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SELECTION OF THE RED FLOUR BEETLE (*TRIBOLIUM CASTANEUM* (HERBST)) FOR RESISTANCE TO A COMBINATION OF PHOSPHINE PLUS CARBON DIOXIDE AND BIOLOGICAL OBSERVATIONS ON THE RESISTANT STRAIN

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ABSTRACT

The main objectives of this work were to study the development of resistance to a combination of phosphine (PH_3) and carbon dioxide (CO_2) in the red flour beetle *Tribolium castaneum* (Herbst) and to investigate some biological characteristics of the resistant strain in comparison with the parental stock.

Sixteen generations of adult *T. castaneum* were exposed in the laboratory for varying exposure periods to a mixture of 40 ppm PH_3 + 46% CO_2 at 26 ± 1°C and 6 ± 1°C in order to select for a resistant strain. Selection pressure was carried out at the median lethal time inducing 50–70% mortality.

Results showed that the lethal time (LT) values recorded to obtain a given mortality were significantly higher at the two test temperatures for the 16th generation of adults than for the parental strain. When compared to the parental stock at the LT_{50} level, resistance to the PH_3–CO_2 mixture increased by 19.4 at 26 ± 1°C and by 18.5 at 6 ± 1°C. This clearly indicated that *T. castaneum* adults have the genetic potential for developing resistance to a PH_3/CO_2 atmosphere. Analysis of the biological characteristics of the resistant strain revealed that it laid significantly more eggs than did the laboratory strain. However, no significant differences were found in either the average pre-oviposition period or the sex ratio. Both the average incubation period and the total developmental period were clearly longer for the laboratory strain than for the resistant strain. The average hatching rate of the eggs and larval mortality were both increased significantly in the resistant strain. The emergence rate of the adults was unaffected, amounting to 100% for both strains.

INTRODUCTION

Some investigators have studied the efficacy of phosphine (PH_3)–carbon dioxide (CO_2) mixtures against stored-product insects, noting the advantages of using such combinations for control (Aliniazae, 1971; Kashi and Bond, 1975; Desmarchelier and Wohlgemuth, 1984; El-Lakwah *et al.*, 1989, 1991b, c, 1992).

A major problem that has developed in many insect control programmes in recent years is the resistance acquired when successive generations are exposed to toxic agents. Survivors of progressive selection can develop characteristics that make the toxicant ineffective or uneconomical.

Resistance to the fumigants methyl bromide (MB), PH_3 (Champ and Dyte, 1976) and ethylene dibromide (Bond, 1973) has been found in field populations of stored-product insects in recent years.

In the laboratory El-Lakwah *et al.* (1991a) developed a PH_3-resistant strain of the red flour beetle *Tribolium castaneum* (Herbst) by exposing successive generations of adults to the median lethal dosage.

The present investigation was conducted in the same way on the same insect both to study the development of resistance to a mixture of PH_3 and CO_2 and to compare the biological characteristics of the resistant strain and those of the parental stock.

MATERIALS AND METHODS

Rearing technique

T. castaneum adults were reared in the laboratory at 26 ± 1°C and 60 ± 5% r.h. The strain had been held in the laboratory at the same temperature without being subjected to any chemical pressure for 6 years before starting the experiments. Approximately 200 7–14-d old adults were added to 50 g wheat flour with 5% dry yeast in a small jar which was then covered with muslin. After 48 h, the adults were sieved out and the cultures then incubated under the above conditions. Newly emerged 7–14-d old adults were used for all stages of this study.

Selection pressure

The adult populations of *T. castaneum* were exposed in the laboratory for the median lethal time (LT) to a controlled atmosphere (40 ppm PH_3 + 46% CO_2) at 26 ± 1°C and at 6 ± 1°C.

