

MAPLE SIRUP. XIV. ULTRAVIOLET IRRADIATION EFFECTS
ON THE GROWTH OF SOME BACTERIA AND YEASTS

Collection of maple sap has undergone drastic changes in the last few years. The conventional spout and bucket system is rapidly being replaced by transparent plastic bags and, more recently, by plastic tubing capable of transporting sap directly from trees to collection tanks.

Maple sap is susceptible to microbial infection, which, when it occurs, lowers the quality of the syrup. Recent studies in this laboratory (14, 18) have shown that sufficient ultraviolet radiation from sunlight is transmitted through plastic bags and tubing to cause a reduction in microbial populations of naturally-contaminated and inoculated maple sap. The advantages of a collection system which permits entrance of sunlight are apparent. It was considered that the use of germicidal lamps for the disinfection of contaminated sap and for keeping stored sap sterile might have even greater potentials in the control of microbial infection.

Since the first observations on the germicidal action of sunlight by Downes and Blunt (12, 13), the effects of solar radiation have been reported for a variety of organisms (2, 3, 22, 24, 25). It is generally acknowledged that the most lethal portion of the sunlight spectrum is in the vicinity of 260-270 m μ (6, 7, 9, 17). The germicidal effect (4, 5, 9, 20, 21) as well as the mutagenic effect (23, 26) of ultraviolet radiation have been reported by several workers.

The beneficial effect of mild heating or slightly elevated temperatures during post-irradiation treatment has been observed by several workers (8, 11, 16). Data are not available, however, comparing recovery rates at low and moderate incubation temperatures.

Extensive studies have not been made comparing the effectiveness of a single dosage of radiation with an equal dosage given in several periods of shorter exposure. Coblenz and Fulton (9) found little if any differences in the survival rates of *Bacterium coli communis* exposed to radiation continuously or intermittently with short and long intervals of rest.

The influence of flow rate of a liquid menstuum during exposure to ultraviolet radiation was investigated by Cortelyou *et al* (10). As anticipated, decreased destruction rates were observed in faster moving fluids.

The purpose of this investigation was to study the effect of ultraviolet radiation on microorganisms in maple sap. The variables tested were chosen from conditions which prevail naturally or which are controllable during sap collection. Representative strains, isolated from naturally-contaminated

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sap, were employed as the test organisms. These included one yeast and two bacterial strains employed in previous studies (15, 19).

In the sugar bush sunlight may exert a continuous effect throughout the daylight hours, or may be discontinuous and even variable in intensity due to intervening night hours, cloudy skies or shadows cast by trees. Previous work in this laboratory (18) had suggested that intermittent exposure to sunlight, during periods of low temperature, caused a greater destructive effect than continuous radiation of similar total dosage. These variable conditions were simulated by use of several dosage intensities and by use of continuous and discontinuous methods of exposure to ultraviolet radiation.

The temperature of maple sap in the sugar bush, during or following sunlight radiation, may vary from near-freezing to moderate. It was important to know whether or not temperature exerted any beneficial or detrimental effect on the recovery and subsequent growth of irradiated cells. Consequently, two incubation temperatures, 41° F and 80.6° F, were employed in the estimation of survivors.

The possibility was investigated of employing a commercial germicidal (ultraviolet) lamp to suppress microbial activity in large quantities of stored sap and to disinfect contaminated sap during handling. For the latter, sap was run over a glass-jacketed germicidal lamp to evaluate the effectiveness of ultraviolet radiation as a disinfectant on flowing sap.

All experiments were designed to permit statistical evaluation of the data so that an unbiased appraisal could be made.

MATERIALS AND METHODS

A. Apparatus and Equipment

1. Ultraviolet source for static system. General Electric 15- and 30-watt, 110 volt germicidal lamps mounted in reflecting fixtures.
2. Ultraviolet source for flowing system. General Electric 30-watt, 110 volt germicidal lamp encased by a concentric glass tube with approximately 0.5 cm space between lamp and tube as shown in Figure 1.
3. Coleman model 14 spectrophotometer.
4. Sterile maple sap, collected and stored in 1-gallon cans at 5° F as described previously (15), was thawed at room temperature and transferred aseptically to sterile flasks.

