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TEMPERATURE AFFECTS THE CLARITY OF HUMMINGBIRD FEEDER SOLUTIONS

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Abstract—Books, magazine articles and the internet suggest that hummingbird feeder solution should be changed frequently to avoid the buildup of bacteria, mold, and fungi. This microbial buildup results in decreased clarity. However, experimental studies on rate at which the clarity declines have not been published. In addition, the microbes that grow in the solution have not been identified and it is unknown if bacteria present in the hummingbird feeder solution may be toxic to hummingbirds. We hypothesized that hummingbird feeder solution clarity is dependent upon sucrose concentration, light intensity, and growing degree days. To test our hypothesis, we measured the clarity of sucrose solution in 12 hummingbird feeders during 30 June - 6 October 2010 in Oklahoma City, Oklahoma. We determined that growing degree days and light intensity had a significant effect on clarity on the sucrose solution, whereas sugar concentration did not have a significant effect. We found that the clarity of the solution declined after 97 growing degree days in full sun and 178 growing degree days in full shade. We found that yeast in the order Sacchromycetales and the bacterium *Methylobacterium extorquens* grew in the hummingbird feeder solution. Neither the yeast nor the bacterium are thought to be toxic to hummingbirds. We concluded that hummingbird feeders should be changed every four to six days during summer, and every 10-18 days during spring and fall.

INTRODUCTION

Feeding hummingbirds is growing in popularity (Williamson 2001) and directions for how often to change hummingbird feeders can be found in books, magazines and online (Williamson 2001, Aziz 2002, West and Butler 2010, Pugh 2011). For example, Stokes and Stokes (1989) and West and Butler (2010) suggest changing the feeder solution weekly if temperatures are less than 15.6°C or every two days if temperatures exceed this limit. Tilford (2009) suggests changing feeder solution every two or three days regardless of temperature.

The clarity of the hummingbird feeder solution declines with time due to an increase in the numbers of microbes (Bringhurst *et al.* 2001). The greater the number of microbes, the greater the opacity of the solution (Bringhurst *et al.* 2001). Some microbes produce toxins, which could be detrimental to hummingbirds (Tortora *et al.* 2007, West and Butler 2010). However, the identity of the microbes in hummingbird feeder solution has apparently never been published.

There are several variables that might influence the rate at which the clarity of the hummingbird feeder solution degrades. For example, sucrose concentration might affect how rapidly microbial populations grow since bacteria and yeasts can use sugar as an energy source (Tortora *et al.* 2007). However, an excess of sugar can inhibit bacteria growth by causing bacteria to undergo plasmolysis, weakening the molecular structure of DNA, and accelerating the production of antimicrobial compounds (Fields 1979, Chirife *et al.* 1983, Parish 2006). For example, Fields (1979) suggests that concentrations of 1 to 10% sucrose will inhibit growth of some microorganisms. Flowers fed upon by hummingbirds range from 16.9%-33.2% sucrose concentration (Demcheck 2003). Williamson (2001) suggests using a 1:4 sugar to water ratio (i.e. a 17% sucrose solution), although ratios ranging from 1:3 (21%) to 1:5 (14%) are acceptable.

Temperature also affects microbial growth (Tortora *et al.* 2007). Mold and bacteria grow and divide faster in the optimal temperature range (Guerrero-Beltran and Barbosa-Canovas 2004). Temperatures below the minimum optimal temperature result in an increased generation time (i.e. bacteria and mold do not grow as rapidly), while temperatures above the maximum optimal temperature cause growth rate to slow and eventually cease (Gunsalus1962). For example, mesophilic bacteria exhibit optimal growth between 20-40^{o}C, but at 42^{o}C the growth rate decreases rapidly (Gunsalus and Stainer 1962, Ryckeboer *et al.* 2003).

