

RELATION OF GROWTH AND ENVIRONMENTAL FACTORS TO
RESPIRATION OF BROCA DO CAFÉ, *HYPOTHENEMUS HAM-
PEI* (FERRARI). ¹

with 5 figures and 19 tables

by

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1. This work was supported in part by a grant from the Rockefeller Foundation to the senior author, and by a grant from the Conselho Nacional de Pesquisas, Rio de Janeiro.

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INTRODUCTION

Broca do Café was first introduced into Brasil in the year 1913, but since that time has spread so widely that at present it occupies an area that includes all of states of São Paulo and Rio, and parts of the states of Espírito Santo, Minas Gerais and Paraná. In this short time it has come to rank as one of the most serious pests of the country, hence it has become the object of considerable study.

The history of the insect, its morphology and biology have been quite thoroughly reviewed by Bergamin (1944-45) and need not be dwelt upon here. Moore recent studies of Broca do Café include the statistical analysis of infestation (Toledo, 1947), the efficacy of several new insecticides in its control (Lepage and Giannotti, 1949-50), and the study of sex determination (Bergamin and Kerr, 1951). The physiology of the insect is practically unknown.

The present study is concerned with the respiratory metabolism during the various stages of the life cycle of Broca do Café, the relation between infested bean and infesting insect, and the influence of various environmental factors upon the respiration. We hope that the results will serve as a basis for further physiological and pharmacological studies upon this most serious pest insect.

MATERIAL AND METHODS

The animals used in the present study were eggs, larvæ, prepupæ, pupæ and adults of the coffee borer, *Hypothenemus hampei* (Ferrari, 1867) (Ipidæ, Scolytoidea, Coleoptera). The borers were obtained in all the stages from infested coffee beans from private farms near Campinas, from the Instituto Agronomico, of Campinas, and the Escola Superior de Agricultura "Luiz de Queiroz" in Piracicaba, São Paulo, during the months of June to September. The coffee beans used were mostly of the varieties *Coffea arabica* and *C. excelsa*.

In the laboratory the animals were reared within coffee beans kept moist in large glass cylinders, or in tied cloth bags suspended from the walls to provide aeration of the cultures and prevent fungus formation. The temperature and humidity of the cultures were allowed to fluctuate with those of the room, which averaged 20°C and 70% RH. Several attempts were made to raise the animals in artificial media, e. g. in agar suspensions of powdered coffee, in ground green coffee, *Drosophila* media, etc., in order to isolate single eggs that the development be observed from the egg stage through to adulthood but all efforts met with failure. The adults survived quite well in several of the media but did not reproduce, nor did the immature forms develop when removed from the natural environment of the coffee bean.

The oxygen consumption and carbon dioxide production of the animals and coffee beans were determined in volumetric microrespirometers (Scholander, 1942a; Scholander and Edwards, 1942) modified to suit the individual experiments. For small numbers of insects the instruments sensitive to 0.06 mm³ per hour were used. For the coffee beans and larger numbers of insects, plastic instruments of sensitivity of 1.99 and 8.15 mm³ per hour (Scholander, 1951) were employed. Due to the smallness of the insects it was necessary to use ten eggs and 5 to 10 immature forms or adults in each vial. Shell vials were used of such sizes as to just fit the insects, thus taking advantage of their thigmotaxis. Ascarite (a commercial sodium hydrate absorbent of CO₂) was placed in the bottom of the vial and covered with a thin layer of cotton, upon which the insects were placed. The animals usually settled down to relative inactivity under these conditions within 15 minutes, thus giving a resting metabolism. With the exception of the experiments on the influence of gas tensions, the respirometer storage bulb and experimental vials were filled with room air. Except for the experiments on the influence of temperature and humidity, experiments were run at 25°C and the humidity produced by Ascarite, which approximates 20% R.H. Experiments were carried on for a minimum of one hour, and usually were of 3 to 4 hours duration to ensure constancy of readings.

Carbon dioxide production was measured indirectly, i. e. by the difference in readings in the presence and absence of CO₂ absorbent. Fresh weights of the animals and beans were taken immediately after each experiment. Dry weight was obtained by desiccating the animals at 110°C for 24 hours and then weighing.

Other techniques employed will be discussed in the individual sections.

RESPIRATORY METABOLISM DURING GROWTH

A. General

Broca do Café is a holometabolic insect undergoing complete metamorphosis with egg, larval, prepupa, pupa and adult stages. As a detailed description of the stages has been presented by Bergamin (1944-45) we will only briefly summarize the relevant facts here. Optimal growth occurs at a temperature of 25°C and relative humidity of 90%. The egg stage varies from 4 to 16 days with an average of 7.5 days. The larval growth occurs in 9 to 20 days with an average of 13.8. Included in the larval stages are two molts for the female and one for the male. The prepupal period varies from 4 to 10 days and averages 7. The complete evolution from oviposition to the emergence of the adult thus averages 27 days at 25°C. The life span of the adult male varies from 78 to 103 days, whereas that of the female ranges from 81 to 282 days. There are, on the average, 7 complete generations in one year. The ratio of males to females is approximately 1 to 9.

Under normal conditions in the field the wingless male spends the entire life cycle within the coffee bean. The female may leave the original bean to infest others or may remain within the original. In the laboratory it was observed that the female often left the beans that became dry, or the cultures that were exposed to light or otherwise disturbed. Infestation of the coffee beans in all stages from immature green to mature brown was observed.

TABLE ONE
OXYGEN CONSUMPTION OF HYPOTHENEMUS HAMPEI IN VARIOUS STAGES (in mm³ O₂ / 10 individuals / hour)

	Eggs	larvæ					prepupæ	pupæ	adults	
		1st instar		2d instar					females	males
		to 1 mm.	1-1.4 mms.	1.5-1.7 mms.	1.8 mms.	2-2.2 mms.				
	0.93 0.82 0.98	1.89 2.10 1.90 2.00	2.04 2.03 3.25 3.08 2.00 3.13 3.10 1.90	2.52 3.80 3.64 2.80 2.72 2.44	4.89 4.62 6.16 4.48 4.20 4.20	6.42 6.52 7.40 6.72 8.60 6.80 8.80 7.50 6.60 7.17	4.08 3.80 3.80 4.18 3.80 3.53 2.33 4.76 3.53	2.44 3.92 2.94 3.26 3.92 2.72 3.08 8.26 2.94 4.22 3.22 2.80	8.70 7.56 8.43 8.12 8.16 8.96 8.43 9.24 8.70 8.94	3.20 2.80 2.71 3.04 2.94
Averages	0.91	1.97	2.56	2.98	4.76	7.26	3.73	3.18	8.52	2.94

TABLE TWO
OXYGEN CONSUMPTION OF HYPOTHENEMUS HAMPEI IN VARIOUS STAGES (in mm³ O₂ / mgm. fresh weight / hour)

	Eggs	larvæ				prepupæ	pupæ	adults	
		1st instar		2d instar				females	males
		to 1 mm.	1-1.4 mms.	1.5-1.7 mms.	2-2.2 mms.				
	1.60	1.69	1.63	1.86	1.67	0.83	0.81	2.10	1.35
	1.63	2.20			1.65	1.08	0.79	1.97	1.33
	1.63	2.37			1.64	0.88	0.72	2.03	1.41
					1.95		0.48	2.01	1.96
					1.45			2.03	1.90
Averages	1.62	2.08	1.63	1.86	1.67	0.93	0.70	2.02	1.59

The oxygen consumption and carbon dioxide production were measured and the respiratory quotient calculated for all stages of *Broca*. The results are given in Tables 1 through 6 and in graphs 1 and 2, and will be discussed in detail below.

B. Eggs

The egg stage lasts an average of 7.5 days at 25°C. The eggs average in linear dimensions 0.599 x 0.314 mms. Due to the fact that it was not possible to isolate and observe the development of single batches of eggs, they were taken at random and their oxygen consumption and CO₂ production measured in lots of 10 each. In summary, it was found that the O₂ consumption per 10 eggs averaged 0.91 mm³ per hour. On the basis of the fresh weight the oxygen consumption was 1.062 mm³/mgm./hr., and on the basis of dry weight 3.06 mm³/mgm./hr. The respiratory quotient

$$(RQ = \frac{\text{vols. CO}_2}{\text{vols. O}_2}) \text{ averaged } 1.3.$$

We can make few comparisons between the eggs of *Broca do Café* and those of the other insects due to lack of control of age. However, it will be seen from the tables and figures that the oxygen consumption of the eggs, on all bases, was lower than that of larvæ and adults, and higher than the prepupæ and pupæ. This agrees well with the data for other insects (Ed-