B. Organisms

Typical isolates from naturally-contaminated maple sap were used.

Pseudomonas-25 (deposited in the Northern Regional Research Laboratory collection and labeled NRRL B-1890 and *Pseudomonas*-11 (NRRL B-1888) were grown in petri plates on tryptone-glucose-yeast extract (TGY) agar for 24 hr at 80.6° F. Surface growth was removed by washing and diluted with sterile sap to obtain the desired concentration. An approximate cell concentration was estimated spectrophotometrically by comparison of the optical density of the diluted suspension with a standard turbidity curve prepared from suspensions of known concentration. Final estimations of the cell concentration were always made by plating and colony counts.

Cryptococcus albidus (NRRL Y-2422) suspensions were prepared in a similar manner from 48 hr cultures grown on Sabouraud agar (adjusted to pH 4.5 with sterile 10% lactic acid) at 80.6° F.

C. Irradiation procedure (static)

Pseudomonas-25. The stock suspension was diluted with sterile sap to a concentration of 1.4×10^8 organisms per ml. Fifteen ml of inoculated sap were transferred to each of

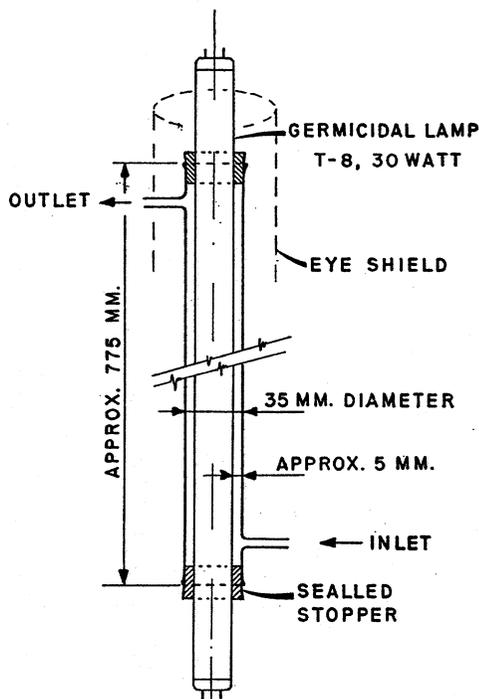


Figure 1. Apparatus used for ultraviolet irradiation of cells suspended in a moving liquid medium.

12 petri plates (3 sets of 4) and subsequently exposed to ultraviolet radiation as described below. The 15-watt Germicidal Lamp was mounted 100 cm above and parallel to the bench surface. All plates were placed in the same position relative to the lamp during exposure. Petri plate covers were removed prior to irradiation and then replaced immediately following exposure. The following methods of exposure were employed.

1. *Continuous irradiations.* Exposure times for this set of plates were 15, 30, 45 and 60 sec, respectively. Immediately following irradiation, the suspensions were diluted and plated with TGY agar. The plates were incubated at two recovery temperatures. Colonies were counted after 3 days at 80.6° F and after 10 days at 41° F. Triplicate plate counts were employed for all dilutions and for both incubation temperatures.

The second set of 4 plates containing inoculated sap was irradiated as described above and then held for 5 hr at 41° F, prior to dilution, plating, and incubation at the two temperatures employed.

2. *Discontinuous irradiations.* Irradiation of the third set of plates consisted of three exposures separated by two holding periods of 2½ hr each at 41° F; each exposure per plate was 1/3 the total time for the corresponding plates irradiated by the continuous method. Following the third exposure period, the suspensions were appropriately diluted, plated and incubated at 41° F and 80.6° F.

All plates were held in the dark at 41° F except during radiation and plating to minimize photoreactivation and possible stimulation of recovery and/or growth by elevated temperatures.

Pseudomonas-11. A suspension containing 1.2×10^8 organisms per ml was prepared and irradiated as described for *Pseudomonas*-25.

Cryptococcus alibidus. A suspension containing 2.3×10^7 organisms per ml was prepared and irradiated by the continuous and discontinuous methods with a 30-watt

germical lamp mounted 35 cm from the bench surface. Survivors were grown on acidified Sabouraud agar, and after incubation periods of 4 days at 80.6° F and 10 days at 41° F colony counts were made.

