Honey Sac Contents: A Technique for Collection and Measurement in Foraging Honey Bees (Hymenoptera: Apidae)[1]

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ABSTRACT

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Workers of *Apis mellifera* L. returning from foraging were collected from the entrances of six colonies, placed by groups of 10 bees into cyanide killing vials, and held for 0, 2, 4, 8, and 24 h. The nectar in their honey sacs was expressed, and the volume and sugar concentration were measured. The controls were measured immediately upon collection without exposure to cyanide. There were no significant differences between the controls and any of the treatments or among the treatments. Bees were trained to feeding dishes containing 50% sucrose syrup, collected after feeding, and measured as before without cyanide and 0 and 24 h after cyanide. The mean volume of syrup from bees held 24 h (63.3 μl) was significantly less than that of the controls (68.9 μl) or 0 h (68.4 μl). There were no significant differences in concentration. Thus, bees with very large loads should be held much less than 24 h before measurement. However, honey bee foragers with more average nectar loads may be collected in the field and held in cyanide killing vials for periods of at least 24 h without causing a significant change in the volume or sugar concentration of the contents of their honey sacs.

For many studies of foraging behavior of the honey bee, *Apis mellifera* L., it is necessary to analyze the contents of the honey sac. It is desirable to have a method of sample collection which can maximize the number of samples collected in a short period of time so that variance resulting from different collection times can be minimized. Also desirable is a method that allows analysis of the samples at a later time in the laboratory but that does not alter the volume or sugar concentration of the honey sac contents, i.e., does not cause regurgitation. Finally, it is preferable to have the sampled bees either anesthetized or dead during analysis so that they cannot sting.

Anesthetizing bees with carbon dioxide (Gary and Lorenzen 1976) or quick freezing (Dietz 1971, Soehngen and Jay 1973, Waller and Martin 1978) are techniques which have been used. However, both of these techniques are inconvenient because they require equipment for storage and transport of Dry Ice.

Here we describe a simple and convenient honey bee sample collection technique for field use that does not cause changes in the volume or sugar concentration of the contents of the honey sac.

Materials and Methods

Killing Vials

In preliminary tests, we found that Owens-Illinois, SP31, 10-dram (ca. 18-g) plastic snap cap vials rapidly lost the potassium cyanide (KCN) charge within a few days after they were made up as killing vials. However, Fisherbrand plastic, 20-ml screw cap scintillation vials maintained effectiveness for a much longer period of time. Killing vials were made from these screw cap vials with layers from the bottom as follows: ±5 mm of KCN, ±5 mm of fine sawdust, fiber glass window screen disc of snug fit, ±5 mm plaster of paris. The first two layers were compacted as they were added with a wooden rod that fit snugly into the vial. The vials were made up the day before their initial use and the tops were left off the first few hours to allow the plaster to dry.

Field Foragers

Wire queen excluders were leaned against the front of the bee hives to block the flight path of returning foragers. As returning bees landed on the excluder, bees without pollen loads were picked up by hand and placed in the killing vials. Honey sac contents from each bee were obtained by decapitation; then the abdomen was squeezed with finger tips to express the contents of the honey sac through the esophagus. The expressed liquid was drawn into a 50μl microsampling pipette. The volume was measured to the nearest 5 μl by measuring the length of the liquid column. Bees yielding less than 5 μl of expressed liquid were not included. A drop of the liquid was placed into a Bausch and Lomb refractometer, and the sugar concentration (percent, wt/wt) was measured (Bolten et al. 1979). Water carriers, i.e., those bees carrying sugar concentrations of 5% or less as measured by the refractometer, were not included.

The controls were measured immediately on collection without exposure to KCN. A 0-h treatment was measured in the field shortly (less than 10 min) after KCN exposure. Other treatments were measured in the laboratory after the bees were held in the killing vials for 2, 4, 8, and 24 h at room temperature or 24 h in a refrigerator at 3° C.

The bees were collected ca. 11:30 a.m. on 6 August 1980. About 14 bees per vial were collected from the entrances of six colonies so that 10 bees from each vial could be recorded that were carrying more than 5 μl of liquid of more than 5% sugar concentration. Thus, a total of 420 bees was measured, i.e., by groups of 10 bees from each of six colonies for each of seven treatments.