The regression line of the laboratory strain (parent stock) was drawn. The LT_{50} of that generation was then extrapolated from the line. When this was done, 240 individuals were exposed to the mixture for the median lethal time, thereby obtaining approximately a 50% kill. Adult insects surviving this selection were reared in turn at 26 ± 1°C and 60 ± 5% r.h. to produce the next generation. The same selection procedure was applied to each generation, rearing being carried out under the same conditions as the laboratory strain. This was done for 16 generations and the susceptibilities of each generation of adults were determined.

Assessment of resistance factor

Mortality results were calculated using the correction of Abbott's formula (1925) and the LT_{50} values of the various generations used to indicate resistance development within successive generations. The increase in resistance was calculated as the ratio between the

LT_{50} values of the selected strain and that of the parental strain, i.e.

$$RR = \frac{\text{The } LT_{50} \text{ of the successive generation}}{\text{The } LT_{50} \text{ of the parental strain}}$$

where *RR* = resistance ratio.

Generation of phosphine

Throughout this work PHOSTOXIN®-pellets (Detia-DEGESCH — Germany) were used to obtain PH_3 gas. Each PHOSTOXIN®-pellet contains approximately 56% aluminium phosphide which reacts with water according the equation:

$AlP + 3\ HOH \rightarrow Al(OH)_3 + PH_3 \uparrow$

A pellet weighs 0.6 g and produces approximately 0.2 g PH_3. The gas was generated by introducing a glass tube (1.5 × 3 cm) containing one pellet and 2 ml water into a Dressel flask connected on one side to a gas reservoir and on the other side to a recirculatory pump (also connected to the gas reservoir). The pump was operated for 0.5 h, after which the flask was detached and the reservoir closed.

Measurement of phosphine concentrations

Concentrations of PH_3 in the gas reservoir were determined using Draeger gas detector tubes (50/a). The required gas concentration was then obtained by diluting the gas, using the Dressel flasks and the recirculatory pump: 1 ppm PH_3 = 1.413 µg/L = 0.001413 mg/L.

Pre-fumigation procedure

Wire gauze 14 × 45-mm cages were filled with about 2 g wheat flour. Batches of 30 *T. castaneum* adults were introduced into each cage which was then covered with rubber stoppers. Three replicates were used in each treatment.

Fumigation procedure

Fumigation experiments were performed at 26 ± 1°C and 6 ± 1°C. The relative humidity during the fumigation was 55–65%. A recirculatory multi-flask fumatorium was set up for the fumigation experiments. To obtain a mixture of 40 ppm PH_3 + 46% CO_2 (v/v), six Dressel flasks were filled with 99% v/v CO_2 and a seventh with 480 ppm PH_3. These flasks were inter-connected in circuit to each other, to a gastight pump and to five additional flasks containing the test insects. The pump was operated for 1 h to circulate the gas mixture inside the flasks.

Post-fumigation procedure

Following exposure to the PH_3–CO_2 mixture, the insects were transferred to glass petri dishes with about 3 g wheat flour and held at 26 ± 1°C and 60 ± 5% r.h. for mortality assessment. Mortality was determined 3 d after fumigation. The percentage mortality was corrected using Abbott's formula (1925).

Effect of selection pressure of PH_3 + CO_2 on some biological parameters of *T. castaneum*

Laboratory experiments were carried out at 30 ± 1°C and 75 ± 5% r.h. to compare some biological aspects of the 12th generation PH_3–CO_2-selected strain with those of the parental stock. In these experiments the following data were recorded: the average number of eggs laid daily by each female, the average total number of eggs laid during 14 d by each female, the pre-ovipostion period, the incubation period of eggs, the percentage of eggs hatching, the developmental periods for the various insect stages, the average duration of larval instars, the average duration of pupal instars and the mean weight of both adults and pupae.

Number of eggs. Tests were carried out to assess how many eggs were laid by the females during a 14-d observation period. Freshly emerged (unmated) males and females were paired in a 3 × 5-cm glass tube containing a small amount of wheat flour; it was then covered with muslin. Every day the number of eggs laid in the flour was counted and the flour replaced. The total number of eggs laid per female during 14 d was recorded. Hatching of eggs was also recorded to determine their viability. Each treatment was replicated four times.