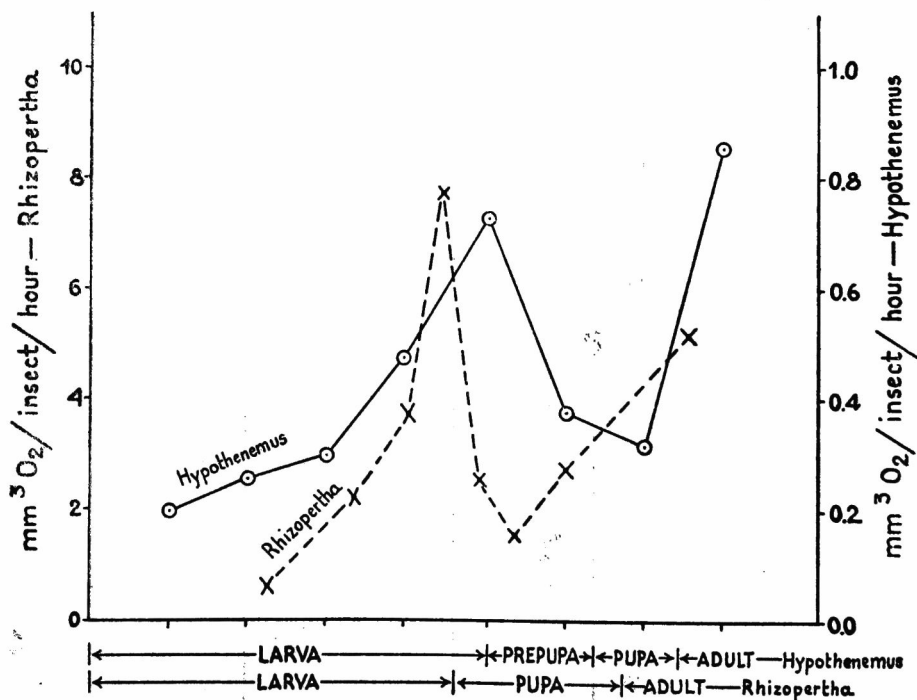


Figure 1. — Average oxygen consumption of *Hypothenemus hampei* during growth. For comparison of form of curve, that for *Rhizopertha dominica* (Birch, 1947) is given on the same grid. Abscissa in arbitrary units to equate stadia.

wards, 1952). The respiratory quotient of an insect egg is generally high. Further, the order of utilization of foodstuffs by the insect embryo is carbohydrate, protein and fat. Thus the RQ of 1.3 found in the eggs of Broca indicates that carbohydrate metabolism was in ascendency in the particular stages used.

C. Larvæ

The respiration of the larvæ was recorded on the basis of 10 individuals per vial. No attempt was made to separate the sexes in the larval stages. However a size distinction was made, i. e., 5 groups were used, those of 1 mm., and 1 to 1.4 mms. in the first instar, and groups of 1.5 to 1.7, 1.8, and 2 to 2.2 mms., respectively in the second instar.

It will be seen from the tables and figures that the oxygen consumption per insect during larval life increased in a regular fashion, and that the curve relating oxygen consumption to size is quite characteristic of a holometabolic insect (Figure 1). The peak of the total oxygen consumption, i. e. oxygen consumption per insect per hour, was reached in the last larval instar immediately preceding prepupation.

On the basis of fresh weight the maximum oxygen consumption per unit of weight was attained immediately after eclosion in the smaller first instar larvæ, throughout the rest of larval life the oxygen consumption remained at a fairly high and constant level. On the basis of the dry weight, however, a more typical picture appears. The oxygen consumption was highest following eclosion, thereafter gradually decreasing with increase in larval age. The measurements were not made at intervals frequent enough to permit analysis of the mode of decrease, but from previous experiments with other insects (Edwards, 1952) it seems likely that the decrease is probably not regular, but rather in the form of a series of successive oscillations.

The difference between the two curves, wet and dry, expressing the relationship between oxygen consumption and age is due, naturally, to the differences in water content of the insect in the different stages. It is generally true that the younger forms have the higher water content; a fact borne out morphologically by the greater flexibility of the smaller animals. The younger insect is more active and has a large amount of actively respiring tissue, i. e. less reserves than the older form. In determining rate of oxygen consumption, or other metabolic process, only the active material should be considered as a basis for calculation, thus the rate on the basis of dry weight is closer to the true picture of metabolic events. On this basis the immature forms showed a greater rate of oxygen consumption, and as previous studies (Edwards and Stafford, 1952) have shown that the maintenance metabolism of an insect is constant in all instars, it is presumed that the higher rate in the early larval Broca is related to activity metabolism, the majority of which is utilized in development.

In early larval life the respiratory quotient declined considerably, thereafter leveling off for the remainder of the larval stages, averaging 0.82 for all larvæ, with a range from 0.73 to 0.87. These figures suggest the utilization of a mixed diet, probably in great part protein and carbohydrate.

D. Prepupæ and Pupæ

In the last larval stage Broca becomes inactive and gradually enters the prepupal stage. Following this short stage of 2 days, molting occurs and the

insect enters the quiescent pupal stage during which the larval tissues are destroyed and the adult tissues formed. As may be seen from the tables and figures, the oxygen consumption of *Broca* drops rapidly during prepupation, to reach a minimum in mid-pupal life. The *RQ* lags somewhat behind, being still high in the prepupa but decreasing gradually in the pupa until the minimum of 0.57 is reached.

The curve relating oxygen consumption and age in the pupa is typical of a holometabolic insect, i. e. U-shaped (Figure 2b), with the maxima at prepupation and emergence respectively and the minimum in the middle of the pupal stage. It is assumed, on the basis of previous studies, that the decrease in oxygen consumption coincides with histolysis and the later increase with histogenesis. The leveling off at the bottom of the curve would thus represent the point at which the two processes are in equilibrium.

It has been previously shown in the pupa of *Vanessa io* (Schwan, 1940) that there is no relation between rate of respiration and change of tracheal form during metamorphosis. Thus, one can assume that the variations during this stage accompany the tissue changes but are not related to changes in diffusion and related processes, i. e. the limiting factors are not availability of oxygen or diffusion, but rather the chemical changes that are occurring during the metamorphosis.

The shape of the respiratory curve during metamorphosis may be influenced by several factors such as temperature change, sex and nutrition. Clarification of the relationship between gaseous exchange, nutrition and metamorphosis of the tissues has been accomplished in part by studies of the changes in *RQ*. In most insects the *RQ* decreases during pupation, but rises again at emergence, e. g. in the Japanese beetle, *Popillia japonica*, the *RQ*'s during the various stages are: larva, 0.97; prepupa, 0.70; pupa, 0.4 to 0.7; and adult, 0.7 to 0.8 (Ludwig, 1931). However, it was noted that in *Broca* the *RQ* does not rise during late pupation. The possible reason for this will be discussed in the next section.

E. Adults

It is to be noted that there are more data for large larvæ, pupæ and adults. This is associated, naturally, with the ease of obtaining animals in these stages in the infested beans. The oxygen consumption of the adults was at about the same level as that of the last stage larvæ; the *RQ* just slightly higher than that of the pupa. The difference between the oxygen consumption of the female adult and that of the male was quite striking. On the basis of entire insect the difference was almost 3 times, on the basis of unit weight the difference became somewhat smaller but still significant. No simple interpretation of the differences can be given but several possibilities exist. In the first place we can note that the difference on the basis of entire insect is largely, but not entirely, due to difference in size. The female *Broca* averages 1.65 mms. in length, 0.73 mms. in width and 0.67 mms. in height, whereas the male is only 1.18 mms. long, 0.55 mms. wide and stands 0.51 mms. high (Bergamin, 1944-45). However, the fact that on a unit weight basis the oxygen consumption of the female is still greater than that of the male leads one to search for other possible explanations.

