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EFFECT OF ABOVE-FREEZING TEMPERATURES ON TEMPORARY STORAGE OF HONEYBEE SPERMATOZOA[1, 2]

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Summary

Honeybee (*Apis mellifera*) semen was diluted 1 : 1 with saline and stored at 5, 10, 15, 20, 25 or 35°C for 2 days, or was not stored (control). Each of 111 queens was inseminated with 4 µl of the diluted semen. The effects of storage temperatures were evaluated by counting the number of spermatozoa that entered the spermatheca. Mean counts were $\leq 3{\cdot}1 \times 10^5$ when spermatozoa were stored ≤10°C; storage at 20°C and 25°C resulted in the highest counts (mean = $1{\cdot}4 \times 10^6$). The response in millions of spermatozoa in the spermatheca (Y) to storage temperature between 10 and 25°C (X) was $Y = -2{\cdot}74 + 0{\cdot}397X - 0{\cdot}00935X^2$ ($r = 0{\cdot}78$, $n = 34$).

Introduction

Various temperatures have been used successfully for the storage of honeybee semen. For short-term storage, Williams (1983) tested diluents while keeping semen at 15 or 35°C for 36 h and Skowronek and Konopacka (1983) kept semen at 12–13°C for 3–6 weeks. For long-term storage, Poole and Taber (1970) stored semen at 13–15°C for as long as eight months, and Harbo (1983) stored it in liquid nitrogen (−196°C) for two years.

This study was designed to find the optimal temperature for storing diluted semen for two days. Such short-term storage is needed if one is to use a single pool of mixed semen to inseminate a group of queens more than once. Multiple inseminations cause a higher proportion of the spermatozoa to enter into a queen's spermatheca; result in a lower variance in the number of spermatozoa in the spermathecae; and make more efficient use of semen than single inseminations (Mackensen & Roberts, 1948; Bolten & Harbo, 1982). Since the interval between inseminations is usually one or two days, storage for two days would allow two or three inseminations from a single batch of semen.

Materials and Methods

Two experiments were conducted in Baton Rouge, Louisiana in October 1985. Experiment 1 comprised six treatments (storage conditions) and 70 observations (counts of spermatozoa in the spermathecae of queens inseminated with the test semen). Experiment 2 comprised five treatments and 41 observations. The experiments were of the same design and differed only in time (they were one week apart) and storage conditions (only the 10°C, 15°C, and unstored controls were included in both experiments).

Semen was collected from drones of unknown age and origin, diluted 1 : 1 with saline, mixed, and stored. The diluent consisted of 1·11 g NaCl; 0·1 g glucose; 0·01 g each L-arginine HCl, L-glutamic acid, and L-lysine; 0·03 g dihydrostreptomycin sulfate; 0·03 g penicillin G-Na; 0·221 g Trizma HCl; and 0·436 g Trizma base in 100 ml total solution (Williams & Harbo, 1982; pH = 8·5 at 25°C, 417 mOsmols/kg). The diluent was sterilized by filtration through a 0·2-µm membrane. After 150 µl of semen had been drawn into a syringe (Harbo, 1985), it was dispersed in an equal volume of sterile diluent in a conical centrifuge tube (3-ml Pyrex No. 8060). The diluted semen was stirred for 3 min with a glass filament, collected into capillary tubes, sealed with petroleum jelly and stored (Harbo, 1979).

The storage temperatures were controlled within ± 0·5°C except for semen stored at 5°C. Tubes of semen were kept at 10, 15 or 20°C by boring holes for capillary tubes in three aluminium blocks which were cooled by Thermoelectric Cold Plates that were kept in a

[1]In co-operation with Louisiana Agricultural Experiment Station.
[2]Mention of a commercial or proprietary product does not constitute an endorsement by the United States Department of Agriculture.

controlled environment chamber set at 25°C. A water bath kept semen at 35°C, and a refrigerator kept it at 5 ± 1°C. Temperatures were monitored with thermocouples.

All the queens used in this experiment were sisters and were stored in cages in one queenless colony. Each queen was randomly assigned a semen treatment and then was inseminated with 4 µl diluted semen.

The spermatozoa that entered the queens' spermathecae were counted. The spermatheca was removed from each queen 3–6 days after insemination. Its contents were dispersed in 10 ml of 0·5-M NaCl, and then measured with a spectrophotometer at 230 nm. The formula, $Y = 15{\cdot}74X - 0{\cdot}41$ was used to convert absorbance units (X) into millions of spermatozoa in 10 ml (Y) (Harbo, 1986).

The two experiments were statistically evaluated separately by means of analysis of variance with mean separations measured by least significant difference (LSD) analyses ($\alpha = 0{\cdot}05$). The four treatment temperatures in Experiment 2 were evaluated as a quadratic relationship.

Results and Discussion

Both experiments showed that many fewer spermatozoa entered the spermatheca when the spermatozoa were stored at 10°C or colder. Sperm migration was significantly greater after storage at 15°C than after storage at any colder temperature ($P<0{\cdot}05$, Table 1). Dilution had little effect on storage at 5°C, for the undiluted semen did not survive significantly better than the diluted semen (Table 1). Therefore, when storing spermatozoa at temperatures above freezing, it is best to keep them warmer than 10°C.

The optimal temperature for 2-day storage was 21°C. This is based on the quadratic response to the 10, 15, 20 and 25°C treatments (Experiment 2, Fig. 1). Two-day storage at 20°C resulted in significantly more spermatozoa in the spermatheca than storage at 15°C ($P<0{\cdot}05$), but not in a significantly different number from that recorded for storage at 25°C or for non-stored controls (Table 1). Poole and Taber (1970) found that for a 13-week storage

TABLE 1. Numbers of spermatozoa ($\bar{x} \pm$ SD, $\times 10^6$) entering spermathecae of queen honeybees inseminated with semen diluted 1 : 1 with saline and stored for two days.

