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Abiotic mortality factors of the coffee berry borer (*Hypothenemus hampei*)

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Abstract

Mortality of the coffee berry borer was studied under controlled laboratory conditions in Tapachula, Mexico. For adult female borers subjected to a range of relative humidities (RH) without food at 25°C, the longest mean survival time (20 days) was obtained at 93.5% RH. Adult borer survival was also studied at a range of temperatures for a fixed relative humidity (93.5% RH); at 20°C mean survival time was 28 days. Fecundity and mortality of borer stages in berries was studied for a range of humidities at 25°C. Maximum fecundity was obtained at 90 and 93.5% RH. Immature stages were ejected from the berry at 84% RH and above, which is interpreted as a form of brood hygiene.

Introduction

The factors that affect mortality of tropical scolytids are poorly understood. Beaver (1977) emphasized the need for quantitative information on population regulation and drew attention to the importance of the moisture conditions of the host. Generally the most studied scolytids have been temperate species where temperature has been the most intensively studied variable (Wagner, 1984). In the tropics however, seasonal temperature fluctuations are often small and variations in humidity caused by seasonal rains become important (Young, 1982).

In the case *of Hypothenemus hampei* (Ferrari) (Coleoptera; Scolytidae), which must be one of the most intensively studied tropical scolytids, almost nothing is known of the factors which regulate populations. This is partly due to the difficulty of studying an insect which spends much of its life cycle inside the coffee berry. The female bores into the ripening endosperm and lays 20 to 50 eggs, normally staying there until the brood gets to the adult stage; the F1 generation then mate incestuously and either start to lay eggs in the same berry or exit to find another (Baker *et al.*, 1992).

In the present study we concentrate on abiotic factors which affect mortality and reproductive potential to provide a) data on optimum rearing conditions; b) some idea of how long a borer can survive after emergence without food.

Materials and methods

The apparatus. For adult survivorship studies (experiments A1 to A6), single adult female coffee berry borers recently emerged from berries, were placed in 5 mm diameter cells drilled in a 10 mm thick acrylic disc and covered by fine nylon or aluminium mesh. Each disc, consisting of 6 or 8 such cells, was suspended horizontally over a saturated salt solution (see below) inside a 30 mm diameter glass screw-top container. The borers were checked daily for survival.

For studies of mortality of immature stages inside the berry (experiments B1 to B4), 40 berries were placed in a rack with a nylon mesh bottom, which was located inside a plastic container with a saturated salt solution in such a way that the berries were suspended about 10 mm above the surface of the liquid. A tight-fitting lid allowed the required humidity to develop.

Preparation of infested berries. To obtain uniform age of immature stages, adult borers were sprinkled over ripening clusters of berries (firm green berries just turn-

ing to yellow, approximately 120–130 days after flowering) on uninfested trees. The day after infestation, newly infested berries were marked by small lengths of coloured cotton thread attached to the stalk. Berries were picked from the trees at times depending on the experiment (see below); the act of removing berries from the trees may have some effect on the subsequent development of the borer population inside it; previous work (Baker *et al.*, 1992), however, suggests that berries removed and placed under the tree provide good conditions for the borer's progeny and that ambient temperature and humidity are likely to be of more importance than any physiological effects.

Picked berries were placed in the racks (see above) and sampled every 4 to 10 days depending on the experiment, they were dissected and the contents recorded. Numbers of emerged stages floating on the surface of the liquid were also counted and removed. Except for temperature experiments and a field trial, all adult borers and berries were incubated at a 25°C (±0.5°C).