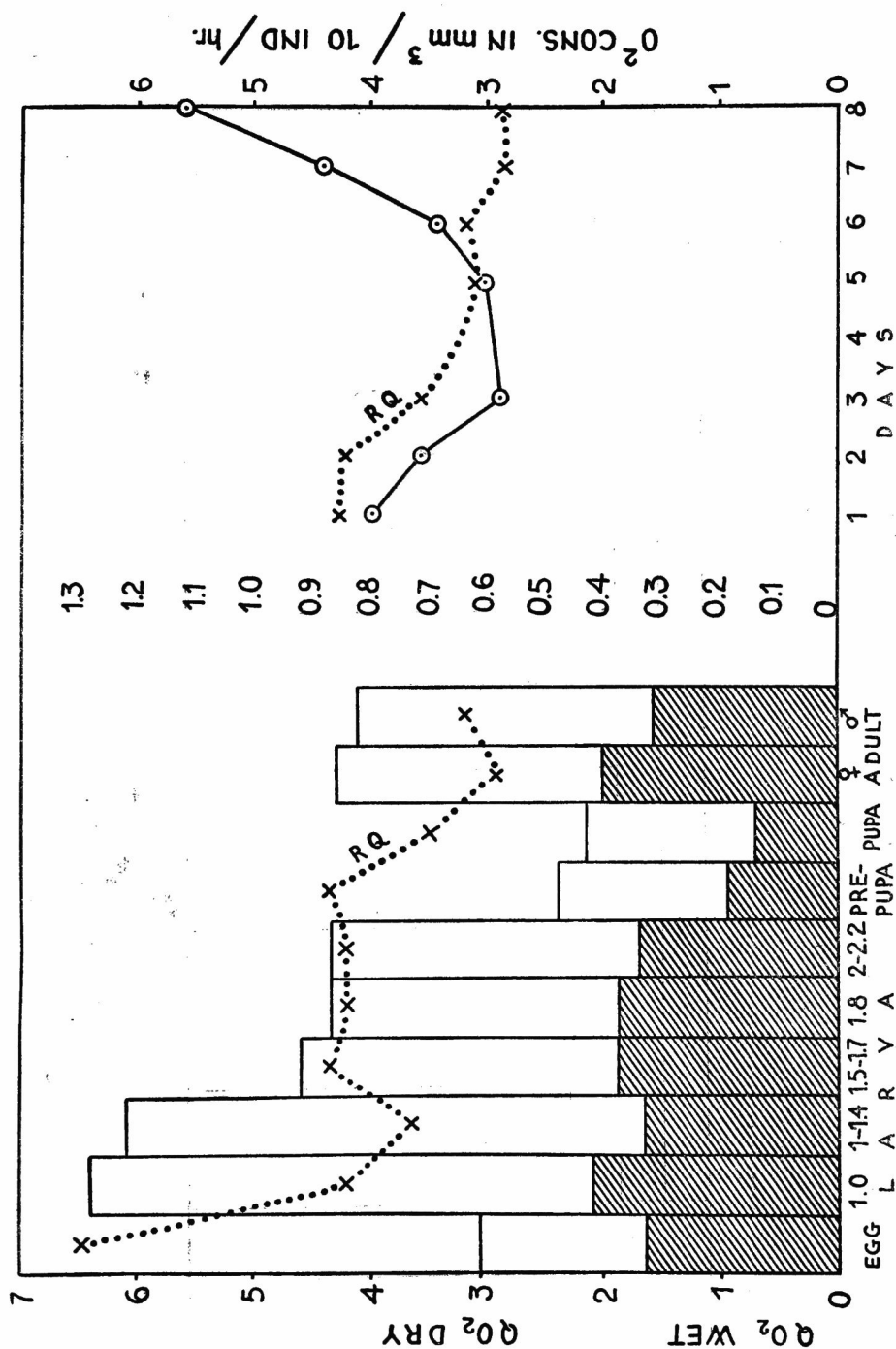


Figure 2. — a. Average oxygen consumption and RQ of *Hypothenemus hampei* in all stages. Cross hatched blocks equal mm³O₂/mgm. wet wt./hr., open blocks equal mm³/mgm. dry wt./hr., dotted line equals RQ. Numbers on abscissa denote length of larvæ in mms. b. Average daily oxygen consumption and RQ of pupæ.

TABLE THREE

OXYGEN CONSUMPTION OF HYPOTHENEMUS HAMPEI IN VARIOUS STAGES (in mm³ O₂ / mgm, dry weight / hour)

	Eggs	Larvæ					prepupæ	pupæ	adults	
		1st instar		2d instar					females	males
		to 1 mm.	1-1.4 mms.	1.5-1.7 mms.	1.8 mms.	2.-22 mms.				
	3.20	6.32	6.53	6.30	5.40	4.66	2.04	2.04	4.14	4.08
	2.73	6.30	6.48	4.53	3.80	4.35	2.80	2.54	3.98	4.00
	3.28	6.60	6.50	3.64	4.40	4.93	1.88	2.38	4.22	4.50
			6.10	3.50	4.48	5.06	2.38	2.29	4.06	3.67
			4.17	3.45	3.77	3.88	2.38	1.94	4.80	4.34
			6.53	6.11	4.20	3.49	2.28	2.71	4.68	
			6.35			4.16	1.94	2.52	4.31	
							1.84	2.72	4.40	
							2.04		4.14	
							1.87		4.47	
						2.48				
						2.00				
						2.48				
Averages	3.06	6.40	6.09	4.60	4.36	4.36	2.39	2.17	4.31	4.12

TABLE FOUR
OXYGEN CONSUMPTION AND RESPIRATORY QUOTIENT OF PUPAE OF HYPOTHENEMUS HAMPEI

Day of pupal life	1	2	3	5	6	7	8 (emergence)
Oxygen consumption	4.35	3.80	2.99	2.72	3.53	3.80	6.52
In mm ³ /10 individuals/hour	3.64	3.36	2.80	3.36	3.36	5.04	5.32
Respiratory Quotient	0.87	0.85	0.73	0.58	0.69	0.57	0.62
	0.84		0.70	0.65	0.58	0.57	0.52

TABLE FIVE
RESPIRATORY QUOTIENT OF HYPOTHENEMUS HAMPEI IN VARIOUS STAGES

	Larvæ					Pupæ	Adults females
	Eggs	1st instar		2d instar			
		to 1 mm.	1-1.4 mms.	1.5-1.8 mms.	2.2 mms.		
	1.30	0.87	0.73	0.84 0.90 0.89	0.84 0.78 0.84 0.83 0.91 0.83 0.85 0.83	0.92 0.89 0.82 0.92	0.62 0.52 0.59 0.63 0.68 0.70 0.66 0.71
Averages	1.30	0.84	0.73	0.87	0.84	0.88	0.64

TABLE SIX
OXYGEN CONSUMPTION AND RESPIRATORY QUOTIENT OF HYPOTHENEMUS HAMPEI IN VARIOUS STAGES

	Eggs	Larvæ					Prepupæ	Pupæ	Adults	
		1st instar		2d instar					Females	Males
		to 1 mm.	1-1.4 mms.	1.5-1.7 mms.	1.8 mms.	2-2.2 mms.				
Oxygen Consumption mm ³ /10 inds./hr.	0.91	1.97	2.56	2.98	4.76	7.26	3.73	3.18	8.52	2.94
	1.62	2.08	1.63	1.86	1.86	1.67	0.93	0.70	2.02	1.59
	3.06	6.40	6.09	3.60	4.36	4.36	2.39	2.17	4.31	4.12
	Respiratory Quotient	1.30	0.84	0.73	0.87	0.84	0.84	0.88	0.70	0.64

In most animals the male has the higher unit metabolism, but among insects many exceptions are found. Females of the wild type *Drosophila melanogaster* have a higher oxygen consumption than the male on the day after emergence (Kucera, 1934). The female *Drosophila willistonii* also has the higher rate (Valente, unpublished). This is also true of the female *Tribolium* (Park, 1936). However, the male silkworm (Raffy, 1934) male housefly (Edwards, 1946) and the male cockroach (Barron and Tahmisian, 1948) have the higher rate. The difference between the two sexes may be changed, however, as in *Galleria mellonella*, by change of temperature (Bell, 1940). Thus the fact that the female Broca has a higher oxygen consumption on the basis of both dry and wet weight is not surprising. The ratio of female to male on the basis of fresh weight was 1.27, whereas on the basis of dry weight was only 1.07. Thus we know that the female has a greater water content. The fact that, assuming equal activity, the difference exists regardless of differences in size and water content leads one to assume that there exists an organizational, hormonal, or enzymatic difference, as in the cockroach (Barron and Tahmisian, 1948). Further study is necessary to elucidate this point in Broca.

The low RQ found in the adult Broca is somewhat puzzling. It is essentially the same as that of the pupa, suggestive of the utilization of reserves. It is known that the adult Broca eats little, particularly the female, thus it is possible that this low RQ represents a true reserve utilization. Another possibility, though remote at the moment, is that, as Broca lives in an environment that may be high in CO₂ content (see section below), it is able to fix CO₂, such a mechanism leading to a low RQ.

RELATION OF COFFEE BEAN TO BROCA DO CAFÉ :

A. Conditions Influencing Infestation

Normally a number of generations of Broca may occur within one coffee bean. The male spends the entire life cycle within the original bean in which it was created, the female may leave the bean after oviposition. Thus one must consider several possibilities in the relationship between infested bean and infesting insect. These are (1) the relation between the physiological state of the coffee bean and infestation, (2) the conditions obtaining within the micro-climate of the bean, such as nutrition, light, temperature, humidity and gaseous diffusion, and (3) the adaptation, if any, made by the infesting insect to these conditions.

The age of the coffee bean seems little to affect the infestation, i. e. Broca will infest the newly developing green bean as well as the older yellow, red or mature brown bean. The age of the bearing tree plays little role also. In addition to these field observations, laboratory tests as follows have shown also that the age of bean does not affect infestation. A series of dishes containing 3 beans each were prepared. In one set of dishes the beans were left attached to the stems, in a second set the beans were permitted to roll freely in the dishes, and in the third the beans were set into 3% agar leaving only the tips exposed. The beans ranged in age and size from newly forming green bean to ripe, brown. Fifty adult, female Brocas were placed in each dish and the dishes placed in a dark drawer at a temperature of 22°C and humidity of approximately 85%. The dishes were inspected each few hours to

determine the amount of infestation, i. e. the numbers of insects still outside the beans. Within the first hour all beans had been perforated, and within 18 hours 90% of the insects in each dish had entered the coffee beans. Entrance appeared to be faster and easier in the dry mature beans.

B. Respiration of Uninfested Coffee Beans

These facts lead to the questions of availability of oxygen and the elimination of CO_2 by the infesting insect in the changing environment that is the developing bean. Let us consider firstly the bean itself. The coffee bean commences as a small, green, turgid affair, reaching almost full size before changing color. As shown in Table 7 the water content in the green stage, in the beans used, was 62.1% on the average. Actually one can easily squeeze water from the immature beans, thus we know that the green bean is essentially a semi-aqueous environment for the insect.

TABLE SEVEN
WATER CONTENT OF COFFEE BEANS IN VARIOUS STAGES

Conditions	no. of beans	mgms. fresh	mgms. dry	% water
green	10	8142.1	3083.2	62.1
yellow	10	6874.8	2870.3	58.3
red	10	8441.1	3825.0	54.7
brown	10	5162.3	2761.9	46.5

As the bean matures, it becomes desiccated, the mature beans used having an average water content of 46.5%. The substance of the seed becomes porous, as does the shell, and the seeds pull away from the shell and inner septum, leaving a gas space of about 1 mm. surrounding the seeds. Thus the diffusion of gases within the bean must change from that of gas through liquid in the green to gas through gas in the mature bean. Concomitant with these changes is a decrease in oxygen consumption in the ageing bean (Table 8). The green coffee bean had an average Q_{O_2} of $0.0903 \text{ mm}^3 \text{ O}_2/\text{mgm.}/\text{hr.}$ on the fresh weight basis and 0.2385 dry., whereas the mature beans consumed only $0.0591 \text{ mm}^3 \text{ O}_2/\text{mgm. fresh weight}/\text{hr.}$ and $0.1106 \text{ mm}^3/\text{mgm. dry weight}/\text{hr.}$ The yellow beans had a lower oxygen consumption than the red, but both were lower than the green beans and higher than the mature brown.