The incubation period and the developmental span. To observe the incubation period of eggs and the developmental periods for the various stages, 1-d-old eggs were placed individually, with a small amount of wheat flour, in a 3 ×-5 cm glass tube which was then covered with muslin. Ten replicates were used for each laboratory and each PH_3–CO_2-selected strain. The incubation period for every egg was recorded. The developmental stages were observed and their developmental periods noted. In addition, the mortality rate for the larvae was calculated.

Statistical analysis

The toxicity data obtained were subjected to Probit analysis (Finney, 1971) using the computer program of Noack and Reichmuth (1978). The biological data were subjected to analysis of variance.

RESULTS AND DISCUSSION

Tables 1 and 2 show the lethal times and parameters of the probit regression-line estimates for different generations of *T.castaneum* adults, selected for resistance to the PH_3–CO_2 mixture at 26 ± 1°C, 6 ± 1°C and 60 ± 5% r.h. The RR's and the lethal response for the various generations at the two test temperatures are given in Table 3 and Figs. 1 and 2. Results showed that the LT needed to obtain a given mortality level was significantly higher for the 16th generation at both temperatures than for the laboratory parent strain. The LT's for 50% kill at 26 ± 1°C were 1.9 h for the parent strain and 36.8 h for the 16th selected generation. Similarly, at 6 ± 1°C the LT's recorded for 50% kill were 4.4 h for the parent strain and 81.2 h for the 16th selected generation

TABLE 1

Lethal times and parameters of probit regression line estimates for adults of different generations of *T. castaneum* (Herbst) exposed to a controlled atmosphere containing 40 ppm PH_3 + 46% CO_2 at 26 ± 1°C and 60 ± 5% r.h.

Generation	Lethal times and their 95 % confidence limits (h)			Parameters of regression line			
	LT_{50}	LT_{90}	LT_{99}	Slope ± SE	*a*	*F*	*R*
Parent (laboratory strain)	1.9 (2–2)	4.2 (4–5)	7.9 (6–10)	3.8 ± 0.35	0.93	4	0.984
2nd generation	3.0 (3–3)	7.7 (7–9)	16.8 (13–12)	3.1 ± 0.18	3.52	6	0.990
4th generation	6.2 (6–7)	11.7 (10–13)	19.5 (16–24)	4.7 ± 0.50	1.29	5	0.849
6th generation	18.8 (16–21)	29.5 (24–37)	42.7 (30–62)	6.5 ± 0.16	–3.30	2	0.965
8th generation	14.7 (11–20)	20.0 (13–30)	25.6 (13–50)	9.6 ± 0.00	–6.22	1	1.000
10th generation	18.5 (15–23)	23.8 (16–35)	29.2 (15–56)	11.7 ± 0.03	–9.85	1	0.993
12th generation	26.0 (23–30)	44.3 (35–56)	68.4 (47–100)	5.5 ± 0.13	–2.82	2	0.981
14th generation	32.7 (28–38)	61.3 (47–80)	102.5 (65–163)	4.7 ± 0.05	–2.10	2	0.990
16th generation	36.8 (31–44)	77.9 (55–111)	144.0 (78–265)	3.9 ± 0.05	–1.16	2	0.988

SE = Standard error of regression line; *a* = axis intercept; *F* = degree of freedom; *R* = correlation coefficient.

TABLE 2
Lethal times and parameters of probit regression line estimates for adults of different generations of *T. castaneum* (Herbst) exposed to a controlled atmosphere containing 40 ppm PH_3 + 46% CO_2 at 6 ± 1°C and 60 ± 5% r.h.