Humidity control. According to the American Society for Testing Materials (1951) cited in Martin (1962), the ratio of chamber volume to surface area of humidity-controlling solution should not exceed 10 in (when the dimensions are measured in inches). In the present study it was 1.2 in for the studies with berries containing broods. For studies on adult mortality, the ratio was about 1. The area of test material exposed should also not exceed the surface area of the salt solution; the surface area of an old coffee berry is about 2.5 cm^2, making about 100 cm^2 of test material per container with 204 cm^2 surface area of liquid. Saturated salt solutions were used to control RH, namely: 20%, CH_3COOK; 33%, $MgCl_2$; 55%, $Mg(NO_3)_2$, 78%, NaCl; 84%, KBr; 90%, $ZnSO_4$; 93.5%, KNO_3; 100%, H_2O (Unwin, 1980).

The experiments. Ten experiments were carried out (summarized in Table 1), six on adults with no berries (A1 to A6) and four on immature stages inside the berries (B1 to B4). Experiments A1 to A4 determined the effects of various humidity regimes on adult female borer survivorship held in cells outside the berry, details can be seen in Table 1. In experiment A2, a small piece of coffee endosperm (a cube with sides of about 3 mm) was added to each cell to provide food along with the borer. Because of the narrow range of humidities, three replicates were made of experiment A3.

In experiment A5 the mortality of groups of borers was investigated because preliminary observations had suggested that borers held in vials survived better in groups than individually. There were two treatments, 1) borer held individually; 2) borer held in groups of seven. Each treatment had two levels, a) 78% RH, b) 100% RH (N=48 for treatment 1), N=336 for treatment 2)). As members of the groups died, they were removed and not replaced, so that the groups became progressively smaller with time.

In experiment A6 the effect of temperature on survival was studied; 48 adult female borers were held individually in cells without food at 93.5% RH at each of the following temperatures: 15, 20, 25 and 30°C.

Four experiments (B1 to B4) were carried out to determine mortality of immature stages inside the berries. For the first test (B1) field-infested berries were picked from the host tree one week after infestation i.e. when a cluster of eggs would be present in most berries. Half of the berries were placed in cages on the ground under the tree (where the ground was moist due to frequent irrigation, mean temperature 26.2 °C) and half removed to the laboratory to be incubated at 25 °C and 78% RH. 20 berries from both laboratory and field were then dissected every 8 days for 80 days. To examine the effect of humidity on brood development in more detail (experiment B2), berries picked 7 days after infestation were allotted to humidities of 78, 84, 90, 93.5 and 100%, at 25 °C (80 berries/treatment). 8 berries were dissected from each treatment every 4 days.

To study mortality in older broods, berries were collected from a tree 40 days after infestation (experiment B3). RH's of 33, 55, 78 and 93.5% were the treatments, and 20 berries per treatment were dissected every 10 days for 80 days. For a final test (experiment B4), berries with mixed brood ages (0 to 40 days post-infestation) were studied under a similar regime to B3 and in this case the mean dry weight of the endosperm (pooled for each sample) was assessed for berries sacrificed for dissection.

Results

Effect of relative humidity on adult survival. In experiment A1, female borers died quickly at low humidities (Fig. 1a). As the data was highly heteroscedastic, the Kruskal-Wallis H-test was employed; the treatments were significantly different ($H=167.9$, $P<0.0001$). Multiple comparisons (Miller, 1966) separated the

Table 1. Synopsis of experimental regimes

Experiment	Stage	Environment	Treatment	Level of treatment	n[1]	Constant
A1	adult	without berry	%RH	20,33,55,78,93.5,100	32	25 ° C
A2	adult	without berry + endosperm	%RH	20,33,55,78,93.5,100	32	25 °
A3	adult	without berry	%RH	84,90,93.5,100	40	25 ° C
A4	adult	without berry	%RH	84,90,93.5,100	40	25 to 30 ° C
A5	adult	without berry	%RH & singles vs groups	78,100	48 336	25 ° C
A6	adult	without berry	temp °	15,20,25,30	48	93.5%RH
B1	all[2]	inside berries	field vs.		200	26.2 ° C (field)
	7d brood		lab conditions		200	78% RH 25 ° C (lab)
B2	all	inside berries	%RH	78,84,90,93.5,100	80	25 ° C
	7d brood					
B3	all	inside berries	%RH	33,55,78,93.5	160	25 ° C
	40d brood					
B4	all	inside berries	%RH	33,55,78,93.5	160	25 ° C
	mixed ages					

[1] n=number per treatment

[2] all=adult female and brood

treatments (at P=0.05) thus (mean days of survival); 20% 2.25a; 33% 2.59ab; 55% 5.47b; 76% 11.72c; 93.5% 19.78d; 100% 16.81cd. (RH treatments followed by the same letter are not significantly different).

