Recognition and duration of the larval instars of banana weevil, *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae), in Uganda

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Analysis of head capsule widths of banana weevil larvae was carried out to determine the number and recognition of instars. The analysis involved model fitting to frequency distributions of head capsule measurements of laboratory-reared and field-collected larvae. The laboratory population revealed developmental polymorphism, with larvae passing through 5–7 instars. The model fitted Gaussian curves with five peaks for laboratory samples and six peaks for field samples. Mean head capsule widths for the first four instars showed close agreement among both laboratory and field-collected populations. Variability appeared greater among field populations, resulting in a higher probability of misidentification. The method of analysis was not sufficiently sensitive to separate later instars. Plotting mean head-capsule widths against instar number showed a geometric curve to be the best fit, but with only approximate conformity to Dyar's rule. The duration of banana weevil immature stages was determined under ambient conditions in three experiments. Most eggs hatched within 5.5–8.0 days. Using a developmental threshold of 12 °C, thermal requirements (92.8–95.9 degree-days) appeared similar to those established for a West African population. Larvae passed through 5–8 instars, with 74 % pupating after six instars. Larvae completed development in 20–41 (most less than 30) days and spent 3–5 days in each instar. The pre-pupal period averaged 4.6 days, while the pupal stage averaged 7.0 days. Overall, the egg to adult period lasted 6–8 weeks. Rearing methods influenced the number of instars and the length of the larval period. Applications of these data for life-table studies are discussed.

Key words: banana weevil, *Cosmopolites sordidus*, developmental polymorphism, Dyar's rule, growth-ratio, head capsule, instar duration, instar recognition.

INTRODUCTION

Banana weevil, Cosmopolites sordidus Germar (Coleoptera: Curculionidae), is an important pest of bananas and plantains in Africa (Persley & de Langhe 1987; INIBAP 1988; Gold *et al.* 1993; *Rukazambuga 1996*). In East Africa, weevil outbreaks have caused widespread plantation failure (Sengooba 1986; Sebasigari & Stover 1988), while sustained attack over several crop-cycles may cause yield losses between 30 and 50 % (Rukazambuga *et al.* 1997) and reduce plantation life (Gold *et al.* 1998).

The banana weevil manifests a 'k' selected life cycle (Pianka 1970) with long a lifespan and low fecundity. Adults are freeliving (not confined to the banana plant) and may live up to four years (N.D. Rukazambuga & C.S. Gold, unpubl.). The female weevil inserts a single egg into a cavity made with its rostrum in the corm or pseudostem (Whalley 1957; Koppenhofer 1993; Abera *et al.* 1998). The larvae tunnel in the corm, killing young plants, delaying flowering, reducing bunch weight and decreasing vigour of followers (*i.e.* ratoon crops) (Rukazambuga 1996).

Egg production is estimated at 1–4 eggs per female per week in the laboratory (Arleu & Neto 1984; Koppenhofer 1993; M. Griesbach & C.S. Gold, unpubl) and 0.5–1.2 eggs per female per week under field conditions (Abera *et al.* 1998). Mortality in the egg and first instar stages appears to be high. Abera *et al.* (1997) found 6–12 times as many eggs as mid- to late-instar larvae during dissections of banana mats. With low rates of oviposition and larval success, population buildup is slow, with most weevil infestations commencing in ratoon crops (Mitchell 1980; Lescott 1988; Rukazambuga 1996).

A thorough understanding of various facets of the weevil's biology, ecology and behaviour is essential for the development of appropriate control measures. The precise number, identification

and duration of larval instars of a pest species are important aspects of life-history studies and may be of practical significance in pest management (Schmidt *et al.* 1977; Daly 1985).

Technical information on larval instars is essential in development of mortality-survivorship studies based on life tables and on population modelling, which each have a bearing on the development of pest management strategies. Conventional life-table work entails proper identification of instars so that life stages affected by biotic and abiotic components of the environment can be determined. Additionally, knowledge of instars may be used in monitoring seasonal development of pest populations such that interventions (*e.g.* biopesticide or insecticide applications) can be optimally timed.

The banana weevil has been variously reported to have five (Cendana 1922; Beccari 1967), six (Koppenhofer *et al.* 1994), seven (Viswanath 1978), 5–7 (Schmitt 1993), and 5–8 (Mesquita *et al.* 1984; Mesquita & Caldas 1986) instars. The variable number of larval instars suggests that banana weevils may display developmental polymorphism, *i.e.* the occurrence of supernumerary instars other than those that are normal for a particular species (Schmidt & Lauer 1977).