It is likely that the temperature of the hummingbird feeder solution will be warmer if the feeder is placed in the sunlight due to due to the greenhouse effect. The greenhouse effect works by preventing heat absorbed via sunlight from leaving the structure (e.g. a hummingbird feeder), which raises the temperature inside the structure (Raval and Ramanathan 1989). The sucrose solutions in full sunlight should therefore be warmer than sucrose solutions in the shade. Consequently, microbes may reproduce more rapidly in hummingbird feeder solutions in full sunlight.

Our objectives were to identify the microorganisms growing inside the hummingbird feeders and to test the hypotheses that hummingbird feeder solution clarity is dependent upon sucrose concentration, temperature, and amount of direct sunlight. The goal of this project was to develop specific recommendations on how often to change hummingbird feeders in Oklahoma.

METHODS

From 30 June 2010 - 6 October 2010 we measured the clarity of sucrose solutions in twelve Perky Pet Popular Pinch Waist Glass Hummingbird Feeders that contained 16 fluid ounces of solution. Our study site was a residential neighborhood in Oklahoma City, Oklahoma (35^{o} 36' 11.44" N, -97^{o} 35' 21.45" W). Six of the twelve feeders contained 21% sucrose solution, which had a ratio of three cups of tap water and one cup of sugar. The other six feeders contained 17% sucrose solution, which had a ratio of four cups of tap water and one cup of sugar. The percent sucrose solution was calculated by dividing the mass of sugar by the total weight of the solution. Six feeders, three with 21% sucrose solution and three with 17% sucrose solution were hung side-by-side outdoors in a constantly shaded area. The remaining six feeders were hung side-by-side outdoors in an area with exposure to sunlight throughout the day. During October and November 2012 temperature differences

between hummingbird feeder solutions in shaded versus sunlight areas were measured and recorded two or three times per day.

Every 48 hours six mL of sucrose solution was taken from each hummingbird feeder and placed into sterile vials. The vials were taken immediately to the University of Central Oklahoma and the clarity of the solution was measured using a spectrophotometer (Spectronic 20 made by Bausch and Lomb), which was calibrated using deionized water. The output from the spectrophotometer was recorded as percent transmittance. We recorded average daily temperatures as reported at Will Roger's World Airport. The hummingbird feeders were taken down after collecting five measurements (ten days worth of data) and cleaned using an autoclave set to 121°C at 15 psi to ensure there was no cross contamination (Tortora *et al.* 2007). A total of six trial runs were conducted.

During September 2010, the microorganisms were identified at the University of Central Oklahoma. The microbes were determined using a combination of gram stains, morphological characteristics, and colonial pigmentation. The microbes were tentatively determined, as rRNA sequencing was not performed.

Statistical Analyses-We used a three-way repeated measures ANOVA model to analyze the effect light intensity had on optical clarity over growing degree days and the effect sucrose concentration had on transparency over growing degree days (Pace 2007). Growing degree days is a measure of temperature (above 10°C) times day and is a method of assigning a heat value to each day (Miller *et al.* 2001). Seasonal effects were confounded by growing degree days, as the temperature was not constant across the months of our study. Consequently, we used growing degree days rather than seasonal effects in our analysis. A paired *t*-test was used to compare temperatures in shaded and sunlit feeders. Results are presented as mean ± standard error.

RESULTS

Temperatures in the shaded feeders averaged cooler than feeders in full sunlight by noon (Table 1). At 0800 hr, both solutions had similar temperatures. Solutions in the shaded feeders (n = 11) averaged 5.2 ± 1.4 °C colder than the ambient temperature, while solutions in full sun (n = 11)

Table 1. Temperature differences between hummingbird feeder solution in shaded versus non-shaded were measured and recorded two or three times per day from 26 October through 5 November 2012. A hyphen (-) indicates that no data were collected.