The experiment was repeated (experiment A2) with a piece of coffee endosperm provided as food in each cell (Fig. 1b). The same test as above produced the following statistics; H=74.2, P<0.0001; 20% 5.56a; 33% 5.75a; 55% 10.63b; 76% 22.69bc; 93.5% 25.00c; 100% 25.38c. It had been expected that all borer would tunnel into the endosperm but this occurred only at the three highest RH's. The characteristic greening of the endosperm (produced by chlorogenic acid) occurred only at the two highest humidities where eggs were also laid. In one case (93.5%) a 1st instar larva was found.

A further experiment (A3) used humidities of 84, 90, 93.5 and 100%. The three repetitions of this experiment at 25 °C were analyzed by a mixed model ANOVA with trials as random effects. Treatments passed Cochran's C test for homoscedasticity (P=0.08); and results were very significantly different (mean survival times (days) followed by the same letter are not significantly different: 84% 7.74a; 90% 14.52b; 93.5% 15.11b; 100% 10.57c. F=58.3, df=3,474, P<0.0001).

An experiment at ambient temperatures (Experiment A4, 25 to 30 °C) again showed very significant differences (84% 3.05a; 90% 4.50b; 93.5% 9.38c; 100% 6.95d. F=31.7, df=3,194, P<0.0001)). In all the trials without food, it was the 93.5% RH that produced the longest mean survival times; mean survival at 25 °C (data taken from experiments A1 to A4 above) is plotted against saturation deficit in Fig. 2.

For the mortality test between individual and groups of borers (experiment A5), the results of survival times were analyzed by the Mantel-Haensel Chi-squared test (Lee, 1980); there was no difference between treatments at 100% RH (Chi-squared=0.132; df 1; P>0.1); however at 78% RH the groups of 7 borer survived significantly longer (Chi-squared=11.75; df 1; P<0.01) suggesting that groups of borers somehow resist desiccation. There were no signs of cannibalism.

Effect of temperature on adult survival. Mean survival times (experiment A6, Fig. 1c) were 15 °C, 25.31 days (SD=15.71); 20 °C, 27.96 days (SD=6.04); 25 °C, 19.42 days (SD=3.58); 30 °C, 12.88 days (SD=3.61). Treatments were significantly different (Kruskal-Wallis H=76.86; P<0.001). By multiple comparisons (Miller, 1966) all four treatments were significantly different from each other at P=0.05).