C. Respiration of Infested Coffee Beans.

The infestation by Broca does not appear to prevent development of the coffee bean but may have an influence upon its respiration. In three freshly picked green coffee beans the oxygen consumption was measured before and after artificial infestation with the results shown in Table 9. One day after artificial infestation the oxygen consumption of the beans showed increases of 56.4, 12.5 and 20.5% respectively. The influence appeared to depend upon the amount of injury. In bean A the infesting Broca had made

TABLE EIGHT

OXYGEN CONSUMPTION OF COFFEE BEANS (*Coffea arabica*) IN VARIOUS STAGES (in mm³/mgm. fresh weight/hour)

Stage	Green	Yellow	Red	Brown
Oxygen consumption .	0.0977	0.0642	0.0780	0.0558
	0.0978	0.0925	0.0486	0.0721
	0.0973	0.0671	0.1067	0.0199
	0.0733	0.0508	0.0869	0.0975
	0.0853	0.0610	0.0761	
			0.1010	
Average			0.0955	
	0.0903	0.0671	0.0847	0.0591

3 holes in one end and 2 in the other. In bean B there was only one hole and the infesting insect had bored only a distance of 3 mms. within. In C the insects had perforated both ends, one perforation each, and were well within the endosperm of the seed. That the effect was one of physical injury is borne out by the fact that drilling through 3 coffee beans with an electric drill of the same size as Broca caused increases of 67.25, and 25% respectively in the oxygen consumption of the beans.

TABLE NINE

INFLUENCE OF INFESTATION UPON OXYGEN CONSUMPTION OF COFFEE BEAN

Experiment Number	A	B	C
Original — mm ³ O ₂ /mgm./hr.....	0.055	0.056	0.083
Infested — mm ³ O ₂ /mgm./hr.	0.086	0.064	0.100
Number Broca entering	8	1	6
% change in QO ₂	56.4	12.5	20.5
Number of Perforations	5	1	2

The infestation appeared to have little influence upon the respiration of the mature bean. Table 11 shows that the oxygen consumption of the tissue of 10, infested, mature beans was 0.060 mm³/mgm./hr., which is identical with that of the average for 10 noninfested beans of the same stage (0.059) from the same trees. Thus it appears that (1) Broca destroys the tissues without completely impeding the growth of the coffee bean, as can readily be observed in the field, but does affect the respiratory rate, and (2) during growth the respiration of the bean reaches a minimal plateau after a certain point in maturity and that this level cannot be influenced by the depredations of the insect.

TABLE TEN
OXYGEN CONSUMPTION OF INFESTED IMMATURE BEANS AND THE INFESTING BROCA

Experiment number	1	2	3	average
Bean and Broca mm ³ O ₂ /hr.	90.6	88.8	130.8	103.4
Bean and Broca mgm. fresh weight.	460.6	320.8	525.2	435.5
Broca alone mm ³ O ₂ /hr.		8.09	17.92	13.01
Broca alone mgm. fresh weight.	2.2	2.6	7.2	4.0
Broca alone mm ³ O ₂ /mgm./hr.		3.1	2.49	2.80
Broca — number infesting bean.	5	7	21	11
Bean tissue mm ³ O ₂ /hr.	58.15			58.15
Bean tissue mgm. fresh weight.	433.1	288.3	480.3	400.6
Bean tissue mm ³ O ₂ /mgm./hr.	0.135			0.135

TABLE ELEVEN
OXYGEN CONSUMPTION OF INFESTED MATURE BEANS AND INFESTING BROCA

Experiment Number	IV	B	1	2	3	4	5	6	7	8	average
Bean and Broca mm ³ O ₂ /hr.	47.8	68.7	326	126.4	206.5	255.0	230.0	324.2	209.5	261.0	205.51
Bean and Broca mgms. fresh	359.0	186.9	633.9	733.1	894.8	571.2	996.3	969.0	817.4	882.8	704.94
Broca alone mm ³ O ₂ /hr.	8.2	21.7	65.2	22.7	19.6	42.4	59.8	26.1	32.6	65.2	36.35
Broca alone mgms. fresh	6.0	24.0	28.0	10.7	7.0	9.9	22.5	18.0	7.6	19.0	15.27
Broca alone mm ³ O ₂ /mgm./hr.	1.36	0.90	2.33	2.12	2.79	4.71	2.66	1.45	4.27	2.43	2.60
Number animals	22	61	60	25	19	30	55	36	16	51	37.5
Bean tissue mm ³ O ₂ /hr.	12.5	12.6	21.8	40.8	27.1	35.4	51.7	62.5	34.3	53.2	35.2
Bean Tissue mgms. fresh.	317.9	143.1	488.8	591.7	779.5	444.3	848.7	916.3	724.5	763.5	601.81
Bean Tissue mm ³ O ₂ /mgm./hr.	0.039	0.088	0.045	0.069	0.035	0.080	0.061	0.062	0.047	0.070	0.060

D. Respiration of isolated Broca and bean tissue

Broca may be in a semi-aqueous environment within the immature, or in a gaseous environment within the porous, mature bean. In immature beans it was observed that fluid could easily be squeezed out with the fingers and that upon opening an infested bean the infesting Broca were often observed to be wet. In this case the oxygen must reach the insect through solution through the fluid filled bean, or by way of the original perforation made upon entry by the insects. In the mature bean we assume oxygen diffusion is less limiting as the gas can enter in gaseous phase through the pore or across the relatively dry coffee bean shell. In either case we wish to know if diffusion is limiting to Broca, i. e. if at any time Broca is anoxic, and if so what effect this has upon the insect. Such a question becomes important practically from at least 2 points of view (1) from that of the penetration of insecticides, and (2) from that of the influence of isolation of the insects from the bean for study of metabolism under various conditions.

A number of experiments were performed to determine the relationship between conditions within the bean and the respiration of Broca, firstly in the green and secondly in the mature coffee bean. Green beans were obtained from Butantan and artificially infested in the laboratory. After 5 days the respiration was measured of (a) intact, infested beans, (b) infesting Broca removed from the bean, and (c) infested bean tissue. Table 10 shows that the intact, infested bean consumed an average of 103.4 mm³ O₂ per hour, and weighed 435.5 mgms. The isolated Broca (average of 11 per bean) consumed 13.01 mm³ per hour and weighed 4 mgms., whereas the isolated bean tissue (433.1 mgms) consumed 58.15 mm³ O₂ per hour.

The isolated insects thus respired at the rate of 2.80 mm³/mgm./hr. and the bean tissue at 0.135 mm³/mgm./hr., the ratio Broca/bean being 20.74. Assuming that the values for Broca isolated from the bean represent the normal respiration of the insect, then Broca accounts for only 1% of the weight but 12.6% of the total oxygen consumed by the infested bean.

E. Diffusion within the coffee bean

Assuming this rate of O₂ consumption for Broca in the bean, and assuming homogeneity of tissue and that the pressure of O₂ at the center of the bean and Broca to be zero, and assuming the coefficient of diffusion through the tissue to be equivalent to that through muscle, we can then apply the formula for a respiring cylinder (Hober, 1945) and calculate the limiting radius of an organism respiring by simple diffusion, as follows:

$$R = \sqrt{\frac{6 DC}{a}}$$

Where: C = pO₂ in atmosphere, 0.21 atms.
 a = oxygen consumption in cc/mgm/ minute = 4.6 x 10⁻²
 D = coefficient of diffusion, 1.6 x 10⁻⁵
 R = radius in cms.

We find thus that the limiting radius is 0.0194 cms. for the insect respiring at the rate given above. The bean is also consuming oxygen, however, at the rate of 0.135 mm³/mgm./hr. so we must consider the requirements to be actually those of bean and Broca, i. e. 5.01 x 10⁻² cc./gm./ minute. Assu-

TABLE TWELVE
NUMBERS OF BROCA DO CAFÉ IN ALL STAGES ISOLATED FROM INFESTED COFFEE BEANS FROM CAMPINAS

Bean number	eggs	larvæ		prepupæ	pupæ	adults	total number insects in all stages in bean
		1st instar	2d instar				
1	40	1	2	2	6	9	60
2		7		3		12	22
3					3	61	61
4						57	60
5		2			1	22	24
6		2	3	12		1	19
7		1		2		27	80
8	50				4	51	55
9					10	26	36
10	30				4	12	46
11		5	3		3	40	51
average	10.9	1.7	0.7	1.7	2.8	28.9	46.7

TABLE THIRTEEN
INFLUENCE OF SEALING PERFORATIONS UPON THE OXYGEN CONSUMPTION OF INFESTED BEANS

Experiment number	1	2	3	4	5	6	7	8	9	average
Bean and Broca mm ³ O ₂ /hr.	13.12	26.21	24.82	6.40	13.58	26.80	7.04	14.38	15.05	17.64
Bean and Broca, perforations sealed mm ³ O ₂ /hr.	8.31	17.72	17.39	1.53	5.91	15.41	4.34	7.67	8.38	10.64
% reduction in O ₂ uptake.....	36.6	32.4	30.0	75.1	56.4	42.5	38.5	46.6	43.3	40.8
Broca alone mm ³ O ₂ /hr.....	12.46	19.00	24.50		13.10	21.10	6.96	14.39	15.70	15.86
Number of infesting Broca.....	12	16	22		10	23	15	16	15	16.1

ming this rate for "a", the limiting radius for which diffusion alone would suffice becomes 0.0187 cms. As the bean is actually of a radius of 0.3 cms. the diffusion alone is insufficient if we assume the infested bean as a closed system.