	Lethal times and their 95 % confidence limits (h)			Parameters of regression line			
Generation	LT_{50}	LT_{90}	LT_{99}	Slope ± SE	*a*	*F*	*R*
Parent (laboratory strain)	4.4 (4–5)	11.2 (9–14).	24.4 (17–34)	3.1 ± 0.31	3.01	5	0.988
2nd generation	6.7 (6–7)	15.8 (13–19)	31.6 (24–42)	3.5 ± 0.34	2.14	5	0.989
4th generation	35.9 (24–54)	54.8 (31–98)	77.6 (32–189)	6.9 ± 0.19	–5.80	1	0.983
6th generation	52.2 (40–68)	69.5 (45–106)	87.8 (46–169)	10.3 ± 0.12	–12.68	1	0.979
8th generation	36.9 (21–65)	63.0 (30–135)	97.4 (22–429)	5.5 ± 0.02	–3.66	1	0.994
10th generation	54.4 (50–60)	75.3 (65–87)	98.1 (79–122)	9.1 ± 0.04	–10.79	2	0.998
12th generation	62.5 (58–67)	93.5 (84–105)	130.0 (108–157)	7.3 ± 0.17	–8.12	3	0.984
14th generation	76.4 (70–83)	130.8 (109–157)	202.9 (151–273)	5.5 ± 0.22	–5.32	3	0.966
16th generation	81.2 (75–88)	129.7 (113–148)	189.9 (152–238)	6.3 ± 0.38	–7.05	3	0.954

SE = Standard error of regression line; *a* = axis intercept; *F* = degree of freedom; *R* = correlation coefficient.

TABLE 3
Resistance ratios (RR) at LT_{50} and LT_{90} levels of different generations of *T. castaneum* adults exposed to an atmosphere containing 40 ppm PH_3 + 46% CO_2 at 26 ± 1°C and 6 ± 1°C

	RR* at 26 ± 1°C		RR at 6 ± 1°C	
Generation	LT_{50}	LT_{90}	LT_{50}	LT_{90}
Parent (laboratory strain)	1.0 (1.1–1.1)	1.0 (0.9–1.2)	1.0 (0.9–1.1)	1.0 (0.8–1.3)
2nd generation	1.6 (1.6–1.6)	1.8 (1.7–2.1)	1.5 (1.4–1.6)	1.4 (1.2–1.7)
4th generation	3.3 (3.2–3.7)	2.8 (2.4–3.1)	8.2 (5.5–12.3)	4.9 (2.8–8.8)
6th generation	9.9 (8.4–11.1)	7.0 (5.7–8.8)	11.9 (9.1–15.5)	6.2 (4.0–9.5)
8th generation	7.7 (5.8–10.5)	4.8 (3.1–7.1)	8.4 (4.8–14.8)	5.6 (2.8–12.1)
10th generation	9.7 (7.9–12.1)	5.7 (3.8–8.3)	12.4 (11.4–13.6)	6.7 (5.8–7.8)
12th generation	13.7 (12.1–15.8)	10.5 (8.3–13.3)	14.2 (13.2–15.2)	8.3 (7.5–9.4)
14th generation	17.2 (14.7–20.0)	14.6 (11.2–19.0)	17.4 (15.9–18.9)	11.7 (9.7–14.0)
16th generation	19.4 (16.3–23.2)	18.5 (13.1–26.4)	18.5 (17–20)	11.6 (10.1–13.2)

*Confidence limits of RR based on confidence limits of parent strain.