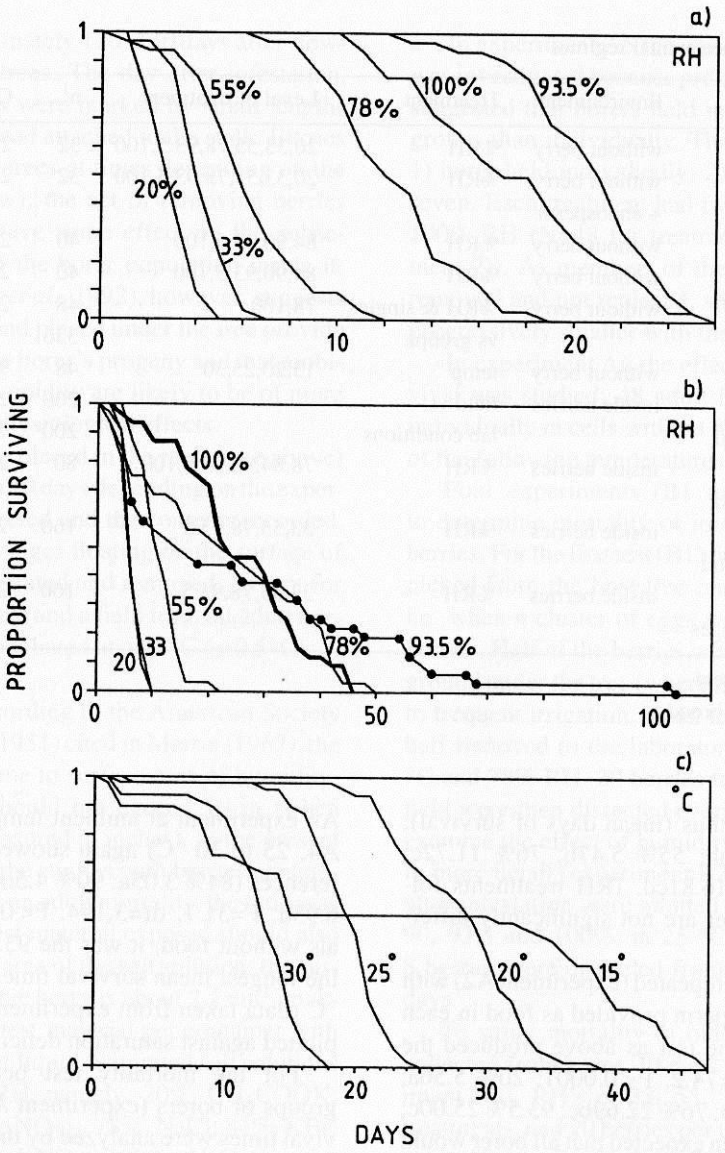

Fig. 1. a) Experiment A1; survival curves for adult female borer incubated with no endosperm at 25 °C with the indicated RH values. b) Experiment A2, same regime as a) but with a small piece of endosperm added to each cell. c) Experiment A6; survival curves for adult females incubated at 93.5% RH at the indicated temperatures.

There was the expected tendency for longer life at lower temperatures, except that at 15 °C, mortality was anomalous, showing initial rapid death followed by a period of very low deaths; this early death may have been due to shock on transferring the borers from a high ambient temperature (30 °C) to such a low temperature.

Mortality of immature stages. Given the difficulty of studying the various stages of the borer outside the berry, experiments were carried out on berries artificially infested by borer under field conditions. The development of the immature borers was studied by destructive sampling of the berries.

For experiment B1, where berries with young borer broods were divided into laboratory (78%RH) and field batches, there were sharply lower numbers of laboratory borer stages as compared to the field berries (Table 2). Kruskal-Wallis H-Tests for significance were made for each sample date for each of egg, larva and female

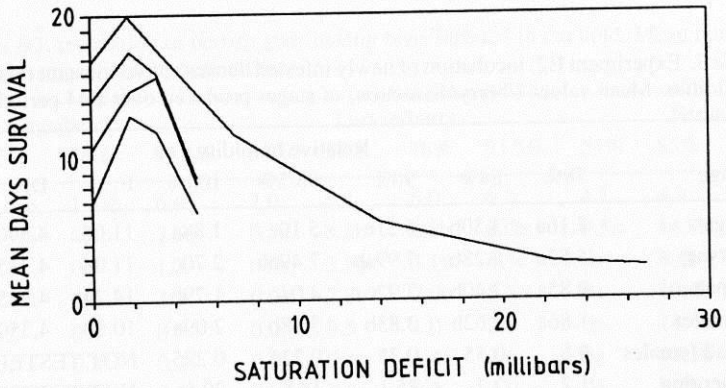

Fig. 2. Mean survival times vs. saturation deficits for 4 independent trials with female borers (data from experiments A1, A3, A4).