Head capsule measurements have been used to determine the number of instars for a wide range of insects (Harman 1970; Surgeoner & Walliner 1975; Beaver & Sanderson 1989; Jobin *et al.* 1992; McClellan & Logan 1994). The most common method is the examination of simple frequency distributions (or modifications thereof) to aid in detecting peaks in a multimodal distribution; each peak representing an instar. This approach assumes that insect growth is discontinuous with major increments in size being limited to the periodic moults. Once the new cuticle sclerotizes, body size is assumed to remain constant during any particular instar. In principle, the technique should present little or no difficulty in instar separation, if there is minimal overlap in instar measurements.

Growth ratios from one larval instar to the next, are often constant as proposed by Dyar's rule (Daly 1985). Dyar's rule, developed in 1890, proposed a geometric progression between mean head-capsule widths in successive instars, with a 28 % constant increment in the size of sclerotized parts of all insects at each moult (Enders 1976). The rule has been revised, such that each species may have a typical, although distinct constant increment in size at each moult (Wigglesworth 1950; Enders 1976). For example, Dyar's constants have been determined as 62–69 % for various caterpillars (Drooz 1965; Dupree 1965); 18.6 % for *Blatella* sp. (Murray 1967); 31 % for *Nemobius* sp. (Nielsson & Bass 1967); 28 % for *Ips* sp. (Balogun 1970) and 31 % for eight species of Coccinellidae (van Emden 1949).

Separation of banana weevil instars would be insignificant in the construction of life tables without additional information on duration of the stages. For banana weevil eggs, Traore *et al.* (1993) found a developmental threshold of 12 °C and a thermal requirement of 89 degree-days for banana weevils in Benin. In India, Viswanath (1978) followed larval cohorts (sample sizes not reported) under ambient conditions at six times during the year. The larvae passed through seven instars with estimated mean durations of 4.1, 5.6, 5.8, 5.8, 5.9, 5.9, and 6.8 days, respectively, with a 2.7-day prepupal period and 7.2-day pupal period. Although the total larval period varied by season (ranging from 36.6–44.4 days), the relative time spent in each instar was fairly constant (9.1–10.9 % in instar 1; 13.4–15.8 % in instars 2–6; and 16.4–17.8 % in instar 7).

Schmitt (1993) and Traore *et al.* (1993) reviewed work on the developmental rates of banana weevil. Most of these studies were conducted at ambient temperatures and revealed a wide variability in stage duration: 4–36 days for eggs, 12–165 days for larvae, 1–4 days for prepupae, 4–30 days for pupae and 24–220 days from egg to adult. While temperature is the most critical factor in determining development rates, relative humidity, cultivar, age of plant, food quality and population density may also exert an influence (Mesquita *et al.* 1984; Schmitt 1993).

The objectives of this study were first to determine the number of instars and to classify instars of banana weevil under laboratory and field conditions, using head capsule widths. Conformity of growth ratios from one instar to another with Dyar's rule was also examined. Second, to determine the relative stage duration for banana weevil immatures under ambient temperatures in the laboratory.

MATERIAL AND METHODS

Rearing of banana weevil was conducted under laboratory conditions at the Kawanda Agricul-

tural Research Institute. Kawanda (00.25N, 32.32E, 1195 m, 12-h daylength throughout the year) is 13 km north of Kampala, Uganda. The site has two rainy seasons (March–May and September–November) with average precipitation of 1180 mm per year. Average daily temperatures are 16 °C minimum and 29 °C maximum.

In Uganda, frequent power cuts preclude the use of controlled temperature regimes. All experiments were therefore carried out under ambient conditions. Temperatures were recorded with a Zeal maximum/minimum temperature gauge placed in the laboratory.

Instar recognition: laboratory studies

Larval rearing and head-capsule measurements (Trial 1)

Adult banana weevils were collected from the field in Bombo, Luwero District, Uganda, using pseudostem traps (Mitchell 1978). The weevils were brought to the laboratory where they were maintained on sections of corm in ventilated 1 l plastic containers for three weeks. The weevils were then exposed for 24 hours to oviposition substrates consisting of fresh suckers (*i.e.* corms and 10 cm of pseudostem) (Atwalira cultivar) placed in ventilated 10 l plastic basins under ambient temperatures (22–27 °C) in the laboratory. Suckers weighed 1–2 kg.

Oviposition occurred below the plant surface in both the corm and pseudostem. The suckers were gently pared with a knife to expose the eggs. A total of 1449 eggs, 0–24-hours old, were extracted using a moistened fine camel's hair brush, transferred onto moistened filter paper in covered Pyrex Petri dishes, and incubated at room temperature. The dishes were monitored daily for hatching. A subsample of 265 newly hatched larvae was reared for head capsule measurement and development rate studies.