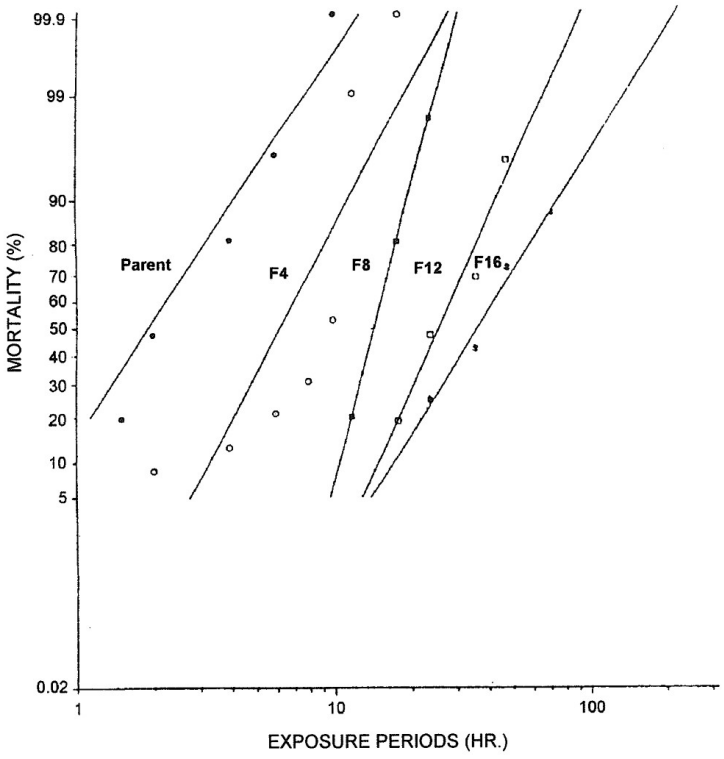

Fig. 1. Lethal response of parent and various strains of *Tribolium castaneum* adults, selected to PH_3–CO_2 at 26 ± 1°C.