Table 2. Experiment B1; differences between borer stages in field berries and those maintained at 78%RH in the laboratory

	Eggs		Larvae		Females	
Day	Lab	Field	Lab	Field	Lab	Field
12	$\overline{8.8}$	$\overline{12.9}$	7.8	3.8	0.9	1.0
20	0.3	5.6	$\overline{8.3}$	$\overline{13.5}$	0.7	1.0
28	0	4.8	4.8	16.4	$\overline{0.4}$	$\overline{1.0}$
36	0.3	6.4	2.9	12.4	0.5	7.5
44	0.2	4.1	1.8	7.4	0.8	9.2
52	0.1	9.0	1.1	10.0	1.0	11.1
60	0.5	2.8	1.1	7.7	1.0	5.1
68	0.1	4.3	1.2	7.2	0.7	2.9
76	0	2.1	0.1	5.2	0.5	3.1
84	0	1.9	0	2.7	0.3	3.0

All field-laboratory stage comparisons below the dashed lines are significanly different (Kurskal-Wallis H tests, $P<0.001$).

stages; pupae and males were too few to be tested. A combined ANOVA was not possible because of severe non-normality and heteroscedasticity. Eggs in incubated berries were significantly less than in field berries from the first sample onwards; laboratory larvae were significantly less from the second dissection onwards; females were significantly lower from the third sample onwards ($P<0.001$ in all cases).

Where young broods were incubated at a range of laboratory-controlled humidities (experiment B2), the results (analyzed by ANOVA) showed that humidities of 90 and 93.5% produced the most progeny (Table 3); differences by the pupal stage were particularly clear. Differences between humidity treatments become very marked after about 20 days of incubation (Fig. 3a). Dead immature stages were not found inside the berries, but many larvae and a few pupae were found outside the berries, floating on the surface of the saturated salt solution; progressively higher numbers were found at higher humidities (Fig. 3b); no external immature stages were found at 78% RH.

In a similar experiment carried out on berries 40 days after infestation (B3), the highest numbers of eggs, larvae and females were found mostly in the 93.5% treatment, though in many cases the 55, 78 and 93.5% categories could not be separated statistically (Table 4).

Pupae counted in experiment B3 were so few that 20-berry samples were pooled. The successive pooled sample values were then analyzed for differences between treatments by the Kruskal-Wallis test and are presented in Table 5 together with pooled data for dead larvae and females found in the berries and emerged females (found mostly alive, floating on the surface of the saturated salt solution); all statistics pertaining to these can be seen in Table 5. The most notable feature of these results is that live pupae were about ten fold more abundant in the 93.5% treatment than any other. Because numbers of live larvae were not so very different between 55, 78 and 93.5% RH's (Table 4), we might conclude that there was a high mortality during the change to pupae or afterward. However, there were only 10 dead pupae found during the whole experiment and no significant differences between levels of dead larvae were found in the three higher humidities (Table 5). We attribute the discrepancy to cannibalism or nest

Table 3. Experiment B2; incubation of newly infested berries at five constant relative humidities. Mean values (/berry/dissection) of stages produced over a 44 period

Stage	Relative humidity 78%	84%	90%	93.5%	100%	F	DF
Eggs	2.16a	3.30b	6.21c	5.10c	1.88a	11.06	4,390
Larvae	5.53a	8.28b	7.99ab	7.49ab	2.70c	11.08	4,315
Pupae	0.85a	2.40b	3.92c	4.04c	1.79b	14.24	4,245
Females	1.86a	3.62b	3.83b	3.68b	2.09a	10.50	4,350
Dead females	0.6	0.55	0.35	0.225	0.225	NOT TESTED[1]	
Emerging females [2]	1.2	2.2	25.1	14.8	29.6	NOT TESTED[1]	

Means within a row followed by the same letter are not significantly different (multiple comparisons by least significant difference method (P=0.05)).
(Means are detransformed from log(x+1))

[1] Not tested because numbers were too low to satisfy parametric statistics

[2] Live females emerged from the berries, values pooled from successive counts and expressed as mean number per berry.