Newly hatched larvae were coded by date of hatching and number. The head capsule of each larva was measured at the widest point (dorsal view) at 40 times magnification with a binocular dissecting microscope fitted with a calibrated ocular micrometer. Larvae were then placed individually in rearing chambers consisting of two corm slices (4 × 4 cm and 0.2 cm thick) with a notch (simulating a gallery) in the lower slice to avoid possible injury to the larva by pressure from the upper slice. The rearing chambers were placed singly in Petri dishes. Head capsule widths were measured daily until the early prepupal stage.

Larvae were retrieved from the corms for daily head-capsule measurements by first examining the corm sections for evidence of tunnelling (*e.g.* presence of holes or frass) and then carefully cutting open the tunnels to expose the larvae. The larvae were removed, measured and placed into new rearing chambers. The size and thickness of the rearing chambers varied according to larval size, with larger larvae requiring sections of up to 0.7 cm.

Numbers of instars and classification

Instar limits were determined by examining daily incremental patterns of the head-capsule widths for each individual. The precise measurement of head capsules of live larvae was, however, reduced by larval movement during measurements. Larvae with head-capsule width varying by three micrometer units (0.0717 mm) on consecutive days were therefore considered to be in the same instar, and mean values were determined for each instar for each larva. Increments greater than three micrometer units were considered to represent the next instar. Moults, where possible, were confirmed by presence of exuviae or pale appearance of the head capsule. The technique facilitated determination of both the number of instars and the stage duration (days) under ambient conditions. Larvae were ultimately classified into categories based on instar number (*i.e.* 5-, 6- and 7-instar groups).

Mean head-capsule width and growth ratio per instar

The mean head-capsule width and standard error were determined for each instar group. Individuals that survived to the fifth instar, but that died in the sixth instar could not be categorized into instar groups, and were not considered in the calculations. Growth ratios, were computed as mean head-capsule width of one instar divided by the mean head-capsule width of the previous instar, and then examined for conformity with Dyar's rule. Ideally the growth ratio data of individuals that successfully pupated and emerged as fit adults should be considered to be more realistic. In this study, however, all individuals that reached the prepupal stage were considered to be representative of their respective polymorphic forms. This was because banana weevils are difficult to rear and only a relatively small number of larvae reached the adult stage.

Head-capsule data analysis

A total of 3871 head-capsule measurements were taken from 265 larvae studied in the laboratory. This constituted the head-capsule width data for which a frequency distribution was compiled for analysis. The method of analysis closely followed that of McClellan & Logan (1994), and involved model fitting by maximum likelihood using Genstat 5 Release 3.2, to detect peaks in a multimodal distribution, conforming to Gaussian curves. The analysis was carried out on the original scale of micrometer divisions rather than on the millimetre scale. Each micrometer division is equivalent to 0.0239 mm. On this scale, head capsule widths, w, are integers between 18 and 120. The model fitted was a mixture of normal distributions with five or six components. With five components the density function is:

$$f(w) = \sum_{i} p\Phi\left(\frac{w - m_i}{s_i}\right),$$

where p_i is the proportion of the sample in the i'th component and m_i and s_i are respectively mean and standard deviation of that component. Φ is the standard normal density function. During model fitting, an observation of w was assumed to come from the range $(w - 0.5, w + 0.5)$. Inspection of the data showed an excess of observations with final digit 0 (*i.e.* $w = 10k$ for integer k) and only a few with final digit 9 ($w = 10k - 1$). Data with final digit 9, 0 and 1 were therefore combined, each observation assumed to come from the range $(10k - 1.5, 10k + 1.5)$.

The five components were assumed to correspond to populations of five instars. An individual was classified as belonging to instar i ($i = 1$–5) if the measured value of w lies in the range (c_{i-1}, c_i). Here $c_0 = 0$, $c_5 = \infty$. The values of c_1 to c_5 were defined so that the estimated chance of an individual of instar i being classified as $i + 1$ was equal to the probability of an individual of instar $i + 1$ being classified as i. That is, ci was defined so that the upper tail area of the distribution of population i above c_i was equal to the lower tail area of the population $i + 1$ below c_i,

$$\Phi\left(\frac{c - m_i}{s_i}\right) = 1 - \Phi\left(\frac{c - m_{i+1}}{s_{i+1}}\right),$$

where Φ is the standard normal integral. These values of c are determined numerically. Misclassification probabilities were then estimated as these tail probabilities that measure the overlap between the distribution of each instar.

Instar recognition: field studies

Larval collection and analysis of head-capsule data

A total of 1337 banana weevil larvae (of various sizes and unknown instars) was obtained by destructive sampling of 236 banana plants (cultivar Atwalira) in a recently completed field trial (planted November 1991) at Kawanda Agricultural Research Institute. Weevil larvae were removed from corm and the first 30 cm of the pseudostem.

Plants were carefully dissected to avoid missing small (*i.e.* first and second instar) larvae that normally feed near the plant surface. Sampling was carried out between 10 July 1996 and 13 August 1996.

