J. Insect Physiol., 1974, *Vol.* 20, *pp.* 2301 *to* 2305. *Pergamon Press. Printed in Great Britain*

ATMOSPHERIC CARBON DIOXIDE REGULATION IN HONEY-BEE (*APIS MELLIFERA*) COLONIES*

THOMAS D. SEELEY

Dyce Laboratory, Cornell University, Ithaca, N.Y. 14850, U.S.A.

(*Received* 18 *February* 1974)

Abstract—Carbon dioxide releases fanning behaviour in the honey-bee. The response is proportional to the atmospheric CO_2 level within the nest and regulates the atmospheric CO_2 concentration between 0·10 and 4·25 per cent in small colonies. Large colonies control atmospheric CO_2 more precisely than small colonies.

INTRODUCTION

VENTILATION of honey-bee nests through fanning activity has long been recognized as a social thermoregulatory measure (HUBER, 1814; LINDAUER, 1961) and as a means of accelerating the nectar concentration process (REINHARDT, 1939). In a little cited work (HAZELHOFF, 1941) it was reported that fanning activity would commence within 1 min of the introduction of a stream of CO_2 into a hive. A physiological basis for this response is suggested by the work of LACHER (1964, 1967) in which it was shown that honey-bees possess a CO_2 receptor and are capable of distinguishing gaseous CO_2 concentrations. This paper reports the nature of the CO_2 released fanning response and its rôle in atmospheric CO_2 regulation within honey-bee nests.

MATERIALS AND METHODS

The primary colony used in these tests consisted of approximately 750 workers, 12 drones, and a fertile queen which were kept in an observation hive. Several Tygon tubes with multiple openings were mounted throughout the hive and allowed simultaneous gas sampling and addition. Mercury thermometers fastened within the hive provided temperature measurements. The number of bees fanning their wings on one side of the observation hive's single comb were counted and are referred to as 'fanners'. Accurate counts of fanners were obtained by dividing the comb surface into small counting regions.

To minimize the perturbation of the atmospheric gas composition within the small hive through frequent sampling, a low sample volume CO_2 analyser using thermal conductivity as the detection mode was designed and built. In operation, a 25 ml gas sample was released into a CO_2 free carrier gas. The flow line was

---

* This study was supported by National Science Foundation Grant No. GB-33692, Reproduction in Honey Bees.

then split into two equivalent lines. From one line the CO_2 was quantitatively removed by passage of the gas through a column of Ascarite while the other flow line was unmodified. The difference in CO_2 content between the two lines created a thermal conductivity difference which was monitored with a GOW–MAC Model 133 thermal conductivity cell, converted to an electrical potential and recorded on a millivolt strip chart recorder. With proper calibration, to allow for the non-linearity of thermal conductivity response, microlitre precision was readily achieved. The CO_2 dynamics of large (35,000 bees) and small (10,000 bees) colonies in identical hives were similarly monitored.

RESULTS

Fanning response to artificial atmospheric CO_2 rise

Initial studies showed that slow CO_2 addition provided a CO_2 concentration rise which was uniform throughout the hive and to which the colony responded by a smooth increase in the number of fanners. A typical response is shown in Fig. 1 where pure CO_2 was added at 100 ml/min uniformly across the top of the hive. Before commencement of CO_2 input the number of fanners was constant and the CO_2 level in the hive was low and steady. Twelve minutes of CO_2 input created a thirteenfold increase in CO_2 concentration which in turn caused a thirtyfold increase in fanning activity. Ventilation involved approximately 40 per cent of the colony population at the point of maximum fanning and air circulation. A powerful stream of air issued from the hive entrance at this time. Within 6 min after CO_2 input ceased, the CO_2 level was reduced nearly to the level observed before the test, and fanning activity had co-ordinately diminished.