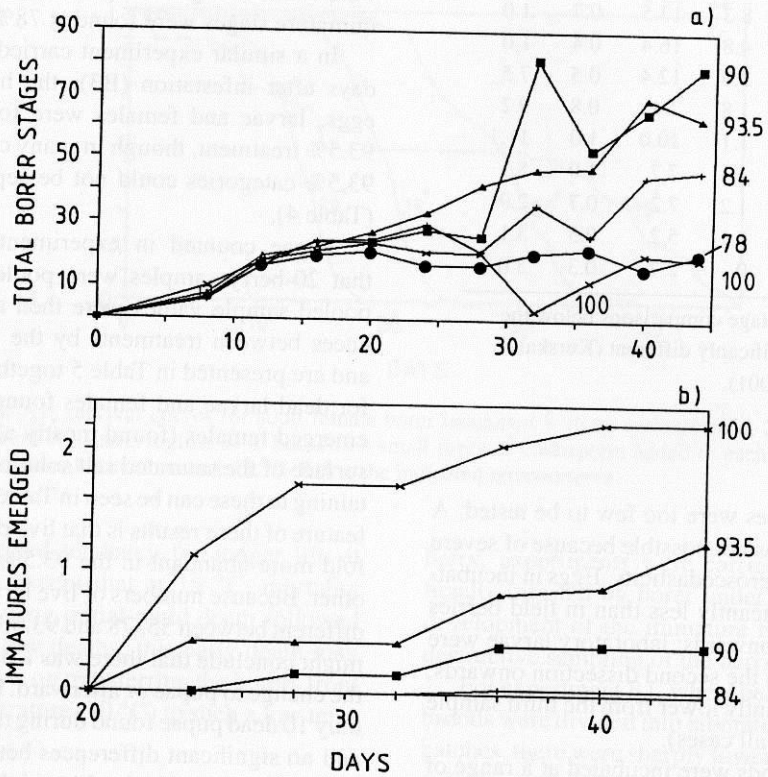

Fig. 3. Experiment B2; a) Total borers found in berries incubated at 25°C and RH's of: 78% (circles); 84% (+'s); 90% (squares); 93.5% (triangles); 100% (x's). b) Cumulative ejected immature stages.

Table 4. Experiment B3; incubation of berries after having been infested in the field. Mean number of borers (egg, larval and adult female stages) at four humidities

	Eggs/berry				Larvae/berry				Females/berry			
Day[1]	33%	55%	78%	93.5%	33%	55%	78%	93.5%	33%	55%	78%	93.5%
10	2.4a	3.4ab	1.3ab	6.6b	2.0	5.1	6.0	6.3	4.1	4.4	5.7	4.5
20	0.0a	2.3ab	4.4b	3.5b	0.5a	8.5b	6.8b	8.9b	0.5a	14.9b	11.0b	7.9b
30	0.0	1.1	0.4	1.3	0.0a	3.3bc	3.1b	8.3c	0a	8.4b	6.0b	16.8b
40	0.0	0.4	0.0	1.8	0.0a	2.3b	2.0b	5.8b	0a	10.4b	12.3bc	29.6c
50	0.0	0.2	0.0	0.5	0.0	0.5	1.0	2.1	0a	14.4b	11.6b	30.0b
60	0.0	0.1	0.3	0.2	0.0	0.1	1.4	2.6	0a	13.5b	8.8b	12.9b
70	0.0	0.0	0.0	0.2	0.0	0.0	0.2	1.0	0a	9.4b	10.0b	5.6ab
80	0.0	0.0	0.0	0.6	0.0	0.0	0.2	0.9	0a	12.2b	10.2bc	4.4ac

For each period for each stage, Kruskal-Wallis H-tests were performed; all rows with letters were significantly different at $P<0.001$. Means within a row within a stage column followed by the same letter are not significantly different, (multiple comparisons (P=0.05) using Millers's test (1966))

[1] Days after start of experiment

cleaning by surviving adults of the brood, especially in the 55 and 78% RH's.