Time	0800 hr			1200 hr			1600 hr		
Date (2012)	Temperature (°C)			Temperature (°C)			Temperature (°C)		
	Outdoor	Shaded	Sunlight	Outdoor	Shaded	Sunlight	Outdoor	Shaded	Sunlight
26 Oct	16.7	18.9	16.7	15.6	13.3	14.4	-	-	-
27 Oct	8.9	3.9	3.9	17.8	15.6	17.8	-	-	-
28 Oct	12.2	3.3	4.4	26.1	10.6	12.2	-	-	-
29 Oct	16.7	5.6	8.3	-	-	-	-	-	-
30 Oct	17.2	8.9	11.1	22.2	17.8	26.1	-	-	-
31 Oct	10	8.9	7.8	21.1	17.8	29.4	22.2	27.2	23.3
1 Nov	18.9	17.2	17.8	22.2	21.7	33.3	29.4	29.4	33.3
2 Nov	19.4	15.6	16.1	24.4	23.3	27.8	23.9	27.2	30
3 Nov	8.9	8.3	11.7	23.3	13.3	18.9	22.2	18.3	18.3
4 Nov	15	6.7	8.3	23.9	13.9	23.9	21.7	19.4	21.1
5 Nov	17.2	6.1	6.1	22.8	19.4	24.4	18.9	18.3	19.4

averaged 4.4 ± 1.2 °C colder than the ambient temperature. The difference between the two temperatures was not significant (paired *t*-test, t = -1.6, df = 10, P = 0.13). By 1200 hr, solutions in the shaded feeders (n = 10) averaged 5.3 ± 1.5°C colder than the ambient temperature, while the temperatures in the sunlit solutions (n = 10) averaged 0.9 ± 2.2 °C warmer than the ambient temperature. The sunlit solutions were significantly warmer by noon than the shaded solutions (paired *t*-test, t = -4.8, df = 9, P < 0.001). By 1600 hr, temperatures in the shaded solutions (n = 6) averaged 0.3 ± 1.4 °C warmer than the ambient temperature, while temperatures in the sunlit solutions (n = 6) averaged 1.2 ± 1.4°C warmer than the ambient temperature. The difference, however, was not significant (paired *t*-test, t = -0.8, df = 5, P = 0.4).

We found that growing degree days had a significant negative effect on clarity (P < 0.001; Table 2). Likewise, solutions placed in full sun became cloudier at a significantly faster rate than solutions put in the shade (P < 0.001; Table 2). Sucrose concentration, however, had no significant effect on the optical clarity of the solution (P = 0.90; Table 2). There was, however a

Table 2. The results of the repeated measures ANOVA model analysis. Growing degree days significantly affected clarity while the sugar concentration and the amount of sunlight did not.

	df	Sum Sq	Mean Sq	*F*	*P*
Growing Degree Days	1	2307	2306.59	45.471	<0.001
Sugar Group	1	1	0.83	0.016	0.898
Light Group	1	662	662.29	13.056	<0.001
GDD:Sugar Group	1	34	34.01	0.671	0.413
GDD:Light Group	1	200	200.33	3.949	0.048
Sugar Group:Light Group	1	257	256.99	5.066	0.025
GDD:Sugar Group:Light Group	1	294	294.04	5.797	0.017
Residuals	330	16,740	50.73		

significant interaction between growing degree days and both the sucrose concentration and the amount of sunlight on the clarity of the solution (Table 2). However, while significant, interactions accounted for only 4% of the observed variation (Table 2). Consequently, simpler, but still highly significant linear regressions were created after discarding the interaction effects. When the data was subset by light versus shade, a significant linear regression ($F_{1,172}$ = 27.56, P < 0.001) for predicting percent transmittance in areas exposed to full sunlight was:

*Percent transmittance (sunlight) = -0.06375*growing degree days + 93.69582.*

And a significant linear regression ($F_{1,172}$ = 17.37, P < 0.001) for optical clarity in the shade was:

*Percent transmittance (shade) = -0.034787*growing degree days + 93.723302.*

Microorganisms identified in the solutions included the tentatively determined bacterium *Methylobacterium extorquens* and a budding yeast species in the order Sacchromycetales.