The head capsule widths of all field-collected larvae were measured using the same procedure as for laboratory-reared insects. However, some large larvae in their late instars could only be observed at a magnification of 25 times. The resulting scores were then converted to the micrometer scale for magnification 40 times, using appropriate conversion constants. Consequently, the head-capsule width, w, ranged from 18–153 micrometer divisions. A frequency distribution of head-capsule data was then plotted and analysed using the procedure adopted for the laboratory sample to determine instars.

Stage duration: laboratory studies

Egg incubation period and thermal requirements

Date of hatching was recorded for egg cohorts used in Trials 1–3 (described below) for five days following first egg hatch. Development times were checked for conformity with thermal requirements of 89 degree-days above a developmental minimum (threshold) of 12 °C as reported by Traore *et al.* (1993) for banana weevils collected from plantain stands in southern Benin (7–8 N, 0–300 m, mean temperatures 27 °C). Daily minimum and maximum temperatures were recorded during the egg incubation period. Degree-days (DD), which represent accumulated heat units above a developmental threshold (Southwood 1978; Dent 1991; Pedigo 1996) were then computed using the formula suggested by Pedigo (1996):

$$DD = \frac{\sum (\text{minimum temp.} + \text{maximum temp.})}{2} - \text{developmental threshold}$$

In calculating degree-days, we assumed that the eggs were an average of 12-hours old upon collection and had hatched at an average of 12 hours before we recorded the day of hatching. Therefore, the calculated degree-days for the first and last day of incubation period were each divided by two, before calculating the accumulated heat units.

Instar duration

Stage duration was calculated in days and percentage time spent in each instar.

Trial 1: Stage duration for larvae measured in head-capsule studies (Part A) was determined by daily observation from hatching to commencement of the prepupal period. The beginning of the prepupal period was reflected by cessation of feeding, slowness in body reactions, stout body and a more rugose cuticle. Commencement of new instars was determined by the presence of exuviae, pale head-capsules or increments in head-capsule widths exceeding three micrometer units (0.0717 mm).

Trial 2: Eggs (540 in a 24-hour period) were obtained on 1 February 1997, as in Trial 1. A subsample of emerging larvae (196) was placed in modified rearing chambers (following the methods of Mesquita *et al.* 1984 and Schmitt 1993) to facilitate observations on moults and the length of the prepupal and pupal periods. The thickness of corm slices used in these rearing chambers was always less than the body girth of the larvae to prevent penetration into the corm. Commencement of new instars was determined as in Trial 1.

Trial 3: Eggs (554 in a one-day period) were obtained on 5 April 1997, as in Trial 1, from which 203 larvae were reared. Thickness of rearing chambers was as in Trial 1 for the larval period and as in Trial 2 for the post-larval stages. Commencement of new instars was determined as in Trial 1.

Data analysis

Analyses were conducted to compare different polymorphic groups for duration (days) and the proportion of time (percentage of larval period) spent in each larval instar. *t*-tests were conducted within trials for stage duration of groups in which n exceeded five individuals. The proportion of time spent in each instar was compared for the 6-instar group across trials. Data involving percentages were square-root transformed to stabilize the variance (Gomez & Gomez 1984); in these cases we present un-transformed data with LSMEANS tests for comparisons of instar groups with unequal numbers of observations.

RESULTS

Instar recognition: laboratory studies

The data indicate the existence of at least four polymorphic groups, with larvae passing through 5–8 instars before pupation. Rearing method influenced the size of the larvae, the number of instars and the duration of the larval period.

Mean head-capsule width and growth ratio per instar

Out of 265 test larvae in *Trial 1*, 117 (44 %) reached the prepupal stage. These passed through 5 (32 %), 6 (67 %) or 7 (2 %) instars. Mean head-capsule widths for each instar indicated that the growth ratio was greatest between the third and fourth instars (Table 1). Thereafter, the growth ratio decreased with increasing instar number. However, plotting the means against instar number showed that a geometric curve is a very good fit, confirming that Dyar's rule is a sound approximation. Fitting the geometric curve gave estimated growth ratios of 1.39 (S.E. = 0.11) for the five-instar group and 1.32 (S.E. = 0.018) for the six-instar group. Sample size in the seven-instar group was too small to check the model.