Actually the bean is open, and as the diffusion in air is 300,000 times that in water, which itself is three times that of the assumed rate above we can assume that oxygen diffusion is not limiting. Often, however, one finds that the perforation and channel in the bean is filled with excreta, dirt and sawdust, thus effectively blocking the entrance of air. To simulate this condition, the pores of three, green, infested beans were blocked with paraffin, effecting an average reduction of 77.7% (74.0, 83.7, and 75.5% respectively) in the total oxygen uptake of beans containing Broca. Thus one can assume that (1) $\frac{3}{4}$ of the required oxygen passes normally through the perforations in the bean, and $\frac{1}{4}$ through the bean shell, and (2) that under certain conditions Broca may be anoxic in the green bean.

Experiments similar to the above were performed also with naturally infested mature beans (Table 11). These beans were obtained in fields in Campinas, and had an infestation rate of 46.7 insects in all stages per bean (Table 12). The intact, infested beans had an average oxygen uptake of 205.51 mm³/hr., and in weight averaged 704.94 mgms. with contained Broca. The isolated Broca showed an oxygen uptake of 36.35 mm³/hr., and averaged 15.27 mgms., giving a QO₂ of 2.60 mm³O₂/mgm./hr. The bean tissue that had been broken up to remove the insects had a QO₂ of 0.060, practically identical with that for uninfested, mature beans (0.059). Thus the weight of the infesting Broca comprised 2.1% of the total and the oxygen consumption 17.68% of the total by the infested bean. The ratio of the QO₂ Broca/QO₂ bean was 43.4, twice that in the green beans, due presumably to the decrease that occurs in the respiratory rate of the bean as it matures. The mature seed is dry and porous and surrounded by a gas space, thus oxygen diffusion cannot be limiting even at this high total rate of oxygen consumption. Nevertheless, it was of interest to determine the amount of oxygen diffusing through the perforations and that through the bean shell. Plugging the pores with paraffin reduced the oxygen consumption of 9 infested coffee beans 40.8% on the average (Table 13). We thus assume that 59% of the oxygen passes through the porous, brittle shell and normally 41% through the openings made by the insects in the mature beans.

INFLUENCE OF ENVIRONMENTAL FACTORS

A. Oxygen tension

1. *Gas content of infested bean.* — To further determine the normal conditions within the beans and their effects upon the insects, micro-gas analyses were made of several beans. The technique was as follows. A hypodermic needle, number 27, was sealed to a micro-plastic syringe and the syringe barrel and needle dead space filled with water. The needle was then inserted into the opening of the infested bean and the space between needle and bean shell covered with plasticene. A sample of the contained gas was then withdrawn from the chambers occupied by Broca within the bean and transferred to a micro-gas analyzer (Scholander, 1942b) for the determination

of O_2 , CO_2 and N_2 . The analyses of 4 infested beans (Table 14) gave an average of 18.00% for oxygen, 2.28% for carbon dioxide and 79.74% for nitrogen. These results can be interpreted in several ways. (1) That diffusion is actually poor, despite the results of the experiments reported above and that carbon dioxide accumulates with concomitant reduction of oxygen within the bean, or (2) that the carbon dioxide production of the bean tissue is high. That this latter may well be the case is indicated by the fact the gas in intercellular spaces in the fleshy tissue of apples, potatoes and carrots is high in CO_2 and low in O_2 (Curtis and Clark, 1950). It cannot be overlooked, however, that in the experiment in which the perforations in the beans were blocked the oxygen consumption of the system of bean plus infesting *Broca* decreased. This suggests, that *Broca*, under some conditions in nature, essentially lives in a closed box in which the oxygen tension may be low. Such partially anaerobic habitats are not unusual for insects, as environments of low oxygen tension may be found also where insects infest various plants, organs or host animals, and in such places as the wood chambers of termites and ants and in the cocoons of Lepidoptera (cf. Wigglesworth, 1939 ; von Brand, 1946).

TABLE FOURTEEN

ANALYSIS OF AIR WITHIN CHAMBERS OCCUPIED BY BROCA IN
INFESTED, MATURE BEANS

A n a l y s i s	% O_2	% CO_2	% N_2
1	19.17	1.83	79.00
2	14.20	3.73	82.07
3	17.70	3.50	78.80
4	20.85	0.05	79.10
average	18.00	2.28	79.74

2. *Influence of exposure to nitrogen upon respiration.* — In general, insects have an oxygen consumption that is independent of oxygen tension. To determine if *Broca* also showed such independence, which could be interpreted as a type of adaptation to conditions within the bean, two sets of experiments were performed. In the first was measured the influence of nitrogen upon the formation of an oxygen debt and the consumption during recovery. In the second the oxygen consumption was measured over a wide range of oxygen tensions.

In the first experiment (cf. Table 15) of 8 trials of 10 animals each, the animals were exposed to pure tank nitrogen for periods of 1, 24 and 72 hours. The technique was to place the animals in cotton in a separatory funnel, flush the funnel continuously with nitrogen for 10 minutes, then close the funnel before stopping the stream of N_2 . The animals became inactive within a few minutes and remained so during the time of exposure. Recovery

TABLE FIFTEEN
INFLUENCE OF NITROGEN ON THE OXYGEN CONSUMPTION OF HYPOTHENEMUS HAMPEI

Experiment number	Hours in N ₂	mm ³ O ₂ /mgm./hr. before exposure	mm ³ O ₂ /mgm./hr. after exposure		mm ³ O ₂ /mgm./hr. excess.		% repayment		minutes for return to normal
			1st hour	2d hour	1st hour	2d hour	1st hour	2d hour	
1.....	1	2.66	2.91	2.19	0.25	-0.47	9.4	-1.8	40
2.....	1	2.78	3.71	2.62	0.93	-0.16	33.4	-5.8	40
3.....	1	2.98	4.18	3.78	1.20	0.80	40.3	26.5	120
4.....	1	3.68	4.54	3.52	0.86	-0.16	23.4	-4.4	40
5.....	24	1.82	2.25	2.19	0.43	0.37	23.6	20.3	75
6.....	24	2.15	2.20	1.34	0.05	-0.81	2.3	-37.6	45
7.....	72	1.64	1.97	1.68	0.33	0.04	20.1	2.5	0
8.....	72	1.64	1.66	1.55	0	-0.09	0	-5.5	0
average		2.42	2.93	2.36	0.51	-0.06	21.5	-2.5	51.4

in both oxygen consumption and activity occurred on the average within 50 minutes after re-exposure to air. The oxygen consumption was measured before and for two hours after the exposure, and the oxygen debt and subsequent repayment calculated from the results. It will be seen that the length of exposure had little effect upon the recovery oxygen consumption. The excess following exposure of one hour varied from 9 to 40%, after 24 hours from 2 to 23% and after 72 hours from 0 to 22% in the first hour. One might expect that if the nitrogen were to have an effect the total excess would be greater for the longer exposure. However, we can see that all but one group of insects were back to normal within the second hour and that there was no significant trend in the results. Assuming that the initial oxygen consumption before exposure represents the amount of oxygen lost during the exposure, that is the oxygen debt per hour, then the oxygen consumed following exposure represents the normal plus the repayment of the debt. Generally, among animals the repayment is high, e. g. in the grasshopper 100% (Bodine, 1928), the termite 50% (Gilmour, 1940a), and *Cryptocercus* 35% (Gilmour 1940b). Thus the repayment by Broca do Café of 21.5% in the first hour, and -2.5% in the second, or a total of 19% is practically insignificant. This suggests that probably Broca does not accumulate

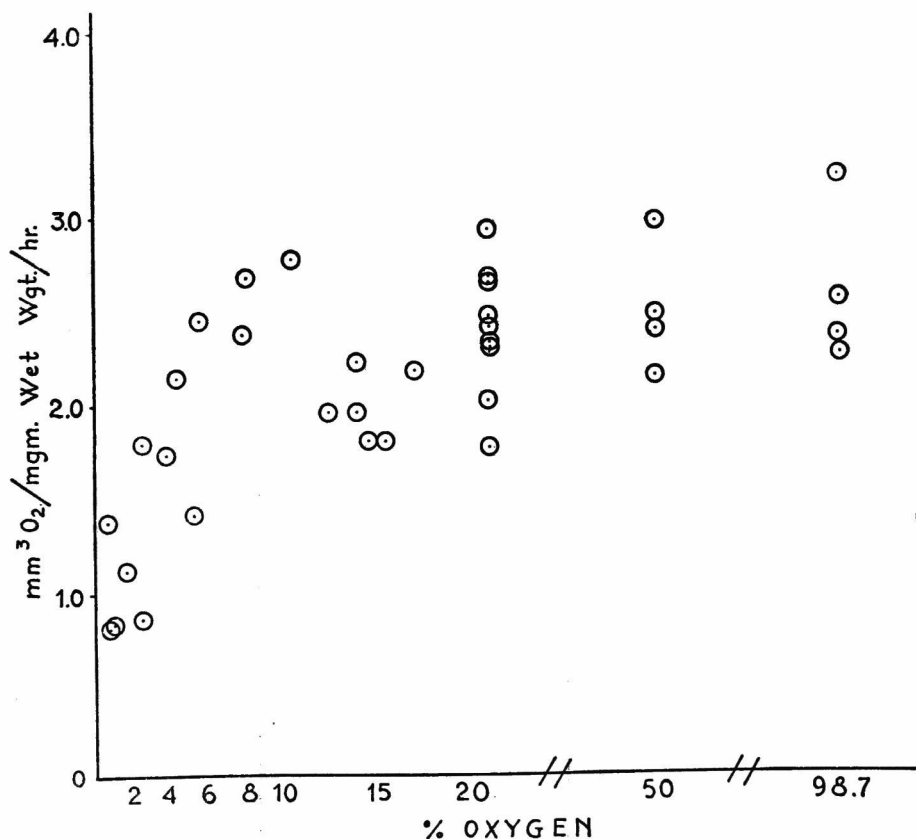


Figure 3. — Influence of oxygen tension upon oxygen consumption of *Hypothenemus hampei*. Note that "break" in curve occurs at approximately 5% O₂. Each point represents average for 10 animals.

