure 1. Effect of collecting sap from a contaminated sap hole in metal sap bucket and plastic bag on the growth of bacteria.

DISCUSSION

The decrease of bacterial numbers in the sap exposed in the plastic bags was undoubtedly the result of the sterilizing effect of the sun's radiation. The lethal action of sunlight on certain bacteria was demonstrated by Downes and Blunt as early as 1877 *(2)*, and since then the effects of ultraviolet radiation on microorganisms have been studied frequently. The older literature has been reviewed by Ellis and Wells *(4)*. The region of bactericidal activity has been reported to lie in the region of wavelengths shorter than 3000 A, the most effective being near 2600 A. Studies of the energy distribution of the solar ultraviolet radiation reaching the earth have been limited by availability of proper instruments. The measurements made by Pettit *(8)* and Stair *(10)* are probably the more exact, and place the short wavelength limit between 2900 and 3000 A. This short wavelength limit is known to vary with the latitude, season, and time of day. This means that the amount of radiation present in sunlight in the region of germicidal activity is only a small and variable fraction of the total ultraviolet component.

A spectral transmission curve of the plastic from bags used in these experiments is shown in Figure 2 (curve I). The plastic transmits from 50 to 80% of the radiation longer than 3100 A with a rather sharp cut-off in the region of 2900 to 3000 A, which coincides with the established limits for the solar ultraviolet. Precautions were taken, in these transmission measurements, to reduce light scattering by the plastic to a minimum by coating the plastic with glycerin and sandwiching between quartz plates. This effect could not be corrected completely, however, and the effective transmission may be somewhat higher than shown. Luckiesh *et al.* *(7)* have determined the relative bactericidal effectiveness of radiation from 2200 to 7000 A in wavelength. Values taken from their curve are plotted in Figure 2 (curve II). It can be seen that the germicidal efficiency of radiation in the region transmitted by the bag is relatively poor (cross-hatched area); nevertheless, over the long period of exposure encountered in these experiments sufficient energy is transmitted to cause the desired germicidal effect.

TABLE 2

Bacterial growth in maple sap as a function of exposure in plastic bags at four locations on the tree

Date	Amt. of sunshine	Ave. temp.	*Exposure*				
			Northerly	Easterly	Southerly	Westerly	Not exposed
March 1952	%	°F.	*Bacteria/ml.*	*Bacteria/ml.*	*Bacteria/ml.*	*Bacteria/ml.*	*Bacteria/ml.*
9	15[e]	40	700	1,000	600	500	500
10	42	48	1,900	1,800	1,800	1,500	3,000
11	10	47	47,000	54,000	58,000	46,000	360,000
12	100	44	1,200,000	900,000	157,000	108,000	8,900,000
13	26	38	1,700,000	910,000	60,000	24,000	14,000,000
14	100	39.5	2,020,000	750,000	5,200	13,600	18,700,000
15	100	39	260,000	260,000	7,600	1,600	17,000,000
16	62	34	....[f]	....	....	....	....
17	87	47	136,000	17,000	800	200	26,000,000
18	91	45	53,000	<100	200	20	39,000,000
19	0	40.5	....	....	....	....	....
20	93	55	31,000	7,000	3,000	60	60,000,000
21	54	58	640,000	327,000	2,600	10	44,000,000
22	0	42	3,900,000	1,500,000	19,300	2,900	53,000,000
23	0	41.5	....	....	....	....	....
24	0	41	4,900,000	3,800,000	870,000	90,000	59,000,000
25	100	50	....	....	....	....	....
26	96	50.6	5,700,000	6,800,000	1,300,000	110,000	73,000,000

[e] Corrected for only one-half day exposure.
[f] .... indicates bacterial count not determined.

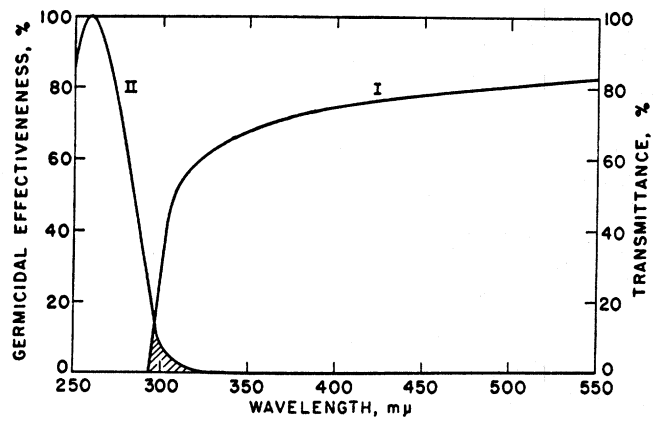

Figure 2. I. Spectral transmittance of sheet (0.012 inch thick) cut from plastic bag, with scattering reduced by sandwiching between quartz plates moistened with glycerin. II. Relative germicidal effectiveness of radiant energy (after Luckiesh et al [7]).