Head-capsule data analysis

Figure 1 shows the head-capsule frequency distribution histogram with the fitted model for the laboratory population. The laboratory sample showed clear separation into five groups that were assumed to correspond to instars. Larval polymorphic groups (Table 1) showed considerable overlap between the fifth and sixth instars and these instars could not be differentiated in a pooled frequency distribution graph. Inspection of the fitted model and observations showed a good fit with no obvious systematic departures between model and fitted data. Although the calculated chi-squared value of goodness of fit was large, most of the contributions come from a few unimportant deviations between the data and the fitted model. For example, the model gives a fitted (predicted) count of 0.06 observations for $w = 26$, but six were observed. This is not unusual when fitting distributions to such large samples since deviations between model and data that are unimportant in practice are detected as 'significant'. The

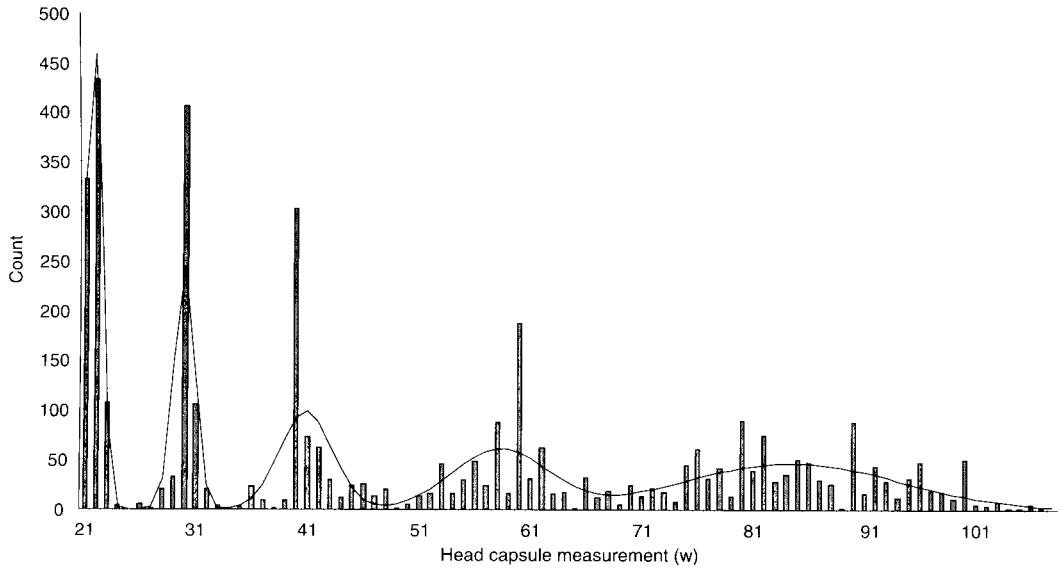

Fig. 1. Frequency distribution of *Cosmopolites sordidus* larval head-capsule widths in micrometer units (mu) and the fitted model for laboratory-reared larvae (1 mu = 0.0239 mm).

Table 1. Empirical mean banana weevil instar head capsule widths in micrometer units (mu): laboratory observations for various instars and associated growth ratios compared among instar groups (1 mu = 0.0239 mm).

Instar No.	Individuals with: Five larval instars		Six larval instars		Seven larval instars	
	Mean ± S.E. (n = 37)	Growth ratio	Mean ± S.E. (n = 78)	Growth ratio	Mean ± S.E. (n = 2)	Growth ratio
1	21.79 ± 0.10		21.64 ± 0.10		21.17 ± 0.16	
2	30.21 ± 0.13	1.44	30.09 ± 0.07	1.39	30.50 ± 0.50	1.44
3	42.13 ± 0.41	1.31	40.73 ± 0.21	1.35	40.00 ± 0.00	1.31
4	60.64 ± 0.51	1.50	57.62 ± 0.45	1.41	60.00 ± 0.00	1.50
5	81.36 ± 0.77	1.24	76.44 ± 0.64	1.33	74.20 ± 1.80	1.24
6			94.56 ± 0.57	1.24	86.50 ± 3.50	1.17
7					95.00 ± 5.00	1.11

standard deviations also increase with group, with remarkably large variations in the fifth group (Table 2). There is no suggestion in the data, however, that this group can be separated into more groupings.

Instar recognition: field studies

Weevils collected from the field trial were of unknown instars. Therefore, the head capsule frequency histogram (Fig. 2) could not be compared with mean ranges for known instars. Additionally, many larvae were larger than those reared in the laboratory, suggesting that reared larvae suffered from food of lower nutritive value or from repeated handling. Fitting the model revealed six groups to be more appropriate than five in this case but the separation into groups was less clear than that for the laboratory sample.