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Table 1.—Field foragers: mean volume and sugar concentration of honey bee honey sac contents after sample collection in potassium cyanide killing vials[a]

Treatment	vol (μl) ($\bar{x}$ ± SE)	Sugar concn (%) ($\bar{x}$ ± SE)	Analysis				
			Source of variation	df	Vol		Concn
					F	P	F P
Controls: no cyanide	24.6 ± 2.3	24.8 ± 1.6	Treatment (T)	6	0.87	0.53	0.67 0.67
Cyanide treated			Colony (C)	5	3.44	0.01	1.86 0.13
0, Measured immediately	26.0 ± 2.3	24.2 ± 1.6	T × C (error)	30	1.15	0.28	1.47 0.06
Held 2 h	25.3 ± 2.3	23.5 ± 1.6	Sampling	378			
Held 4 h	24.2 ± 2.3	24.0 ± 1.6					
Held 8 h	28.3 ± 2.3	23.1 ± 1.6					
Held 24 h	24.3 ± 2.3	25.7 ± 1.6					
Held 24 h refrigerated	23.8 ± 2.3	24.9 ± 1.6					

[a]For each treatment 10 bees from each of six colonies were sampled (n = 60). None of the treatment means for volume or concentration were significantly different at the 5% level by Duncan's multiple range test.

Table 2.—Feeding dish foragers: mean volume of honey bee honey sac contents after sample collection in potassium cyanide killing vials[a]

Treatment	Vol (μl) ($\bar{x}$ ± SE)	Analysis			
		Source of variation	df	Vol	
				F	P
Controls: no cyanide	68.9 ± 1.7	Treatment (T)	2	22.24	0.002
Cyanide treated		Colony (C)	3	2.52	0.15
Measured immediately	68.4 ± 1.7	T × C (error)	6	0.44	0.85
Held 24 h at room temp	63.3 ± 1.7[b]	Sampling	108		

[a]For each treatment, 10 bees from each of four colonies were sampled (n = 40).
[b]This mean is significantly different from the other two at the 1% level, by Duncan's multiple range test.

Feeding Dish Foragers

Bees were trained to a glass petri dish containing 20 ml of 50% (wt/wt) sucrose syrup. This dish was 15 cm from the hive and was placed on a surface permitting bees returning to the hive from the feeder to walk. Foragers that had completed feeding at the dish and were returning to the hive were picked up by hand and treated, as were the field foragers.

The controls were measured immediately without exposure to KCN; one set of samples was measured in the field shortly (less than 10 min) after KCN exposure, and a second set was measured after 24 h of exposure to KCN at room temperature.

The bees were collected between 1:00 and 2:30 p.m. on 29 October 1981. Bees were collected from four colonies, all of which were different from the six field forager colonies. The total number of bees recorded was 120, i.e., by groups of 10 bees from each of four colonies for each of three treatments.

Data were analyzed by using a randomized complete block design with blocks representing colonies. Hypotheses were tested by F tests and Duncan's multiple range test.

Results and Discussion

Field Foragers

None of the treatments had a significant effect on either the volume or the sugar concentration (Table 1) of the contents of the honey sac of the sampled bees. Therefore, honey bees may be collected in KCN killing vials and held for at least 24 h without a significant effect on the contents of their honey sacs.

This provides a rapid and convenient method for field collection of honey bees. The samples can then be held until the honey sac contents can be measured in the more suitable conditions of the laboratory. The speed of this collection method permits nearly simultaneous collection from a number of colonies with a reasonably small collecting crew. For example, a collecting crew of eight people (using this technique) was able to collect 30 bee samples from each of 35 colonies in ca. 20 min.

An important requirement of this method, which we learned in earlier tests, is that the killing vials must kill the bees quickly. Occasionally, vials had lost most of the KCN or were improperly made, so that some of the bees were still active when the vial was opened. In these cases, significant regurgitation of the honey sac contents had occurred. Properly made vials killed bees within ca. 30 sec. Even with rapid sample collection, at any time only a few collected bees were still active and capable of leaving the killing vial.

Feeding Dish Foragers

The recorded volume (Table 2) of the bees held 24 h ($\bar{x}$ = 63.3 μl) was significantly less than that of the controls ($\bar{x}$ = 68.9 μl) or that of the bees measured shortly after KCN exposure ($\bar{x}$ = 68.4 μl). These latter

two means did not differ significantly. Thus, exposure to KCN did not in itself cause a change in the volume of honey sac contents, even with these very large volumes. Concentration of honey sac contents was not affected by these treatments, since concentrations remained at 50% in all cases. These results contrast with those of Gary and Lorenzen (1976), who found that some bees anesthetized with CO_2 regurgitated during or after anesthesia. Their study showed that the number of bees regurgitating increased from none of 20 when fed 10 μl to 6 of 20 when fed 50 μl of 49% honey solution.

Therefore, honey bee field foragers carrying nectar loads of 20 to 30 μl can be collected and held in KCN killing vials for periods of at least 24 h without significant changes in the volume or sugar concentration of the honey sac contents. Such loads are reasonably typical in volume for many foraging honey bees (Rinderer 1982). However, bees with loads around 60 μl should be measured within a holding period of much less than 24 h to avoid significant changes in volume.

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