DISCUSSION

We hypothesized that higher sucrose concentration, temperature, and light intensity would have a significant effect on clarity of the sucrose solutions. However, while growing degree days and amount of sunlight significantly affected clarity, sucrose concentration in our study did not show a significant effect. Liebig's Law of the Minimum states that growth rates are limited by the scarcest nutrient (Stilling 2012). It seems likely, therefore, that the sucrose concentrations were not a limiting factor for microbial growth in the solutions.

The formulas derived above to describe percent transmittance can be used as a guide for how often to change the solution in a hummingbird feeder. In our experience, hummingbirds did not visit feeders as often once the percent transmittance dropped below 88% (pers. obs.). So, for feeders placed in full sun, the feeders should be changed after 97 growing degrees days. If the average high temperature was 20°C (68 °F), then the feeders would need to be changed after approximately 10 days (97°C*days/(20 °C - 10°C) = 9.7 days, where 10°C is the base for growing degree days). These recommendations are summarized in Table 3.

Average High Temperature (oC)	# of Days Full sun	# of Days Full shade
15	20	36
20	10	18
25	7	12
30	5	9
35	4	8
40	4	6

Table 3. Recommendations for how frequently to change hummingbird feeder solutions. Feeders in full sun should be changed after 97 growing degrees days, while feeders in full shade should be changed after 178 growing degree days.

The microorganisms found in the feeders were *Methylobacterium extorquens* and yeast in the order Sacchromycetales. For mesophilic bacteria and yeast, such as *Methylobacterium extorquens* and Sacchromycetales, the optimal growth temperature range (temperature at which microbe grows the best) was 20-40°C (Gunsalus and Stainer 1962, Arthur and Watson 1976, Tortora *et al.* 2007) and the fastest growth rate is near the top of the optimum growth temperature (Gunsalus and Stainer 1962, Tortora *et al.* 2007). We suggest that one reason for the relatively rapid rate at which the transmittance declined in sunlit solutions was due to the greenhouse effect causing elevated temperatures in these solutions. The warmer temperatures (within the mesophilic range) allowed the bacteria and mold to reproduce more rapidly.

Yeasts in the order Sacchromycetales are not known to produce toxins that are harmful to hummingbirds (Frobisher and Fuerst 1973, Eng *et al.* 1984) although it should be noted that the yeast *Candida albicans* (in the order Sacchromycetales) causes infections in humans (Ryan and Ray 2004). *Methylobacterium extorquens* is pathogenic, but of low virulence (Anesti *et al.* 2004). *Methylobacterium extorquens* and Sacchromycetales commonly grow on phyllospheres, the aboveground portion of plants, which provide habitats to bacteria and yeasts (Davey *et al.* 2009, Knief *et al.* 2010). Therefore, it is likely that hummingbirds and/or insects are inoculating feeders with *Methylobacterium extorquens* and Sacchromycetales. It appears

that *Methylobacterium extorquens* and Sacchromycetales have a commensal relationship in the sucrose solution because Sacchromycetales consumes sucrose in order to produce methanol and *Methylobacterium extorquens* consumes methanol in order to produce energy (Vorholt *et al.* 2000, Dyer 2003, Batista *et al.* 2005, Vuilleumier *et al.* 2009, Alvarenga *et al.* 2011).

In conclusion, we found the rate at which the clarity of the sucrose solution declined depended upon both growing degree days and whether the feeder was placed in full sunlight or full shade. Feeders in full sunlight during the hottest days of summer may need to be changed as often as every four days. However, there are still several issues that should be studied in the future. We did not gather detailed information on visitation rates at the feeders, and so it is possible that hummingbird visitation rates may decline before the transmittance rate of the solution reaches 88%. It is also possible that feeders which are routinely visited by Hymenopterans (such as ants and bees) may show a more rapid decline in optical transmission rates as the presence of these organisms in the solution could result in an increase in potentially limiting factors such as proteins and carbohydrates. Finally, it would be worthwhile to expand the scope of this study beyond central Oklahoma to determine whether additional microbes may be present at other locations as well as using molecular approaches to firmly identify these microbes.

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