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The Effect of Larval Density, Photoperiod and Food Change on the Development of *Gnatocerus cornutus* (F.) (Coleoptera: Tenebrionidae)

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Abstract—Larval density, photoperiod and periodic changing of the food supply were investigated as possible causal factors in the retardation of development of the mature larvae of *Gnatocerus cornutus* (F.). Four larval densities, 1, 2, 4 and 8 larvae per gram of food, were exposed to photoperiods of 0, 12, 15 and 24 h per day. The effect of changing the food at 3-weekly intervals was investigated with 2 and 4 larvae per gram of food. The mean times for development from larval to adult stages, and survival rates revealed that increased larval density significantly increased development time, mortality and the incidence of cannibalism. Changes in photoperiod and food had little effect, showing that the effect of larval density is independent of lighting conditions and changing of the food medium.

Key words—*Gnatocerus cornutus*, development, cannibalism, photoperiod, crowding.

INTRODUCTION

The broad-horned flour beetle *Gnatocerus cornutus* (F.) is a stored product insect with a cosmopolitan distribution. It is of tropical origin and, as a result, its incidence as a pest of stored products may be related to its susceptibility to cold conditions. Its lower limit for development is 10°C (Hill, 1978). In warmer parts of the world it is found on commodities such as cassava root and cotton, but in the more temperate climate of the U.K. it is associated with flour and provender mills, where it is protected from the cold. It is found predominantly in mill machinery, where it feeds on milled products and causes fouling with its excreta and quinones (Kendall, 1974).

The presence of this insect in flour and provender mills has facilitated its spread to bakeries (Turner, 1979) and animal rearing premises (O'Connor, 1987). Turner (1979) found that *G. cornutus* was present in more than half of the bakeries surveyed and occurred in all sections except the bread cooling areas. In 1973 it was most commonly found in the "dry dough" areas (56% of areas sampled) but by 1979 was found predominantly in the "wet dough" areas (39% in 1973, 66% in 1979). O'Connor (1987) reported problems in piggeries in Northern Ireland, where *G. cornutus* was found causing extensive damage by tunnelling inside polystyrene insulation blocks. It appears that the spread from the mills to other premises is via sacks used to transport and store flour and feedstuffs (Hardy-Smith, 1971).

The fact that this beetle is found in food production sites, combined with its potential to spread between premises, has made it a candidate for inclusion in tests on control of storage pests. During fumigation tests at the Central Science Laboratory (CSL), problems were encountered in rearing

this insect in large numbers beyond the larval stage at 25°C, 60% r.h. Previous studies have quoted development times, from egg to adult, for this beetle of 57 days, at temperatures between 24°C and 30°C (Pimentel, 1949) and 47 days at 30°C (Tsuda and Yoshida, 1984). In tests at CSL the cultures yielded an abundance of larvae and few adults after more than 90 days. The studies by Pimentel (1949) and Tsuda and Yoshida (1984) were based on the rearing of single larvae and their development times did not take into account any effect of larval interaction. The problems encountered in rearing *G. cornutus* to beyond the last larval instar indicate that larval density could be a major factor controlling the rate of development.

Photoperiod is also often cited as a factor which can affect development in insects. For example, the development of the yellow mealworm beetle *Tenebrio molitor* L. has been shown to be delayed as photoperiod increases (Tyshchenko and Sheyk Ba, 1986). The effects of larval density, photoperiod and periodic changes in food on the development of mature larvae of *G. cornutus* were investigated in an attempt to successfully rear this insect.

METHODS

Experimental technique

All rearing and experiments were conducted at 25°C, 60% r.h. Insects were reared in 3-l glass jars, on a diet consisting of 190 g rolled oats, 100 g fishmeal, 95 g wholewheat flour and 15 g de-bittered, dried brewer's yeast. Each jar was seeded with 80 adults. After 3 months there was an abundance of final instar larvae (approx. 8 mm long) which were used for the experiments.

Glass tubes measuring 5 cm in depth and 1.2 cm in diameter were used, with 1 g of food added to each. Different larval densities were obtained by adding 1, 2, 4, or 8 last instar larvae to each tube. A small soft-haired brush, cut down to 3 or 4 bristles, was used to transfer the larvae in order to minimize mechanical damage.