TABLE SIXTEEN
INFLUENCE OF HUMIDITY UPON WEIGHT OF HYPOTHENEMUS HAMPEI

D a y	room air	ascarite	weight in milligrams			
			80% RH	50% RH	20% RH	
0	4.5	3.9	4.3	4.5	4.6	
1	4.6	3.4	4.0	4.2	4.0	
2	3.1	2.0	3.4	3.3	3.5	
4	2.3	1.9	3.3	2.8	2.3	
loss in mgms.	2.2	2.0	1.0	1.7	2.3	
% loss in weight	48.8	51.2	22.7	37.8	50.0	
condition at end	poor	poor	good	fair	poor	

lactic acid during the debt period and/or that secondary respiratory systems are in use during this period obviating the recovery oxidation of coenzyme one.

The exposure had little influence upon the recovery oxygen consumption and also upon recovery time. Generally, among invertebrates recovery requires approximately ten minutes per hour of anaerobiosis (von Brand, 1946). Broca however, showed no such relationship; the recovery from one hour exposure being as long as, or in one case longer than, the recovery time for 72 hours exposure, with an average of 51.4 minutes for all exposures. We can conclude from these experiments that Broca is quite well adapted in its oxygen consumption to possible anaerobic conditions within the bean.

3. *Influence of oxygen tension upon oxygen consumption.* — In a second test the oxygen consumption of a number of animals was measured in mixtures of air containing from 0.5% to 98.7% oxygen. As demonstrated in Figure 3 the oxygen consumption was constant between 5% and 98.7% oxygen in the respired air. Below 5% the oxygen consumption decreased rapidly. Such an independence of oxygen consumption and oxygen tension is characteristic of not only those insects such as Broca, that live in an oxygen poor atmosphere. *Tenebrio* pupæ respire at a constant rate between 5 and 97% oxygen (Gaarder, 1918), both diapausing and non-diapausing eggs of *Melanoplus* show a constant rate between 60 and 160 mms. Hg. of oxygen (Bodine, 1934) and the blowfly a constancy between 12 and 158 mms. Hg. of oxygen. However, such an independence of the amount of oxygen in the environment must be considered as one of the factors leading to the resistance of Broca to attack and enabling it to live within the essentially closed confines of the coffee bean.

B. Humidity

1. *Humidity and weight loss* — It has been observed in the field and laboratory, that when the coffee bean becomes dry the infesting Broca leave the bean in search of a more humid one. Further, Bergamin (1944-45) found that the larvæ will not eat at humidities less than 15%, that optimum growth occurs at humidities approaching 100%, and that the female chooses only humid beans for oviposition.

A number of experiments were performed to determine the influence of humidity upon the respiratory metabolism of Broca outside the bean. In the first the influence upon loss of weight and general condition was observed. Five vials were arranged containing in order (1) room air only, (2) Ascarite (equivalent to 20% RH), (3) 100% KOH equivalent to 20% RH, (4) 50% KOH equivalent to 50% RH, and 20% KOH equivalent to 80% RH. On a platform in each vial were placed 10 adult, female Broca. Initially and daily thereafter for four days the animals were weighed and their general condition noted. The results are given in Table 16. Within the first day the greatest weight losses (13.1 and 12.8%) occurred in the animals over Ascarite and 20% RH and the least in the vial containing room air only. The losses in 80 and 50% were practically identical. Over a period of four days the greatest loss occurred in those animals in the vials containing room air, ascarite, and 20% RH respectively and the least in the ani-

mals in 80% RH. Visually it was determined that those animals in 80% RH were in good condition, those in 50% fair and the rest in poor condition at the end of the experiment.

2. *Humidity and 24 hour oxygen consumption* — The one day oxygen consumption of Broca, 10 animals per vial, was determined using Ascarite, and KOH solutions to give 20, 50 and 80% RH respectively. As shown in Table 17, over a period of one day there was little influence of relative humidity upon oxygen consumption, the rates in the above conditions being 2.83, 2.99, 2.87 and 2.79 mm³/mgm./hr. respectively. Thus it is assumed that humidity change has little immediate effect upon the oxygen consumption of Broca. Further, it was found that for short term experiments, as previously discovered, Ascarite serves as an efficient and harmless absorbent of CO₂.

TABLE SEVENTEEN
INFLUENCE OF HUMIDITY UPON RESPIRATION OF HYPOTHENEMUS HAMPEI

Conditions	(oxygen consumption in mm ³ O ₂ / mgm. fresh weight / hour)			
	ascarite	20% RH	50% RH	80% RH
Oxyg. consumpt.	1.36	2.40	2.87	1.54
	0.90	2.89	2.87	2.35
	2.33	3.70		2.57
	2.12			3.13
	2.79			
	4.71			
	2.66			
	1.45			
	4.28			
	3.43			
	3.57			
	2.27			
Average	2.83	2.99	2.87	2.79

3. *Influence of humidity and starvation upon respiration* — The long term influence of humidity upon the oxygen consumption of starving animals was determined in two groups of animals. In the first, two groups of 10 adult females were kept in room air; in the second, two similar groups in vials of 80% RH. The oxygen consumption of each group was measured daily at the same hour over a period of some days. From the results given in Table 18 it can be seen that in starving animals humidity had a profound effect upon the oxygen consumption. In the two groups in room air the oxygen consumption remained constant for the first three days. On the fourth day the animals in one vial died, and the others showed a considerable decrease in oxygen consumption. On the fifth day the second group of animals in room air were dead. The two groups of insects in 80% showed a gradual decrease in oxygen consumption, although the animals appeared to be in good condition.

TABLE EIGHTEEN
INFLUENCE OF STARVATION AND HUMIDITY UPON OXYGEN CONSUMPTION OF HYPOTHENEMUS HAMPEI

	(oxygen consumption in mm ³ O ₂ / mgm. fresh weight / hour)							
	control	1st day	2d day	3d day	4th day	6th day	7th day	12th day
A. In room air								
	2.33	2.17	2.22	2.37	dead			
	2.61	2.59	2.46	2.43	1.80	dead		
B. In 80% RH								
	2.01		2.05	1.69	1.69	1.66	1.84	1.79
	2.03		2.65	1.65	1.60	1.73	1.31	0.96

In general, humidity changes have no direct influence upon the respiration of insects, but indirectly may modify metabolism through changes in temperature, activity, nutrition, water content and utilization of tissues. The effect appears to be dependent mainly upon water content, as evidenced for example by the greater effect upon larvæ than adults. In some insects, e.g. larvæ of *Rhizopertha dominica* humidity has no effect upon the oxygen consumption (Birch, 1947). In others, e. g. *Tenebrio* larvæ subjected to various temperatures and humidities, it is found that there is no variation in CO₂ output with variation in humidity but that rate of utilization of reserves depends upon temperature alone (Mellanby, 1936). In cockroaches the higher oxygen consumption in moist air can be explained by increased activity and increased body temperature (Gunn and Cosway, 1942). In starved larvæ of *Popillia japonica*, in which tissue utilization is rapid, lowered respiration and death follows no matter what the water content of the animal or the saturation of the air (Bellucci, 1939). That neither water content alone or tissue utilization alone is the limiting factor is indicated by experiments on *Phormia regina*. Normal flies show little change in oxygen consumption with changes in humidity. Wet DDT'ed flies show a 5 fold increase in oxygen consumption whereas dry poisoned flies show a three fold increase. The results are the same on both basis of weight at time of experiment or referred to original weight, hence it appears that in the wet poisoned flies the "respirable substrate" fraction of the total solid must decrease more rapidly than water, whereas in the dry, poisoned flies the water is lost faster than substrate and there is a greater water loss in proportion to the oxygen uptake (Buck and Keister, 1949).

In view of the foregoing discussion we interpret the results from the experiments with *Broca* as follows. (1) That changes in humidity do not normally directly affect the oxygen consumption. (2) That changes in humidity have an influence on the rate of water loss over a period of some days. (3) That the balance between water loss and respirable substrate loss is affected by changes in humidity. The results of the starvation experiments at the different humidities can be interpreted as above for *Phormia*. In low humidity the loss of water is greater than loss of respirable substrate, but that at higher humidity both water and respirable substrate diminish during starvation but there is a more rapid loss of respirable substrate.