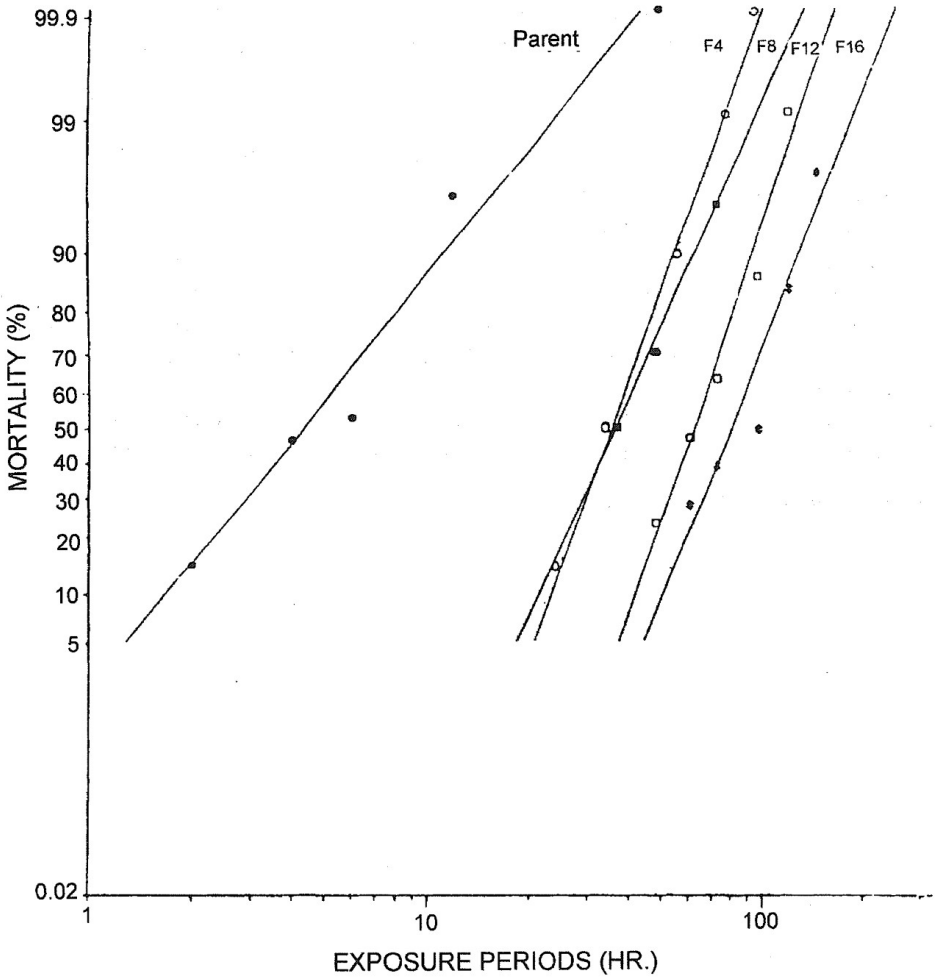

Fig. 2. Lethal response of parent and various strains of *Tribolium castaneum* adults, selected to PH_3–CO_2 at 6 ± 1°C.

(Table 2 and Fig. 2). The same trend occurred at both temperatures at LT_{90} and LT_{99} levels.

At the LT_{50} level, the RR's at 26 ± 1°C, increased from 1.6 (the first generation) to 19.4, and at 6 ± 1°C to 18.5, at the 16th generation (Table 3). These results clearly indicated that *T. castaneum* adults have the genetic potential to develop resistance to an atmosphere consisting of 40 ppm PH_3 + 46% CO_2. They corroborate the findings of other investigators: stored-product insects have the genetic potential to build up resistance to modified atmospheres (Bond and Buckland, 1979; Navarro *et al.*, 1985; El-Lakwah *et al.*, 1995).

Biological characteristics of the selected strain in comparison with the parental stock

The biological parameters of the selected strain of *T. castaneum* examined at the 12th generation are summarized in Tables 4, 5, 6 and 7 and Fig. 3.

Data showed that there was no significant difference between the laboratory and the PH_3–CO_2-selected strain in the average pre-oviposition period or the sex ratio (Table 4). The total developmental period was significantly longer for the laboratory strain (29.167 d) than for the resistant strain (24.076 d). It was also observed that the emergence rate of the adults was unaffected (100% for each strain). The average incubation period was significantly longer in the laboratory strain (4.2 d) than for the selected strain (2.67 d). The mortality rate of the larval instars was significantly higher for the selected strain than for the laboratory strain. The average total duration of the larval instars and the average duration of pupal instars were significantly longer for the laboratory strain than for the selected strain.

During the 14-d observation-period, the average number of eggs laid per female per day was significantly higher for the selected strain than for the laboratory strain (Table 5). The average hatching rate of the eggs significantly declined from about 96% for the selected strain to 81% for the laboratory strain.

A comparison of the average number of eggs laid daily per female by the laboratory strain and the selected strain during an observation period of 14 d is given in Fig. 3 and Table 5. From them it is clear that the selected strain laid an average of 172 eggs during the observation period, significantly higher than the average of 55.8 eggs laid by the parent strain.

TABLE 4

Some biological parameters for the laboratory strain and the PH_3–CO_2-resistant strain (F12) of *T. castaneum* at 30 ± 1°C and 75 ± 5% r.h.

Parameters	Laboratory strain (L)	PH_3–CO_2-resistant strain (F12)	Probability
Average pre-ovipostion period (d)	5.33 ± 0.33[1]	6.33 ± 0.33	0.059
Average no. of eggs per female per day	3.80 ± 0.409	12.40 ± 3.44	0.000**
Average total no. of eggs per female during 14 d	55.83 ± 1.1	172.0 ± 2.44	0.000**
Average hatching rate (%)	81.67 ± 3.34	96.67 ± 1.76	0.015*
Average incubation period (d)	4.17 ± 0.310	2.67 ± 0.213	0.002**
Average duration of larval instars (d)	19.00 ± 0.317	16.40 ± 0.509	0.002**
Average duration of pupal instars (d)	6.00 ± 0.0	5.00 ± 0.0	0.000**
Total developmental periods (d)	29.167 ± 4.68	24.076 ± 4.25	0.000**
Mortality for larval instars (%)	0.0	40	
Emergence rate (%)	100	100	
Sex ratio	1:1	1:1	

* = Differences significant at the 5% level; ** = differences significant at the 1% level. [1]±SD.