D. Irradiation procedure (flowing)

Three other suspensions of *C. albidus* in sap, containing 1.9, 2.5, and 4.6 x 10⁶ cells per ml, were prepared and irradiated while flowing as a 0.5 cm layer over a 30-watt germicidal lamp (Fig. 1). Three different flow rates, 26, 33, and 40 ml per second, were employed. After the system had been equilibrated (suspension flowing for a few minutes), samples of the eluate were taken for estimation of the number of survivors. Aliquots of the sample were mixed with acidified Sabouraud agar after dilution and plating, and survivors counted after 4 days at 80.6° F.

RESULTS AND DISCUSSION

A wide range of survival values was required which could be treated statistically. The dosages which gave this spread of data were established empirically. For the two *Pseudomonas* strains it was found that a 15-watt germicidal lamp mounted 100 cm from the petri plates gave the desired range in data when exposures of 15, 30, 45 and 60 sec were employed.

Table 1 shows the effects of the variables tested (duration of irradiation, method of exposure, holding period subsequent to irradiation, and incubation temperature) on destruction of *Pseudomonas*-25 by ultraviolet radiation. An analysis of variance of these data is given in Table 2 which shows that,

TABLE 1

The effects of method of irradiation, holding for 5 hours at 41° F, and incubation temperature on the reduction in numbers of reproducing cells of *Pseudomonas*-25 exposed for various lengths of time to a 15-watt germicidal lamp

Method of irradiation	Hours held at 41° F	Incubation temperature ° F	Irradiation time, seconds			
			15	30	45	60
			Percent reduction			
Continuous.....	0	41	9.3	66.8	88.6	97.8
	0	80.6	28.3	60.9	82.1	92.3
	5	41	26.8	72.8	89.1	97.1
	5	80.6	32.0	63.9	82.4	91.3
Discontinuous.....	5 ¹	41	13.8	39.6	69.9	51.7
	5 ¹	80.6	19.7	35.1	64.1	87.2

¹ Two—2½-hour periods.

TABLE 2

Results of the analysis of variance of data obtained with ultraviolet-irradiated *Pseudomonas*-25 cells (table 1)

Source	Degrees of freedom	Sum of squares	Mean square	F
Dosage (D).....	3	15,255.39	5,085.13	66.84 ¹
Method (M).....	2	2,177.67	1,088.84	14.31 ¹
Temperature (T).....	1	10.20	10.20	0.13
D x M.....	6	463.78	77.30	1.02
D x T.....	3	360.66	120.22	1.58
M x T.....	2	143.66	71.83	0.94
D x M x T (error).....	6	456.50	76.08
Total.....	23	18,867.86		

of the main effects, dosage and method were statistically significant at the 1% level, having F values of 66 and 14, respectively. Since the analysis of variance shows that no interactions exist between main effects, the effects of dosage and method are independent and clear-cut. As anticipated, increases in exposure times (dosage) had the most pronounced effect on the reduction of numbers of reproducing cells, regardless of the irradiation method employed. Table 1 also shows that a more effective reduction in cells was accomplished by the continuous method of exposure to ultraviolet than by the discontinuous method. Further, it can be seen that no significant influence resulted from holding survivors at 41° F for 5 hr following radiation.

Since temperature of incubation had no significant effect on the reduction of reproducing cells of *Pseudomonas*-25, it can be concluded that survivors recovered equally well at either low (41° F) or moderate (80.6° F) incubation temperatures.

Table 3 shows the data obtained when cells of *Pseudomonas*-11 were irradiated and the analysis of variance of these data is given in Table 4. As observed with *Pseudomonas*-25, only dosage and method of irradiation (main effects) had statistical significance at the 1% level on the reduction in numbers of reproducing cells (Table 4). Some statistical interaction is noted between dosage and method. As was the case for the *Pseudomonas*-25,

TABLE 3

The effects of method of irradiation, holding for 5 hours at 41° F, and incubation temperature on the reduction in numbers of reproducing cells of *Pseudomonas*-11 exposed for various lengths of time to a 15-watt germicidal lamp

Method of irradiation	Hours held at 41° F	Incubation temperature, ° F	Irradiation time, seconds			
			15	30	45	60
			Percent reduction			
Continuous.....	0	41	36.8	64.9	84.6	96.0
	0	80.6	37.4	69.3	84.3	94.0
Discontinuous.....	5	41	26.9	56.0	79.3	89.5
	5	80.6	28.6	60.9	80.9	89.1
	5 ¹	41	19.2	39.6	55.2	79.3
	5 ¹	80.6	27.1	42.6	59.1	74.5

¹ Two 2½ hour periods.