C. Temperature

1. *Influence upon oxygen consumption* — Under natural conditions *Broca do Café* is apparently quite sensitive to changes in temperature (cf. Bergamin, 1944-45, for complete studies on the influence of temperature upon the life cycle). It has been observed that the optimum temperature for growth and reproduction is 25°C. Above 30°C *Broca* does not reproduce. When infested beans are dried in the sun and the temperature reaches 40°C the animals begin to leave the beans and at 45°C the animals die inside the drying beans. Such sensitivity would be expected in an animal that lives within the relatively constant environment within a fruit.

There is a remarkable parallelism between the response of *Broca* in its oxygen consumption to change in temperature and the behavioral responses noted above. From 15°C to 30°C the oxygen consumption of *Broca*

TABLE NINETEEN
INFLUENCE OF TEMPERATURE UPON THE OXYGEN CONSUMPTION OF HYPOTHENEMUS HAMPEI

Temperature °C	15	20	25	30	35	40 1st hr.	40 2d hr.	45 1st hr.	45 2d hr.
Oxygen consumption in mm ³ / mgm. / hr.	1.18 1.21	2.24 2.71	4.80 4.21 4.68 4.44	7.70 7.20	6.72 5.57 6.60 7.48	10.60 9.20	7.80 9.32	17.38 17.86	4.40 2.10
average	1.19	2.48	4.53	7.45	6.59	9.90	9.56	17.67	3.25
Temperature range	15-25	20-30	25-35	30-40	35-45				
Q ₁₀	3.81	3.01	1.46	1.33	2.68				

increases in regular fashion (cf. Table 19 and Figure 4). At 35°C the oxygen consumption is lower than at 30°C, but rises once more to a higher level at 40°C, reaching its maximum within the first hour at 45°C. The thermal death point appears to be close to 45°C, i. e., the oxygen consumption at this temperature is extremely high in the first hour of exposure, but drops radically within the second hour and within the third hour the animals die. Thus there is a "break" in the metabolism — temperature curve at the temperature at which, in nature, reproduction ceases, and there appears to be a good correlation between the temperature at which maximum oxygen consumption occurs and that at which the animals become injured or die in nature.

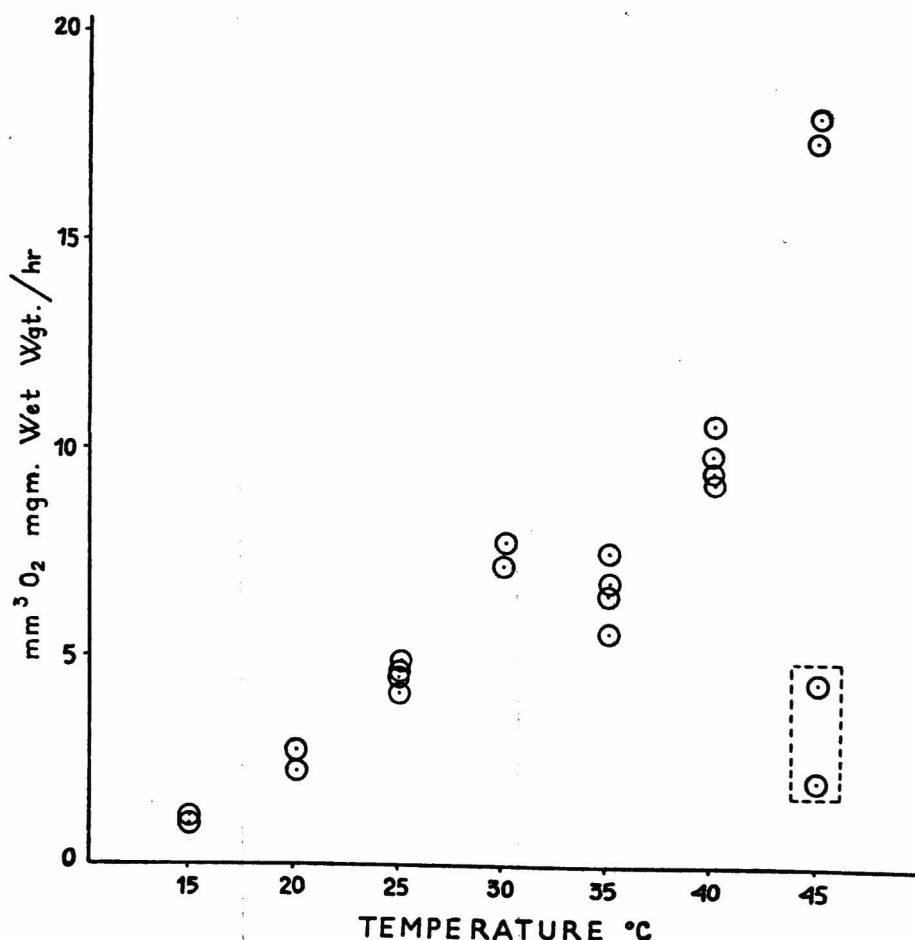


Figure 4. — Oxygen consumption of *Hypothenemus hampei* at various levels of temperature. Note deviation at 30° C and approximation of thermal death point at 45°C Enclosed points denote 2d hour oxygen consumption.

2. Q10. — The radical shift in the metabolic response to temperature change is further reflected in the Q10, i. e. the rate at T°C divided by the rate at T — 10°C. Q10's typical of biological processes lie between 2 and 3,

hence Broca is quite typical from 15 to 30 degrees. However, Broca becomes atypical in that generally the Q_{10} shows a gradual decrease with increasing temperature, but in the case of Broca shows a secondary increase.

3. *Thermal increments.* — A better understanding of the metabolic events as affected by temperature may be obtained by calculation of activation energies by treatment of the data by the Arrhenius equation, as modified by Crozier (1924-25):

$$u = 4.58 \left(\frac{\log k_1 - \log k_2}{\frac{1}{T_2} - \frac{1}{T_1}} \right)$$

A plot of the values thus obtained (Figure 5) shows linearity from 15° to 30°C and thereafter extreme departure from linearity. Thermal increments of 11,000 and 21,000 cal. may be obtained from the curve, indicative

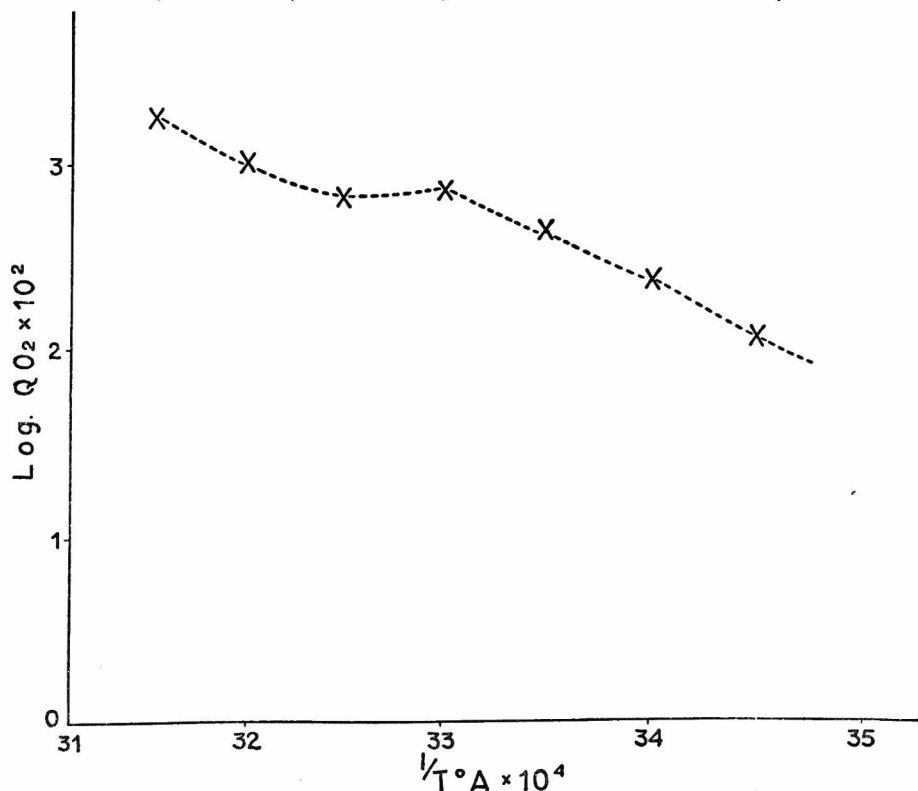


Figure 5. — Arrhenius plot of oxygen consumption — temperature data. Deviation from linearity occurs at 30°C. For straight line portion of curve μ equals 21,000 cal., concave portion can be interpreted as μ equals 11,000 cal.

of the existence of two, concurrently operating, rate limiting reactions whose maxima are attained above and below 30°C respectively. The thermal increment of 11,000 cal. is a common one encountered in cell respiration and has been previously reported from *Melanoplus* and *Drosophila* (Crozier and

Stier, 1924-25 ; Orr, 1924-25), among the insects. Higher values, comparable to that found for Broca below 30°C have been found in *Pollenius* at temperatures below 10.5°C (Argo, 1939). The thermal increments may be utilised in the interpretation of the type of enzyme systems in use (cf. Hoagland, 1935). The deviation from linearity can be interpreted as irreversible enzyme inactivation or the gradual dominance of a physical system over a chemical one.