Instar boundaries from fitted models for laboratory and field samples

Instar distribution demarcated from the fitted model of laboratory data (Fig. 1, Table 2) closely approximated size intervals determined for known instars (Table 1). The estimated instar means for field-collected larvae (Table 3) were

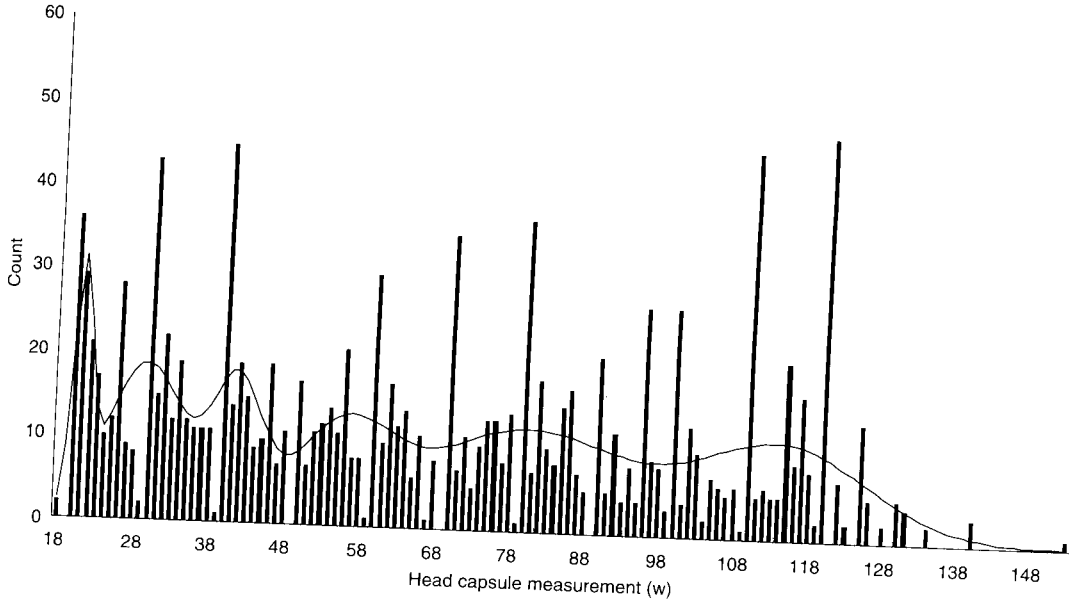

Fig. 2. Frequency distribution of *Cosmopolites sordidus* larval head-capsule width in micrometer units (mu) and the fitted model for field-collected larvae (1 mu = 0.0239 mm).

Table 2. Laboratory sample fitted model: banana weevil mean larval head-capsule widths estimates in micrometer units (mu) for different groups and associated standard errors (S.E.), growth ratios, standard deviations (S.D.) and proportions (1 mu = 0.0239 mm).

Group (Instar)	Mean ± S.E.	Growth ratio	S.D. ± S.E.	Proportion ± S.E.
1	21.7 ± 0.03		0.71 ± 0.03	0.24 ± 0.01
2	29.9 ± 0.06	1.38	0.96 ± 0.03	0.15 ± 0.01
3	40.9 ± 0.11	1.37	2.33 ± 0.77	0.15 ± 0.01
4	58.2 ± 0.23	1.42	4.27 ± 0.19	0.17 ± 0.01
5	84.5 ± 0.37	1.45	9.71 ± 0.35	0.30 ± 0.01

Table 3. Field sample fitted model: banana weevil mean larval head capsule-widths estimates in micrometers units (mu) for different groups and associated standard errors (S.E.), growth ratios, standard deviations (S.D.) and proportions (1 mu = 0.0239 mm).

Group (Instar)	Mean ± S.E.	Growth ratio	S.D. ± S.E.	Proportion ± S.E.
1	20.9 ± 0.23		1.14 ± 0.39	0.06 ± 0.02
2	29.6 ± 0.88	1.42	5.12 ± 1.61	0.18 ± 0.04
3	41.6 ± 0.93	1.41	3.26 ± 0.98	0.09 ± 0.04
4	55.7 ± 1.49	1.34	6.39 ± 2.28	0.13 ± 0.07
5	80.1 ± 2.47	1.44	13.06 ± 4.85	0.29 ± 0.10
6	114.4 ± 2.86	1.43	11.44 ± 1.64	0.24 ± 0.05

Table 4. Estimated separation points in head capsule micrometer units (mu) for different larval instars: laboratory and field samples (1 mu = 0.0239 mm).

Instar No. (Group)	Cut points	
	Laboratory sample	Field sample
1	25.2	22.6
2	33.1	36.9
3	47.0	46.4
4	66.3	63.7
5		98.3

very similar to the corresponding instars in the laboratory sample (Table 2). The standard deviations of the field sample were, however, much larger. For example, the fifth and sixth instars alone had such large standard deviations that 53 % of the population fell into these two groups.

The estimated separation points for the different instars of the laboratory and field samples are presented in Table 4, while the derived misclassification probability estimates are provided in Table 5. The misclassification probabilities reflect what can be seen from Figs 1 and 2. Using head capsule data from laboratory-raised insects to estimate instar gives a low error but field-sampled insects have a chance of up to 19 % of being misclassified by this method (Table 5).