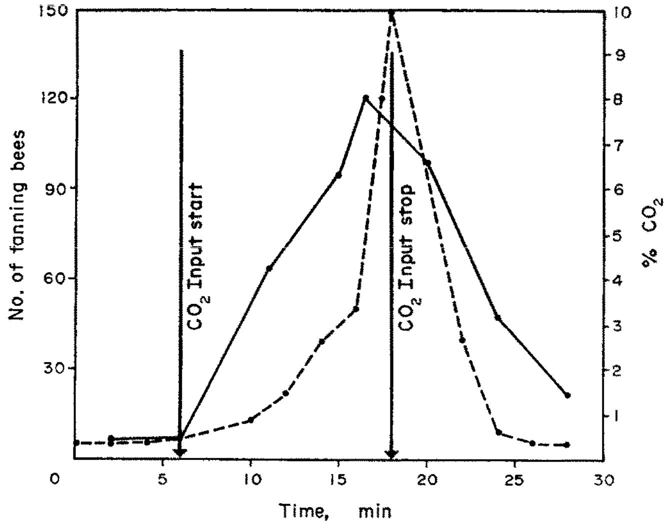

FIG. 1. Fanning response to artificial nest atmosphere carbon dioxide rise. - - -, Number of fanners; ——, per cent carbon dioxide.

During the period when fanning increased, the temperatures within the nest dropped slightly as cool air was drawn into the hive. Reduction of fanning caused a slight temperature rise.

Fanning response substitution of nitrogen for CO_2

To show that fanning was not merely a response to oxygen depletion within the nest, the colony was repeatedly observed when the atmospheric oxygen was displaced by nitrogen. As is shown in Fig. 2, throughout the period of gradual oxygen displacement, colony fanning activity underwent no significant change. Oxygen depletion caused narcosis of the colony with fanning activity ceasing altogether. Until narcosis, the temperature within the hive remained constant with minor (±0·1°C) fluctuations.

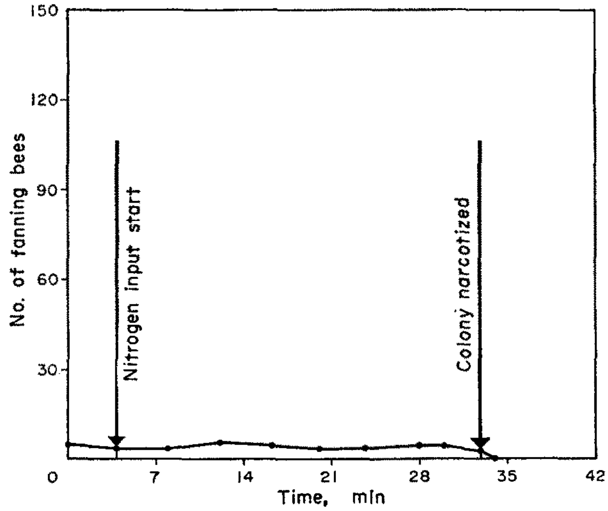

FIG. 2. Fanning response to oxygen depletion.

Natural fanning and CO_2 level in the hive

CO_2 content in the hive atmosphere, number of fanners, and temperature were simultaneously monitored at 7 min intervals throughout four afternoons of normal flight activity. Typical data for one afternoon's dynamics are shown in Fig. 3. Hive temperature was essentially stable throughout the observation period, but the CO_2 level and fanning activity underwent strong variation. The CO_2 fluctuations were almost exactly duplicated by the changes in fanning activity, though the two phenomena were slightly out of phase with fanning rises trailing carbon dioxide rises by a few minutes.

Air samples were withdrawn from the centre of the brood nest of large and small natural colonies at 40 min intervals. Fifty-two hours of both day and night measurements were made in four observation periods. Resultant data are given in Table 1.

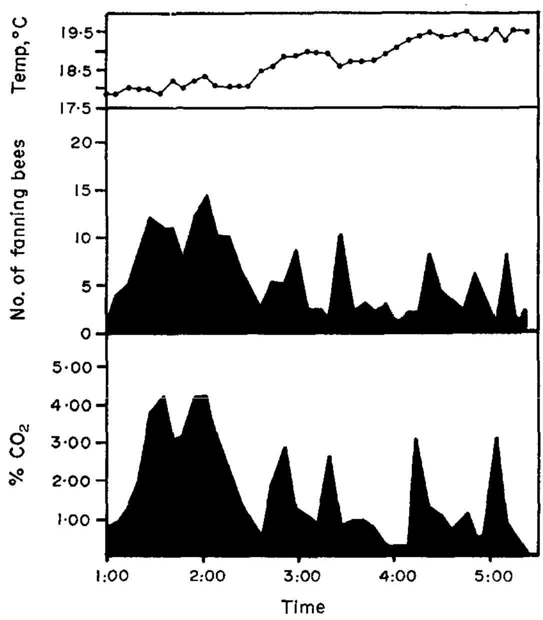

FIG. 3. Comparative plot of simultaneously measured per cent atmospheric carbon dioxide, fanning activity and temperature in a small colony.