Experiments 1 and 2 were analysed separately. Means followed by different letters are significantly different at the 0·05 level (LSD mean separation).

Experiment no.	*Storage temp*	*No. queens*	*No. spermatozoa*
1	5*	12	0·15 ± 0·12*a*
	5	10	0·07 ± 0·07*a*
	10	12	0·21 ± 0·16*a*
	15	12	0·80 ± 0·37*c*
	35	12	0·53 ± 0·32*b*
	not stored	12	1·00 ± 0·56*c*
2	10	8	0·31 ± 0·22*a*
	15	9	1·07 ± 0·22*b*
	20	8	1·50 ± 0·45*c*
	25	9	1·33 ± 0·50*bc*
	not stored	7	1·14 ± 0·30*bc*

*As semen used in this treatment was not diluted or stirred the volume was 2µl instead of 4µl.

period spermatozoa survived better at 15 than at 24°C. Therefore the optimal storage temperature may vary with different lengths of time in storage.

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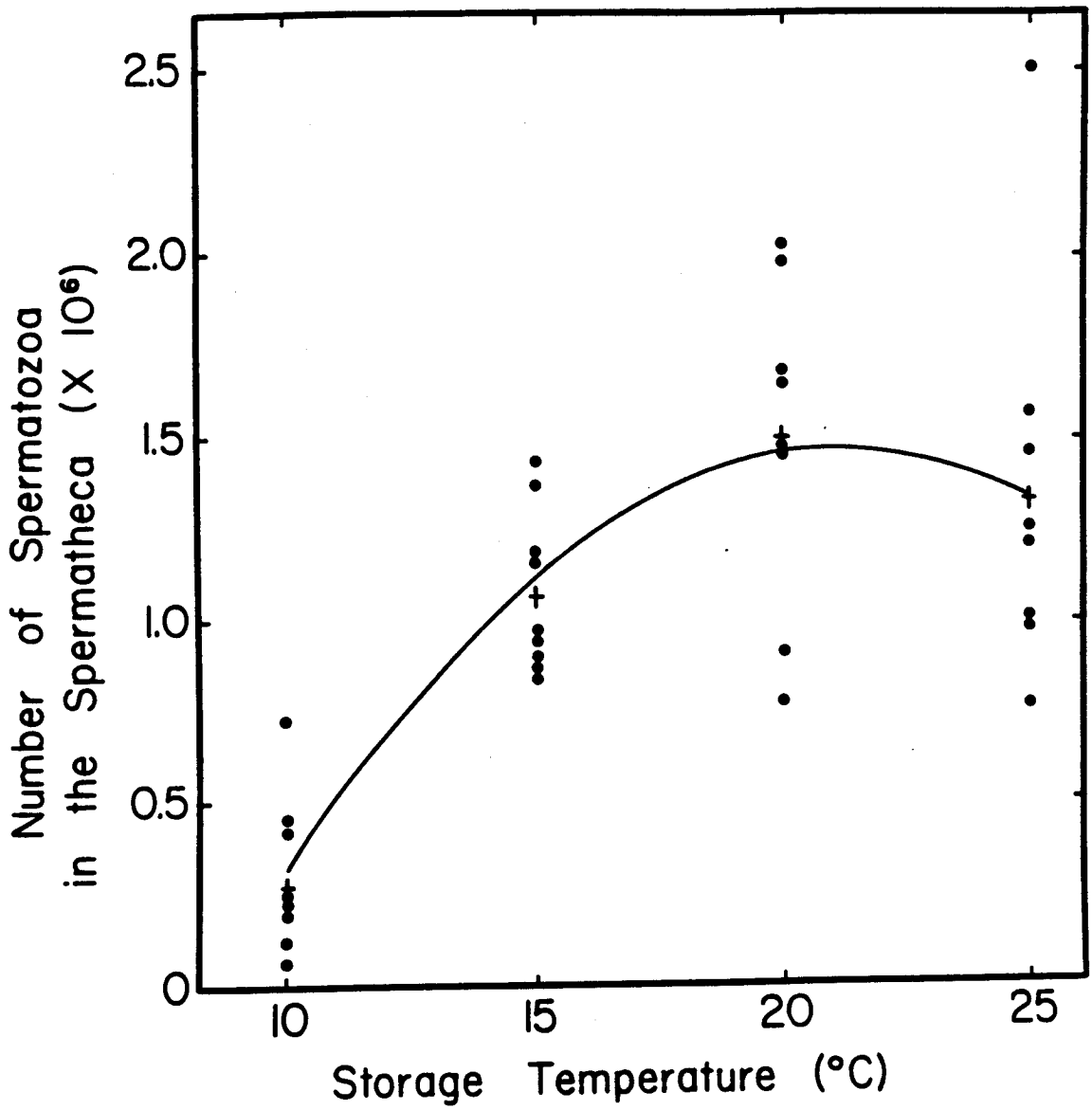

FIG. 1. Data from Experiment 2 evaluated as a quadratic trend. The equation $Y = -2{\cdot}74 + 0{\cdot}397X - 0{\cdot}00935X^2$ for the above curve explained a significant amount of variation ($r = 0{\cdot}78$, $F = 24{\cdot}6$, df = 2 and 31, $P = 0{\cdot}0001$). Insemination dosage was *c.* 17 million cells; Y = millions of spermatozoa entering the spermatheca; X = storage temperature between 10 and 25°C. Treatment means are represented by (+).

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