TABLE 5
Average daily number of eggs laid per female of *T. castaneum* during 14 d for the laboratory and the PH_3–CO_2-resistant strain (F12) at 30 ± 1°C and 75 ± 5% r.h.

Day	Average number of eggs laid per female per day	
	Laboratory strain (L)	PH_3–CO_2-resistant strain (F12)
1	1.7 ± 0.219[1]	10.2 ± 0.280
2	1.3 ± 0.366	11.2 ± 0.684
3	1.5 ± 0.280	12.5 ± 0.326
4	2.7 ± 0.275	16.0 ± 0.335
5	3.2 ± 0.109	14.5 ± 0.440
6	4.3 ± 0.219	13.5 ± 0.369
7	5.5 ± 0.281	15.2 ± 0.663
8	7.3 ± 0.366	15.5 ± 0.939
9	6.8 ± 0.313	12.7 ± 0.626
10	6.3 ± 0.404	13.8 ± 0.262
11	3.8 ± 0.313	10.2 ± 0.313
12	4.2 ± 0.201	11.7 ± 0.219
13	4.2 ± 0.393	8.7 ± 0.139
14	3.0 ± 0.238	8.0 ± 0.478
Average total number of eggs laid during 14 d	55.8 ± 1.1	172.0 ± 2.44**

**Differences significant at the 1% level. [1]±SD.

From Table 6 it can be seen that eight larval instars were recorded for the selected strain whereas there were only seven larval instars for the laboratory strain. This table shows that the laboratory-strain first, second, fifth and sixth larval instars continued for a significantly longer time than did those of the selected strain; the total duration of the larval instars was 19 d for the laboratory strain and 16.4 d for the selected strain, this difference being significant.

The mean weights of adults and pupae of the various *T.castaneum* strains are given in Table 7; the adults and pupae of the PH_3–CO_2-resistant strain were significantly lighter than those of the laboratory strain and of PH_3-resistant and CO_2-tolerant strains reared in the laboratory over several years.

Summary

The PH_3–CO_2-selected strain laid significantly higher numbers of eggs than the laboratory strain, and their hatchability was higher, but their incubation period was lower. Larval mortality was significantly higher in the selected strain. The duration of the 1st, 2nd, 5th and 6th larval instars was significantly shorter for the selected strain, and the