DISCUSSION

Each aspect of the work has been discussed in some detail in the individual sections throughout the course of the paper. We will, therefore, limit the discussion here to generalities.

In the metabolism during growth and metamorphosis Broca appears to be a typical holometabolic insect, showing the characteristic high oxygen consumption immediately following eclosion, the gradual decrease with increase in larval age, the U-shaped pupal curve, and the adult level similar to that of the last larval stage. The energy seems to go into growth and storage of reserves in the larva and into reproductive activities in the adult. Broca appears to differ from many other insects in two respects. The female has the higher oxygen consumption on the basis of unit weight, a fact which cannot be explained on the bases of activity and size, but must be due to hormonal or other fundamental difference between the sexes. Secondly, Broca shows an average change in RQ throughout the immature and pupal stages but then differs from the usual in the adult stage. The possibility exists that this represents an adaptation chemically to the high CO₂ content of the bean. However, further work is necessary to determine if this is so, or if this represents a minimal feeding and hence reserve utilization in the adult. We further need to know the difference between the sexes in regard to RQ.

Broca appears to be well adapted to the conditions obtaining in its micro-environment within the bean. Oxygen appears not to be limiting normally, but even under conditions where diffusion may be impeded Broca appears to be adapted in that it has an oxygen consumption independent of oxygen tension and appears not to derive an oxygen debt from anaerobic exposure. The coffee bean is essentially a constant environment, insofar as temperature and humidity are concerned. Thus it was not surprising that Broca, in its oxygen consumption, showed sensitivity to changes in these factors. Indeed the correlation between the field and laboratory observations is extremely good as regards the influence of temperature and humidity upon behaviour and metabolism, and lends some insight into the fundamentals of the normal behaviour of the borer.

From the practical point of view, the major point of interest is that Broca appears to be well adapted to live a protected life within the coffee bean, the adult female leaving only if conditions are unfavorable for oviposition. Thus, in control measures, one must consider how to prevent the entrance of the insect into the bean, how to penetrate the bean with chemicals or to bring about conditions that will cause the insect to leave itself exposed outside the bean. The results of studies on the influence of certain chemicals upon the metabolism of Broca, with some considerations of control measures, will be presented in the following paper in this series.

SUMMARY AND CONCLUSIONS

The oxygen consumption of *Hypothenemus hampei* (Broca do Café) has been measured in the various stages of growth of the insect from egg through adulthood. The influence of various environmental factors upon the oxygen consumption has been determined, as well as certain aspects of the relationship between the infested coffee bean and the infesting insect studied.

During growth, the oxygen consumption of the egg stage is low, reaching a maximum immediately after eclosion. The rate decreases gradually with increase in larval age, and decreases rapidly during prepupation and pupation, reaching a minimum in mid-pupal life. The adult level is comparable to that of the last larval stage. The oxygen consumption of the adult female is greater than that of the male.

The respiratory quotient is greater than unity in the egg stage, averages 0.84 in the larva, declines in the pupa to values between 0.57 and 0.70, and remains at a low level in the adult. It is presumed that this represents a carbohydrate metabolism in the egg, a mixed diet in the larvæ, utilization of reserves in the pupa, and/or fixation and retention of CO₂ in the pupa. Several possible explanations of the low RQ in the adult are discussed.

With maturity the coffee bean shows a decrease in water content and oxygen consumption and changes in character from semi-aqueous to porous-solid thus changing the environment for the contained Broca. The infesting insect influences the oxygen consumption of the immature, but not that of the mature coffee bean. In both types of beans, diffusion of oxygen through the bean shell and perforations caused by the insect seems to be sufficient to care for the needs of both insect and bean. If the perforations are blocked there is a large reduction in oxygen consumption in the infested, immature bean and a smaller reduction in that of the mature, infested bean, suggesting the relative importance of the two routes in the beans.

The gas content of the infested mature beans is relatively high in CO₂ and low in oxygen, suggesting that Broca is exposed to partially anaerobic conditions. However, the oxygen consumption of Broca appears to be independent of oxygen tension, and exposure to nitrogen does not cause a large oxygen debt and excess repayment. The recovery in oxygen consumption and behaviour appears to be independent of length of anaerobic exposure. The oxygen consumption appears to be independent of oxygen content of respired air between 5 and 98.7% O₂. Below 5% oxygen in the air the oxygen consumption decreases rapidly.

Changes in humidity do not affect the short term oxygen consumption of Broca. Over a longer period of time, 4 to 12 days, humidity has an influence on both oxygen consumption and weight loss. It appears that the affect of humidity is not direct, but that the primary affect is one of rate of tissue utilization, which is greater in lower humidities.

Increase in temperature from 15 to 30°C causes a regular increase in oxygen consumption. Above this temperature deviation from regularity occurs, which is reflected in the deviation of Q₁₀ and deviation of activation energies from linearity. The maximum oxygen consumption occurs at 45°C.

The thermal death point appears to be close to this temperature. Thermal increments of 21,000 calories below, and 11,000 cal. above 30°C may be obtained from the Arrhenius expression of the data, indicative of at least two rate limiting reactions occurring simultaneously.

The results of these studies on the metabolism of *Broca* in the laboratory confirm and extend field observations. They should serve as a basis for the future study of the physiology and pharmacology of *Broca do Café*.

SUMARIO E CONCLUSÕES

O consumo de oxigênio de *Hypothenemus hampei* (*Broca do Café*) foi medido em vários estágios do desenvolvimento do inseto, desde a fase de ovo até a de adulto. Determinou-se também a influência de vários fatores do ambiente sobre o consumo de oxigênio, assim como certos aspectos da relação entre o grão do café infestado e o inseto infestante.

Durante o desenvolvimento da *Broca*, o consumo de oxigênio é baixo no estado de ovo, atingindo o máximo imediatamente após a eclosão. A taxa do consumo decresce gradualmente com o aumento da idade da larva, e decresce rapidamente durante a prepupação e pupação, atingindo o mínimo no meio do estágio pupal. A taxa de consumo no adulto eleva-se a um nível comparável ao do último estágio larval. O consumo de oxigênio na fêmea é maior que no macho.

No estágio de ovo, o quociente respiratório é maior que a unidade; na larva, mantém-se ao redor de 0,84, declina na pupa até valores entre 0,57 e 0,70, e permanece em um nível baixo no adulto. Presume-se que os valores acima representem um metabolismo de carboidratos nos ovos; uma dieta mixta nas larvas; uma utilização de reservas, ou talvez uma retenção e fixação de CO₂ na pupa. Diversas explicações possíveis para o baixo QR no adulto são apresentadas.

Durante a maturação o grão de café mostra um decréscimo no conteúdo de água e no consumo de oxigênio, e uma mudança do estado semi-aquoso a solido-poroso. Assim, para a *Broca* há uma mudança no ambiente. O inseto infestante tem influência no consumo de oxigênio do grão imaturo, mas não após a maturação do mesmo. Em ambos os tipos de grãos de café a difusão de oxigênio através da casca do grão e das perfurações feitas pelo inseto parece ser suficiente para responder às necessidades de ambos, inseto e grão. Se tais perfurações são bloqueadas mostra-se uma grande redução no consumo de oxigênio do grão infestado imaturo e uma redução menor se o grão infestado está maduro, fato esse que sugere a importância relativa da casca e das perfurações no papel da difusão do oxigênio.

O conteúdo de gas do grão infestado é relativamente elevado em CO₂ e baixo em O₂, sugerindo que a *Broca* está exposta parcialmente a condições anaeróbicas. Contudo, o consumo de oxigênio da *Broca* parece ser independente da tensão de oxigênio e, além disso, a exposição do inseto a nitrogênio não causa um débito de oxigênio com consequente pagamento no período da recuperação.

A recuperação da taxa do consumo de oxigênio e o comportamento normal do inseto parece ser independente da duração das condições anaeróbicas.

O consumo de oxigênio é constante, quando a concentração de oxigênio do ar respirado varia de 5 até 98,7%. Abaixo de 5% de oxigênio no ar, o consumo de oxigênio decresce rapidamente.

Trocãs de umidade não afetam o consumo de oxigênio em períodos curtos, mas tem importância em períodos longos, como de 4 a 12 dias. Neste caso, a umidade tem influência tanto no consumo de oxigênio como na perda de peso. Parece que o efeito do fator umidade não é direto, mas faz-se sentir através da taxa de utilização de tecidos, taxa essa que aumenta com o decréscimo da umidade.

O aumento da temperatura de 15 a 30°C causa um acréscimo regular no consumo de oxigênio. Acima de 30°C observa-se uma saída dessa regularidade o que se reflete no desvio do Q10 e na linearidade da curva que expressa as energias de ativação. O consumo máximo de oxigênio ocorre a 45°C. O ponto termico letal parece ficar no redor desta temperatura. Incrementos termais de 21,000 calorias, abaixo, e de 11,000 calorias, acima de 30°C podem ser deduzidos da expressão de Arrhenius, indicando, nos nossos resultados, ao menos duas reações limitantes concorrentes.

Estes resultados sobre o metabolismo da Broca do Café, obtidos no laboratório, confirmam e alargam as observações feitas no campo e poderão servir como base para estudos futuros sobre a Fisiologia e Farmacologia da Broca do Café.

ACKNOWLEDGEMENT

The authors wish to express their gratitude to Dr. Paulo Sawaya, for his facilitation of these studies and his continous interest and helpful criticism. We would like to thank also Dr. Jacob Bergamin for material and information.

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