After addition of the larvae, the tubes were stoppered with muslin squares, secured with short lengths of polyethylene tubing and placed in the appropriate lighting regime. The tests ran over a period of 2 years and included photoperiods of 12, 15 and 24 h (all at 800 lux intensity) and continuous darkness. Following the initial results, tests also included an investigation into the effect of food shortage and whether the effect of larval density was associated with conditioning of the food. In batches of tubes containing 2 and 4 larvae the food in the tubes was therefore replaced with a fresh supply every third week.

In all experiments the contents of the tubes were inspected weekly for the presence of pupae and adults, the latter being removed to avoid the possibility of mating and egg-laying. The observations continued until all larvae had died or emerged as adults, with the exception of the test having 8 larvae per tube, where the counts were stopped after 90 days with some larvae remaining. When a new adult was detected the time of emergence was estimated as the mid-point between the previous date of observation and the date of its detection.

Table 1. Total number of larvae tested in each of 4 tests, over a 2 year period, in each larval density and photoperiod

Test no.	Light regime	Larval density per gram of food					
		1	2	2[a]	4	4[a]	8
1	Dark	30	—	—	60	—	—
	12 h	30	—	—	60	—	—
	15 h	30	—	—	56	—	—
2	Dark	30	60	—	—	—	—
	12 h	30	60	—	—	—	—
	15 h	30	60	—	—	—	—
3	Dark	15	60	60	116	112	240
	12 h	15	60	60	104	120	232
	15 h	15	60	60	120	120	240
4	Dark	15	60	—	116	—	—
	12 h	15	60	—	96	—	—
	15 h	15	60	—	116	—	—
	24 h	15	58	—	116	—	—

[a]Food changed every 3 weeks.

Table 2. Development and survival of larvae in relation to larval density, food change and photoperiod

Photoperiod (hours light)	No. larvae per tube	Total no. of larvae tested	% Larvae and pupae cannibalised	% Mortality not due to cannibalism	Total adults emerged (%)	Mean time (SE) to adult emergence (days)	Last adult emerged or larva/pupa died (days)
0	1	90	0	0	99	15 (1.44)	23
	2	180	1	17	82	23 (3.06)	54
	2[a]	60	2	7	91	20 (0.72)	38
	4	292	19	19	62	40 (9.85)	103
	4[a]	112	20	16	64	35 (1.55)	84
	8	240	29	42	29[b]	>54 (2.93)	>90
12	1	90	0	2	98	17 (0.63)	31
	2	180	0	11	89	25 (2.85)	65
	2[a]	60	5	8	87	25 (1.13)	46
	4	260	17	26	57	42 (9.28)	103
	4[a]	120	7	38	55	26 (0.94)	54
	8	232	34	32	34[b]	>81 (1.75)	>90
15	1	90	0	4	96	17 (0.65)	23
	2	90	<1	12	88	23 (1.67)	54
	2[a]	60	0	7	93	20 (0.53)	38
	4	292	9	37	54	30 (4.18)	103
	4[a]	120	12	33	55	29 (1.07)	61
	8	240	56	15	29[b]	>65 (3.47)	>90
24	1	15	0	0	100	18 (0.27)	22
	2	58	0	21	79	18 (0.39)	33
	2[a]	—	—	—	—	—	—
	4	116	3	59	38	39 (1.89)	113
	4[a]	—	—	—	—	—	—
	8	—	—	—	—	—	—

[a]Tubes which had food changes every 3 weeks.
[b]Includes some larvae still viable at the end of the test.

The actual numbers of larvae tested in each of 4 successive experiments, at the various photoperiods and larval densities are given in Table 1.

Treatment of data

Results from the 4 chronologically separated tests were subjected to an analysis of variance to check whether any differences in mean development times, or mean percentage survival, were significant for similar larval densities and photoperiods. A logarithmic transformation of mean development time and an arcsine transformation of percentage emergence were carried out, to satisfy the constraints of the ANOVA test, which assumes a normal distribution. Generally, each test thereafter represented one replicate for each set of conditions. For the single larvae however, because of the small batch size, tests 3 and 4 were combined into one replicate. Additionally, for the test including 8-larvae each batch of thirty tubes was split into 3 groups of 10, giving 3 replicates for each photoperiod (Table 1). The results were tested, using further analyses of variance, for effect of larval density, photoperiod and food change on the rate of development and on emergence. When combining the results, to give overall mean development times, the small differences in batch size in each experiment were taken into account. For each set of conditions the following operation was carried out:

$$\frac{(m_1 x b_1) + (m_2 x b_2) + (m_3 x b_3) + (m_4 x b_4)}{(b_1 + b_2 + b_3 + b_4)}$$

where m_n = mean development time in test "n" and b_n = batch size in test "n".