Stage duration: laboratory studies

Laboratory ambient temperatures were 22–27 °C during all three trials.

Egg stage

Egg hatching rates ranged from 60–70 % for the three trials. Most egg failure resulted from mould developing on the filter paper. In Trial 1, mean egg development time was 6.9 days for Trial 1, 6.2 days for Trial 2 and 7.7 days for Trial 3. Using a minimal developmental threshold of 12 °C (Traore *et al.* 1993), accumulated heat units ranged from 83.9–85.3 degree-days for the first hatch and 92.8–95.9 for mean egg development times. These results suggest conformity to development periods established for West African banana weevil populations by Traore *et al.* (1993).

Larval stage: instar number and duration

In Trial 1, 117 larvae reached the prepupal stage, of which 93 were successfully reared to adult. These passed through five, six or seven instars (Table 6a). Mortality from egg to adult was 65 %, much of this presumably being accounted for by the disturbance of the larvae during measurement of head capsules. Stage duration was similar among polymorphs for each of the first four instars. Larvae passing through six instars had an extended development period, but spent less time in the fifth instar than those pupating after five instars. Total duration of the larval period was significantly longer for the sixth-instar group than that of the fifth-instar group. Overall, the larval period averaged 23.2 days (range 20–27 days).

In Trial 2, 55 larvae (28 %) reached the prepupal stage, of which 42 successfully emerged as adults. Rearing methods resulted in a greater number of instars and prolonged the length of the larval stage compared to Trials 1 and 3 (Table 6b). In this trial, larvae passed through six (26 %), seven (67 %) or eight (7 %) instars. Stage duration was similar among polymorphs for the first five instars, but differed in the sixth instar (Table 6b). Overall, the larval period averaged 33.2 days (range 24–41

Table 5. Estimates of misclassification probability for different banana weevil larval instars for the laboratory and field samples based on their head capsule width.

Instar (Group)	Probability of misclassification					
	Laboratory sample			Field sample		
	Lower	Higher	Total	Lower	Higher	Total
1	—	0.000	0.000	—	0.084	0.084
2	0.000	0.000	0.000	0.084	0.075	0.159
3	0.000	0.004	0.004	0.075	0.072	0.144
4	0.004	0.030	0.034	0.072	0.105	0.177
5	0.030	—	0.030	0.105	0.080	0.185
6				0.080	—	0.080

Table 6. Banana weevil instar duration (days ± S.E.) for instar groups (IG) across Trials 1–3.

Group	*n*	L1	L2	L3	L4	L5	L6	L7	L8	Total
A. Trial 1										
IG5	37	3.4	3.0	3.7	4.5	7.6				22.2
IG6	78	3.6	3.1	3.7	4.3	4.7	4.3			23.6
t-value		1.72	0.90	0.20	1.36	13.33*				5.96*
IG7	2	3.5	3.0	4.0	4.0	6.0	3.0	1.5		25.0
B. Trial 2										
IG6	18	3.4	3.1	5.6	4.7	4.5	8.0			29.3
IG7	33	3.6	3.4	5.2	4.8	5.2	4.8	6.2		33.1
t-value		0.69	1.65	0.72	0.25	1.67	5.68*			4.32*
IG8	4	4.0	3.8	4.5	6.0	3.5	5.0	5.8	5.5	38.1
C. Trial 3										
IG5	2	4.0	3.0	6.0	4.0	10.5				27.5
IG6	50	4.0	3.1	3.7	4.0	4.3	6.7			25.8
IG7	3	4.3	3.3	2.7	4.0	4.0	4.7	2.7		25.7

*$P < 0.01$.

days), while the prepupal period averaged 4.0 days (range 1–8 days) and the pupal period 5.7 days (range 3–8 days). Overall, the egg to adult period lasted 49.4 days (range 40–58 days).

In Trial 3, 55 larvae (27 %) were reared to the prepupal stage, of which 42 successfully emerged as adults. These passed through five (4 %), six (91 %) and seven (5 %) instars (Table 6c), with a mean larval period of 25.9 days (range 23–30 days). Since only five individuals passed through five or seven instars, no analyses were run for stage duration. The prepupal period averaged 5.1 days (range 2–10 days), while the mean pupal period was 8.0 days (range 6–10 days). Overall, the egg to adult period averaged 45.9 days (range 40–48 days).

In Trial 1, mean instar duration expressed as percentage total larval period was similar among polymorphic groups for instars 1–3 (Table 7a), while there was a small but significant difference in the percentage of development spent in instar 4 and a large difference for instar 5. Larvae spent 34 %, 38 % and 42 % of their development period in the fifth and higher instars for the five-instar, six-instar and seven-instar polymorphs, respectively.