Rentschler *et al. (9)* and Luckiesh *et al. (7)* while reinvestigating the Bunsen-Roscoe reciprocity law noted that low intensities of germicidal energy are effective if permitted to act over a long time. The sensitivity of the microorganisms was found to vary at different stages of the growth cycle, the more rapidly growing organisms being more susceptible. Evidence was also obtained that sub-lethal doses of irradiation greatly retarded the development of the organisms. This is in agreement with the results of Coblentz and Fulton *(1)* who found that germicidal action of radiation was cumulative, and that an intermittent exposure had the same effect as a continuous exposure for an equivalent length of time. Examination of results in Table 1 reveals that almost 2 days' irradiation was required before a pronounced drop in bacterial count was obtained in the sap. It should be recognized that during these prolonged periods of exposure which extend over several generation times, growth as well as destruction of bacteria are occurring simultaneously, and the bacterial count at any given time depends on which process was favored by the climatic conditions such as temperature and intensity of radiation. Thus during warm, cloudy weather the bacteria will grow faster than they are killed and the bacterial count will increase, but even so the apparent growth rate will be considerably less than if the sap were not exposed to daylight. During cool sunny weather the bactericidal effect of the sunlight will be more pronounced and the bacterial count will decrease. Of course there will be times when growth and destruction will occur at equal rates and the bacterial count will appear to be stationary.

Although Luckiesh *et al. (7)* found that radiation of wavelengths longer than 3000 A was relatively ineffective, yet the possibility exists that it may also exert appreciable influence when long exposures are used. Swart-Füchtbauer and Rippel-Balden *(11)* recently investigated the bactericidal activity of sunlight and found an appreciable effect even in the visible region at exposures of 12 to 16 hours. By exposing maple sap in plastic bags we are making use of a property of sunlight which has not been sufficiently appreciated and which as yet is not thoroughly understood. Although the experiments presented here demonstrate the sterilizing power of sunlight, it must be kept in mind that the results were obtained with only one type of micro-organism in the controlled experiments, and during only one season. However, the sterilizing effect was demonstrated with sap contaminated by natural flora during the normal period of maple sap collection.

In recent years, there has been a definite increase in consumer demands for the lighter grades of maple sirup. An important factor in the production of this light grade sirup is delivering to the evaporator only sap that is free of microbial spoilage. The use of plastic bags or other containers transparent to sunlight, together with improved sanitary practices, should reduce microbial growth and help increase the production of the desired higher quality maple sirup.

SUMMARY

The sterilizing power of sunlight was found to be effective in reducing the bacterial content of maple sap when exposed in plastic bags, transparent in the ultraviolet region.

Both artificially inoculated and naturally contaminated maple sap became practically sterile upon exposure to bright sunlight during cool weather.

Evidence was obtained that in exposed bags the growth and destruction of bacteria occur simultaneously and that the predominance of one process over the other is dependent on such factors as temperature, intensity of radiation and time of exposure.

Acknowledgment

The authors wish to express their appreciation to Dr. B. A. Brice for his interest and advice during the course of this study, and to A. Turner, Jr., for making the transmittance measurements on the plastic.

LITERATURE CITED

1. Coblentz, W. W., and Fulton, H. R. A radiometric investigation of the germicidal action of ultra-violet radiation. U. S. Bur. Standards *Sci. Papers,* **19**, No. 495, 641-680 (1924).
2. Downes, A., and Blunt, T. P. Researches on the effect of light upon bacteria and other organisms, *Proc. Roy. Soc.,* **26**, 488-500 (1877).
3. Edson, H. A., Jones, C. H., and Carpenter, C. W. Microorganisms of maple sap. Vermont Agricultural Expt. Sta. *Bull.,* **167** (1912).
4. Ellis, C., and Wells, A. A. *The Chemical Action of Ultraviolet Rays.* 1941, Reinhold Publishing Corporation, New York.
5. Hayward, F. W., and Pederson, C. S. Some factors causing dark-colored maple sirup. New York State Agr. Expt. Sta. *Bull.,* 718 (1946).
6. Holgate, K. C. Changes in the composition of maple sap during the tapping season. New York State Agr. Expt. Sta. *Bull.,* **742** (1950).
7. Luckiesh, M., Taylor, A. H., and Knowles, T. Killing air-borne respiratory micro-organisms with germicidal energy. *J. Franklin Inst.,* **244**, 267-290 (1947).
8. Pettit, E. Spectral energy-curve of the sun in the ultraviolet. *Astrophys. J.,* **91**, 159-85 (1940).
9. Rentschler, H. C., Nagy, R., and Mouromseff, G. Bactercidal effect of ultraviolet radiation. *J. Bact.,* **41**, 745-774 (1941).
10. Stair, R. Ultraviolet spectral distribution of radiant energy from the sun. *J. Res. Natl. Bur. Standards,* **46**, 353-357 (1951).
11. Swart-Füchtbauer, H., and Rippel-Baldes, A. Die baktericide Wirkung des Sonnenlichtes. *Archiv. f. Mikrobiologie,* **16**, 358-362 (1951).
12. Willits, C. O. (Unpublished data.)