A final experiment (B4) repeated the treatments of the second experiment but with berries of mixed ages and with dry weight measurements of endosperm every 10 days (Fig. 4a). The 93.5% RH maintained the percentage dry weight at markedly lower values for the first 20 to 30 days of the experiment. Eggs, larvae and daily emergence were all higher at 93.5% RH (Fig. 4b, c, d).

Table 5. Experiment B3; incubation of 40-day-old field infested berries. Mean values of various stages/berry found during 80-day experiment. 20-berry samples pooled for analysis because of low numbers

	Relative humidity			
Stage	33%	55%	78%	93.5%
Dead larvae	1.03a	0.04b	0.09b	0.02b
Dead females	2.02a	0.44b	0.25b	0.16b
Live pupae	0.07a	0.10a	0.13a	1.74b
Females emerged[1]	2.1a	3.6a	2.5a	32.6b

Multiple comparisons by Miller's method. Means within a row followed by the same letter are not significantly different (P=0.05).

[1] found outside the berry floating on the saturated salt solution.

Discussion

The work presented here is the first detailed study on the mortality of a tropical scolytid. The results show that the adult berry borer is very sensitive to relative humidity; raising the humidity from 84 to 93.5% can double the mean survival time. Development of the borer is also highly dependent on ambient humidity; numbers of immature stages were 2 to 5 times higher when incubated at 93.5% than 78% RH. Despite rapid death at low humidities (20 to 55% RH) females did not bore into endosperm even thought this might have retarded desiccation.

Mortality factors inside the berry are more difficult to study because berries can only be destructively sampled. Experiment B3 with 40-day-old infestations suggests that by this stage the borer can withstand desiccation to 55% RH though at the cost of lower production of offspring. The discovery of immature stages outside the berry indicates that there is some form of control being carried out inside the berry; the first indication of this was 24 days after infestation, which means that the founder female was responsible. The presence of pupae outside the berry is significant because they must have been pushed rather than have jumped. We conclude that the phenomenon is a form