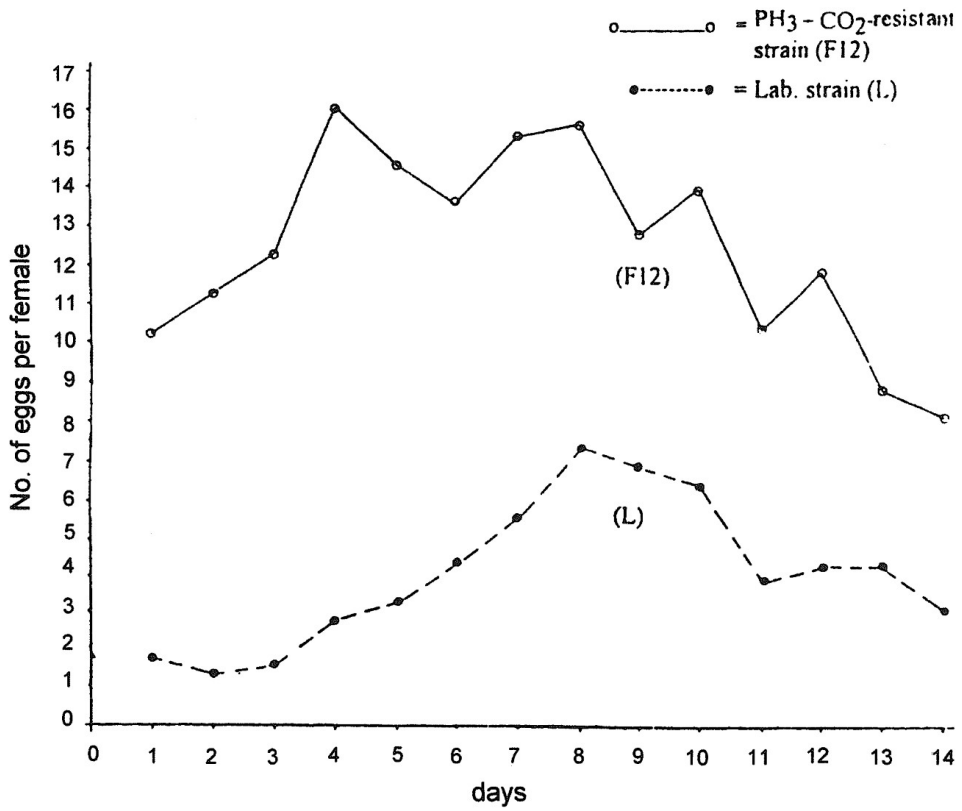

Fig. 3. Average number of eggs laid daily per female by a laboratory strain and a PH_3–CO_2-selected strain of *Tribolium castaneum* at the 12th generation.

average number of larval instars was significantly higher. No significant differences between the two strains in the other biological parameters were found.

These findings agree with those of Abdel-Salam and Nasr (1967), Spratt (1979), El-Sayed (1981) and El-Lakwah *et al.* (1991a). Abdel-Salam and Nasr (1967) observed an increase in egg laying by *Spodoptera littoralis* exposed in the laboratory to sublethal doses of insecticides. El-Sayed (1981) mentioned that resistance to fenitrothion was associated with a significant decrease in all tested biological aspects of *Callosobruchus maculatus* except the average total number of eggs laid per-female, which was significantly larger than that of the laboratory strain.

El-Lakwah *et al.* (1991a) found that the females of a PH_3-resistant strain of *T. castaneum* laid a significantly higher number of eggs during an observation period of 14 d than those of the parent stock. The hatchability of eggs for the PH_3-resistant strain declined and their incubation period was considerably prolonged. No other significant biological differences were found between the two strains.

TABLE 6
Average duration and total period of the larval instars for the laboratory strain (parent) and the PH_3–CO_2-resistant strain (F12) of *T. castaneum* at 30 ±1°C and 75 ± 5% r.h.

Larval instars	Average duration of larval instars		Probability
	Laboratory strain (L)	PH_3–CO_2-resistant strain (F12)	
First	2.60 ± 0.377[1]	1.00 ± 0.0	0.003**
Second	3.80 ± 0.375	2.40 ± 0.246	0.014*
Third	2.00 ± 0.0	1.80 ± 0.201	0.346
Fourth	2.00 ± 0.0	1.80 ± 0.201	0.346
Fifth	2.60 ± 0.246	2.00 ± 0.0	0.040*
Sixth	4.80 ± 0.580	2.40 ± 0.246	0.005**
Seventh	1.20 ± 0.969	2.80 ± 0.491	0.179
Eighth	0.0	2.20 ± 1.02	0.063
Average no. of larval instars	6.40 ± 0.2	7.60 ± 0.2	0.008**
Average total period for the larval instars (d)	19.0 ± 0.317	16.40 ± 0.509	0.002**

** = Difference significant at the 1% level; * = difference significant at the 5% level. [1]±SD.

TABLE 7
Mean weight of the adults and pupae of the various *T. castaneum* strains

Strain	Weight (mg)	
	Adults	Pupae
Laboratory strain	2.3	2.9
PH_3-resistant strain	2.5	2.9
CO_2-tolerant strain	2.3	2.5
PH_3–CO_2-resistant strain	2.0	2.3

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