No further allowances were made for differing batch sizes between treatments in the analysis of variance.

RESULTS

Significant differences between the 4 tests were not observed for either development time ($P = 0.377$) or survival ($P = 0.177$) in similar treatments.

The results in Tables 2 and 3 show a reduction in the proportion of adults emerging as the larval density increases. An increase in larval density caused an increase in mean development time and in the number of days taken for the last adult to emerge or last larva to die (Table 2 and 3). The photoperiods tested had no effect on the development period of the larvae and neither did a change

of food at intervals of 3 weeks (Table 3). The reduction in development time observed for the 12-h photoperiod with 4 larvae per tube, from 40 days with no food change to 26 days with the food change, was however statistically significant ($t = 7.54$, $P < 0.001$).

The weekly inspection of the tubes revealed that many larvae and pupae became completely or partially eaten in the tubes containing 2, 4 and 8 larvae and this cannibalism increased with larval density, often occurring before any adults had emerged (Table 2). Any individual missing or found partly eaten was classed as cannibalised, although it was impossible to ascertain whether individuals had been preyed upon while alive or were already dead when cannibalised. Other dead individuals were included in the figures for mortality.

Changing the food every 3 weeks did not affect results with respect to mortality ($t = 0.72$, $P = 0.51$) or incidence of cannibalism ($t = 0$, $P = 1.00$). In the tubes with 4 larvae and a 12-h photoperiod, however, there was a significant decrease in cannibalism from 17% to 7% ($t = 5.07$, $P < 0.001$) associated with food change.

DISCUSSION

It is clear from the results in Tables 2 and 3 that the development time, from mature larvae to adult, was extended as the larval density increased, as shown by the mean development time and the time taken for the last larva to emerge or die. In the tubes with a 12-h photoperiod the mean development time rose from 17 days for the single larvae to more than 80 days at a density of 8 larvae per gram of food. Results in all the other photoperiods showed similar trends.

The relationship between density and rate of development could be due to tactile stimulation between larvae, as is the case with some other Tenebrionids (Tschinkel and Willson, 1971), or possible hormonal conditioning of the food medium, as in *Tribolium confusum* (J. du V.) (Park, 1938). The present result did not show that changing the food directly affected development time, and so hormonal conditioning of the food medium is unlikely to be the explanation for the delay in *G. cornutus*.

Tsuda and Yoshida (1985) suggested that delays in development might be due to competition for pupation sites on the sides and bottom of the glass tubes. During culturing however, and during this study, pupae were often found loose in the food indicating that distinct sites are not critical for pupation to occur.

The missing and partially eaten immature stages noted during the experiment confirm that this beetle is cannibalistic. The only factor that has been investigated in relation to cannibalism in the past is water supply, which was found to have no effect (Welch, 1964). It appears that, in this study, pupae and larvae were being eaten by larvae and that the incidence of this increased with density. This contrasts with findings by Daly and Ryan (1983) who found that, in *T. confusum*, predation of pupae was due mainly to adults and the small proportion due to larvae was inversely density-dependent. One reason for the current results may be due to the depletion of some dietary component where several larvae are reared together. However, the results show that changing the food every 3 weeks had little effect on the incidence of cannibalism, except in the tubes with 4 larvae and a 12 h photoperiod where a significant reduction in cannibalism occurred when the food was

Table 3. *P*-Values generated by the analyses of variance

	Source of variance	Development time[a]	Survival
Data from all the results except the 24-h[b] data:	Larval density	<0.01	<0.01
	Photoperiod	0.561	0.165
Data from test 3 which examined the effect of food change with 2 and and 4 larvae per tube:	Larval density	<0.01	<0.01
	Photoperiod	0.299	0.613
	Food change	0.526	0.821

[a]Due to the poor survival rate in the tubes containing eight larvae there was insufficient data to include in the analysis for development time.

[b]Only one test included a 24-h photoperiod and was omitted from the analysis due to the lack of replication.

changed ($t = 5.07$, $P < 0.001$). The reason for this one exception is not apparent from the current data.

In summary, an increase in larval density slowed the rate of development and increased mortality and cannibalism in *G. cornutus*, while changes of photoperiod or food had no consistent effect. The results suggest that the delay in development beyond the last instar is a response to repeated tactile stimulation between individuals.

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