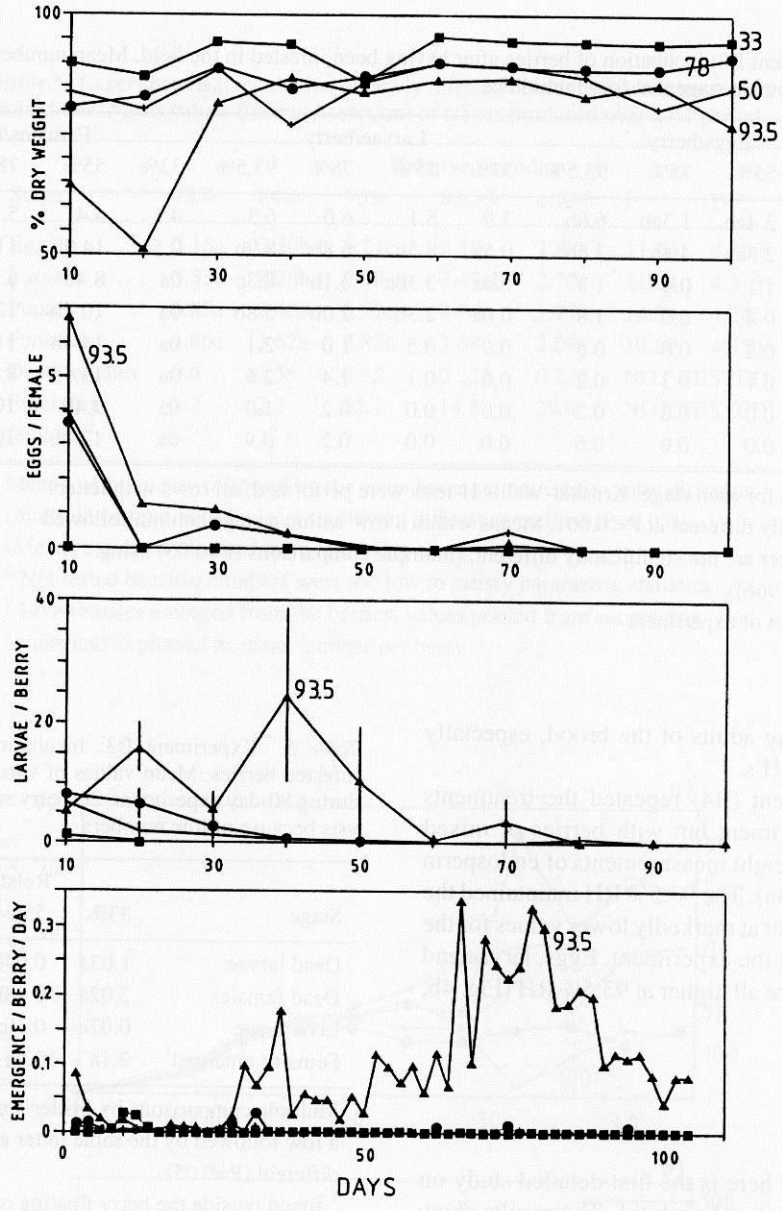

Fig. 4. Experiment B4; data from dissections of berries maintained at RH's of 33% (squares), 50% (crosses), 78% (circles), 93.5% (triangles). a) Mean dry weights of endosperm; b) number of eggs counted per female present per berry; c) mean live larvae per berry; d) number of borer emerging per berry per day.

of brood hygiene; because more ejected stages were found at high humidities, they may have been infected by humidity-induced pathogenic infections. In the low humidity treatments of experiment B3, live larvae or pupae were scarce and more dead larvae should have been present than were recorded; they may have been ejected from the berry though none were noted. Another possibility is cannibalism (thought to occur in scolytids, Entwhistle, (1972); Beaver (1974)); no remains such as mandibles were detected however, though due to their small size they could have been overlooked.

Thus the borer has an optimum humidity range for development and survival somewhere between 90 and 95% RH at 25 °C. This humidity seems high for a seed borer, but is understandable in the context of its natural habitat in the understorey of African equatorial forest; fallen berries would be well protected

from drought in a deep soil litter; dead stages would be quickly removed in order to limit infections and avoid the attentions of ants etc. attracted by putrefying cadavers. Such maternal functions of the founder are consistent with the life style of an insect exploiting a rare (in the natural state) and limited resource with relatively few offspring bearing 100% of the maternal genes. The susceptibility to even quite high humidities suggests that post emergence mortality may be marked in some circumstances. Richter (1983) found dry season RH values of <50% between 1200 and 1800 h, 1.5 m above ground level in a coffee plantation in Chiapas. Mexico during the dry season; wet season values were 75 to 80% RH. If the borer cannot quickly find a berry, does it have some alternative temporary refuge where it can find protection and food? Perhaps field cage studies could answer this question.

The practical consequences of this study are that great care should be taken to control humidity when culturing the borer for parasite rearing etc.; the precise RH would depend on levels of pathogenic fungi etc. that might be present, though about 90% RH would be a good initial choice; dry weight determinations and ejected immature stages would give early warning of problems arising. Any behavioural or LD_{50} tests should have standardized RH's in order to avoid anomalies. In the field, irrigation schemes and cultural control measures should take account of the susceptibility of the borer to desiccation; we believe that the scarcity of borers found in low altitude plantations in Chiapas, Mexico may be due in part to the long and often intense dry season (Baker *et al.*, 1989). Finally, it should be admitted that the disappearance of dead stages from the berry presents a problem for the field population ecologist.

Acknowledgements

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