TABLE 4

Results of the analysis of variance of data obtained with ultraviolet-irradiated *Pseudomonas*-11 cells (table 3)

Source	Degrees of freedom	Sum of squares	Mean square	F
Dosage (D).....	3	11,265.04	3,755.01	1,009.41 ¹
Method (M).....	2	1,892.46	946.23	254.36 ²
Temperature (T).....	1	17.51	17.51	4.71
D x M.....	6	214.08	35.68	9.59 ²
D x T.....	3	38.19	12.73	3.42
M x T.....	2	3.50	1.75	0.47
D x M x T (error).....	6	22.34	3.72
Total.....	23	13,453.12		

TABLE 5
Results of the analysis of variance of the comparison between data obtained for strains 11 and 25 of *Pseudomonas* (Tables 1 and 3)

Source	Degrees of freedom	Sum of squares	Mean square	F
Strains (S).....	1	3.26	3.26	0.89
Dosages (D).....	3	26,252.04	8,750.68	2,397.45 ^a
Methods (M).....	2	3,830.17	1,915.09	524.68 ^a
Temperature (T).....	1	27.76	27.76	7.61 ¹
S x D.....	3	268.38	89.46	24.51 ^a
S x M.....	2	239.94	119.97	32.87 ^a
S x T.....	1	0.41	0.41	0.11
D x M.....	6	448.98	74.83	20.50 ^a
D x T.....	3	151.62	50.54	13.85 ^a
M x T.....	2	83.12	41.56	11.39 ^a
S x D x M } S x D x T } S x M x T } (error).....	23	838.55	3.65
D x M x T }				
S x D x M x T }				
Total.....				

¹ Significant at 5% level.
^a Significant at 1% level.

continuous exposure resulted in the greatest reduction of cells, and the effect of holding at 41° F following irradiation did not appreciably increase the number of survivors. Likewise, incubation temperature had no effect on the number of survivors of irradiated cells. The data of Tables 1, 2, 3, and 4 indicate that there may be an effect due to bacterial strain difference. Since all conditions tested were identical, a statistical comparison was made of the data for the two bacterial strains and is given in Table 5. Since the F value for strains is not significant, it was concluded that strains 11 and 25 were equally susceptible to ultraviolet radiation.

Preliminary experiments showed that *C. albidus* was much more resistant to ultraviolet radiation than were the bacteria. Therefore, a stronger light source, 30-watts, mounted only 35 cm above the petri plates was used.

The results and the analysis of variance of ultraviolet-irradiated *C. albidus* cells are given in Tables 6 and 7. In contrast with the pseudomonads, the

TABLE 6
The effects of method of irradiation, holding for 5 hours at 41° F, and incubation temperature on the reduction in numbers of reproducing cells of *Cryptococcus albidus* exposed for various lengths of time to a 30-watt germicidal lamp

Method of irradiation	Hours held at 41° F	Incubation temperature, ° F	Irradiation time, seconds			
			15	30	45	60
Percent reduction						
Continuous.....	0	41	64.5	73.9	81.5	87.2
	0	80.6	75.4	81.1	89.0	95.2
	5	41	63.2	73.2	86.0	88.9
	5	80.6	74.9	82.4	92.5	96.1
Discontinuous.....	5 ¹	41	67.6	71.8	85.8	91.4
	5 ¹	80.6	77.2	78.9	90.8	94.6

TABLE 7
Results of the analysis of variance of data obtained with ultraviolet-irradiated *Cryptococcus albidus* cells (Table 6)

Source	Degrees of freedom	Sum of squares	Mean square	F
Dosage (D).....	3	1,770.68	590.23	766.53 ^a
Method (M).....	2	8.14	4.07	5.28 ^a
Temperature (T).....	1	361.15	361.15	469.02 ^a
D x M.....	6	31.95	5.32	6.90 ^a
D x T.....	3	20.29	6.77	8.79 ^a
M x T.....	2	7.11	3.56	4.62
D x M x T (error).....	6	4.61	0.77
Total.....	23	2,203.93		

^a Significant at 5% level.