TABLE 1—COMPARATIVE ATMOSPHERIC CARBON DIOXIDE LEVEL AND DEGREE OF VARIATION IN LARGE AND SMALL COLONIES

Colony size	Mean CO_2 concentration (%)	Standard deviation (%)	Maximum deviation (%)
Large	0·44	0·16	0·32
Small	0·78	0·34	1·21

DISCUSSION

The results reported here indicate that honey-bee fanning behaviour is released when the CO_2 content of the nest atmosphere increases. Oxygen depletion alone evokes no fanning response. Under natural conditions CO_2 increase and oxygen depletion would occur simultaneously. Carbon dioxide appears to be the quality criterion which is regulated through the behavioural response of fanning.

The colony, whose fanning dynamics are shown in Fig. 3, did not need ventilation for the purpose of temperature regulation, and fanning followed changes in CO_2 content of the nest atmosphere. The regulation of the CO_2 level in a small experimental colony appeared relatively coarse with CO_2 variations of 1 to 3%. This magnitude of variation matches the dimension of the honey-bee's ability to differentiate between CO_2 concentrations (LACHER, 1967).

Large colonies exhibit a better homeostasis in atmospheric CO_2 possibly in consequence of the nearly continuous thermoregulatory ventilation.

In contrast to the honey-bee's active regulation of the CO_2 concentration in the hive atmosphere, the termite *Macrotermes natalensis*, whose nest CO_2 reaches almost 3%, achieves this control passively through an elaborate nest design which facilitates air circulation and exchange (LÜSCHER, 1961).

It has been shown that even a few per cent CO_2 in the atmosphere causes significant inhibition of succinic dehydrogenase, an enzyme of the Krebs tricarboxylic acid cycle (BENDALL *et al.*, 1960; KASBEKAR, 1966). Also the physiological problems of optimal pH maintenance and water retention are greater in a high CO_2 environment. Therefore, active and passive reduction of CO_2 in the nest atmosphere is important and mechanisms to this effect may prove common to insect societies with high population densities existing within enclosed nests.

REFERENCES

BENDALL D. S., RANSON S. L., and WALKER D. A. (1960) Effects of carbon dioxide on the oxidation of succinate and reduced diphosphopyridine nucleotide by *Ricinus* mitochondria. *Biochem. J.* **76**, 221–225.

HAZELHOFF E. H. (1941) De Luchtverversching van een Bijenkast gedurende den zomer. *Maandschr. Bijent.* **44**, 1–16.

HUBER F. (1814) *Nouvelles Observations Sur Les Abeilles.* II. (New observations on bees, I and II.) Translation (1926) Dadant; Hamilton, Ill.

KASBEKAR D. K. (1966) Effect of carbon dioxide–bicarbonate mixture on rat liver mitochondrial oxidative phosphorylation. *Biochem. biophys. Acta* **128**, 205–208.

LACHER V. (1964) Elektrophysiologische Untersuchungen an einzelnen Rezeptoren für Geruch, Kohlendioxyd, Luftfeuchtigkeit und Temperatur auf den Antennen der Arbeitsbiene und der Drohne (*Apis mellifica* L.). *Z. vergl. Physiol.* **48**, 587–623.

LACHER V. (1967) Verhaltensreaktionen der Bienenarbeiterin bei Dressur auf Kohlendioxid. *Z. vergl. Physiol.* **54**, 74–84.

LINDAUER M. (1961) *Communication among Social Bees.* Harvard University Press, Cambridge.

LÜSCHER M. (1961) Air-conditioned termite nests. *Scient. Am.* **205**, 138–145.

REINHARDT J. F. (1939) Ventilating the bee colony to facilitate the honey ripening process. *J. econ. Ent.* **32**, 654–660.