^b Significant at 1% level.

only main effects statistically significant at the 1% level, and with no accompanying interactions, are incubation temperature and dosage. At the 5% level, methods as well as two interactions (between dosage and methods and between dosage and temperature) were statistically significant. Even so, the important main effects are dosage and temperature. Also, the 5 hr holding period at 41° F after continuous exposure had no effect on the number of survivors.

The striking feature which distinguishes these results from those observed with the bacteria was the effect of incubation temperature on survivors. With *C. albidus* a consistent and significantly lower percent reduction was observed at the lower temperature, 41° F. This is consistent with the enhanced recovery of ultraviolet-irradiated *E. coli* under conditions suboptimal for the growth of unirradiated cells reported by Alper and Gillies (1).

The effectiveness of the destruction of *C. albidus* by irradiation when the liquid in which they are suspended is caused to flow at different rates over a 30-watt germicidal lamp is given in Tables 8 and 9. The effects of only two variables, flow rate and concentration of cells, were studied. Of these only flow rate (time of exposure) had statistical significance. Table 8 shows that the germicidal effect decreases as the flow rate increases. Initial cell concentration had no effect on percent reduction (Table 9).

From the above it is immediately suggested that ultraviolet radiation can be used both to disinfect sap as it is put into storage and to suppress growth during storage. The practical application of ultraviolet radiation would be to flow all sap over a germicidal lamp as it is being run into storage tanks and to have a germicidal lamp mounted in the tank to keep the sap sterile,

TABLE 8
The effect of flow rates and initial concentration on reduction in numbers of *Cryptococcus albidus* cells irradiated with ultraviolet in a moving liquid

Flow rate per second	Initial concentration per ml		
	1.9 x 10 ⁸	2.5 x 10 ⁸	4.6 x 10 ⁸
	Percent reduction		
26 ml.....	52.6	52.4	55.6
33.....	47.4	50.4	49.8
40.....	34.8	33.7	39.3

TABLE 9
Results of the analysis of variance of data obtained with *Cryptococcus albidus* cells irradiated with ultraviolet in a moving liquid (Table 8)

Source	Degrees of freedom	Sum of squares	Mean square	F
Rate of flow (R).....	2	504.55	252.28	97.03 ¹
Concentration (C).....	2	18.69	9.35	3.60
R x C (error).....	4	10.38	2.60
Total.....	8	533.62		

¹ Significant at 1% level.

or low in microbial count, during storage. It should be emphasized that if the contaminating load is high at the time the sap is collected, the quality of the sap will not be improved by ultraviolet irradiation, since the undesirable effects resulting from microbial activity would have occurred. The utility of germicidal lamps lies in its use for the disinfection, prior to storage, of slightly or moderately contaminated sap and in suppressing growth and deleterious fermentations during storage.

SUMMARY

A study was made of the effects on the reduction of living cells of two bacterial and one yeast strain suspended in maple sap when exposed to ultraviolet radiations of different intensities and for different lengths of time.

The two bacterial strains were equally sensitive to destruction by ultraviolet irradiation but were more sensitive than the yeast.

As the time of exposure to irradiation increased, there was a corresponding increase in the reduction of living cells for all organisms, regardless of the method of irradiation employed.

The two bacterial strains exposed to ultraviolet radiation by the discontinuous method were less effectively killed than when they were exposed by the continuous method, demonstrating that the total effect of mild radiation dosages is not cumulative. Destruction of the yeast strain was the same, however, by both methods of static irradiation.

Recovery from radiation damage during a 5 hour holding period at 41° F did not result in any significant increase in number of survivors for the yeast and bacteria.

The temperature of incubation, 41° F or 80.6° F, following irradiation had no effect on survivor plate counts to the two bacterial strains. The yeast strain, on the other hand, showed a higher recovery when incubated at the lower temperature.

In the flowing system, greatest reduction of the yeast occurred with the slowest flow rate (increased dosage), irrespective of initial cell concentration.

The practical significance of these results is discussed.

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