In Trial 2, the percentage of the larval period spent in each instar was similar between the six-instar and the seven-instar groups for instars 1, 2, 4 and 5 (Table 7b). A significant difference was found between the two groups for the third and

Table 7. Banana weevil instar duration, expressed as percentage of total larval period, for instar groups (IG) of Trials 1–2.

Group	*n*	L1	L2	L3	L4	L5	L6	L7
A. Trial 1								
IG5	37	15.3	13.5	16.7	20.3	34.2		
IG6	78	15.2	13.1	15.6	18.1	19.8	18.1	
t-value		0.23	0.70	1.64	3.0**	16.81**		
B. Trial 2								
IG6	18	11.6	10.6	19.1	16.0	15.4	27.3	
IG7	33	10.8	10.2	15.7	14.5	15.7	14.5	18.7
t-value		1.60	0.81	2.53*	1.06	0.06	7.43*	

*$P < 0.05$; **$P < 0.01$.

Table 8. Comparison of larval instar duration expressed as percentage time of total larval period (days) for 6-instar group across Trials 1–3.

Trial	*n*	L1	L2	L3	L4	L5	L6
1	78	15.2 a	13.1 a	15.6 b	18.1 a	19.8 a	18.1 b
2	18	11.6 b	10.6 b	19.1 a	16.0 ab	15.4 b	27.3 a
3	50	15.5 a	12.0 b	14.3 c	15.5 b	16.7 b	26.0 a
F-value		23.36*	18.76*	10.53*	8.10*	11.26*	34.84*

*$P < 0.01$.

Means within a column followed by the same letter are not significantly different ($P < 0.05$); contrast by LSMEANS.

sixth instars. Overall, larvae spent 27 %, 33 %, and 43 % of their development period in sixth and higher instars for the six-instar, seven-instar and eight-instar polymorphs, respectively.

For larvae passing through six instars before pupation, the mean larval period ranged from 23.6 days in Trial 1 to 29.3 days in Trial 2 (Table 6). These differences largely reflected the length of time spent in the last instar. Moreover, the proportion of time spent in the different instars also differed between the three trials (Table 8).

Rearing methods appeared to influence larval size, as well as number of instars and developmental period. Larval head-capsule widths in Trial 2 (where the weevils could not form galleries) were 10–17 % smaller than those of comparable instars in Trials 1 and 3, suggesting reduced food uptake in this trial.

DISCUSSION

In this study, banana weevil larvae reared in the laboratory passed through 5–8 instars. The phenomenon of developmental polymorphism occurs naturally in many insect species. Schmidt & Lauer (1977) found that nutritional quality influenced the proportions of instar groups in *Choristoneura viridis* and *C. occidentalis* (Lepidoptera: Tortricidae) but not the kinds of instar groups within a given species. Other factors known to affect the number of insect instars include photoperiod (Fantinou *et al.* 1996), temperature and relative humidity (Schmidt & Lauer 1977). This suggests that developmental polymorphism for a given insect may have both a genetic and an environmental basis.

For the laboratory population, the growth ratios in the fifth and sixth instar-groups provided only an approximate conformity to Dyar's rule. This was also true for the growth ratio from the fitted model for both laboratory and field samples. Similar discrepancies have been reported for other insects (Schmidt *et al.* 1977; Jobin *et al.* 1992; Fantinou *et al.* 1996; McClellan & Logan 1994). This would imply that Dyar's rule is not always applicable, and consequently continues to be controversial. The constant geometric relation, in the strict sense, is not a fundamental feature of insect growth (Daly 1985).

The use of frequency-distribution curves to determine the characteristic number of instars of a species is most useful in insect species with little or no overlap in head capsule width between adjacent instars.

Head-capsule width cannot therefore be used directly to determine the developmental stage of individual larvae for species with considerable overlap between successive instars. Various statistical methods have consequently been developed (Caltagirone *et al.* 1983; Got 1988; Beaver & Sanderson 1989; McClellan & Logan 1994) to classify larvae whose head capsule measurements fall in the overlapping areas.

Developmental polymorphism further complicates interpretation of data. Instar determination using head-capsule-width frequency distribution has been found unsuitable for some insects with multiple instar groups such as *Choristoneura* species (Lepidoptera) (Schmidt *et al.* 1977), pine bark weevil borer (Kishi 1971) and Ephemeroptera (Daly 1985). In banana weevil, however, mean head-capsule widths for the first four instars were similar among groups ultimately passing through five, six, and seven instars.

Schmidt *et al.* (1977) indicated that the use of head-capsule-width frequency distribution for instar determination may not always be reliable. These authors observed that the curves only

provide clear results when the insects are fairly homogenous with regard to rate of development and numbers of instars. Insect species exhibiting developmental polymorphism, such as banana weevil, would therefore give rise to complex and less clear frequency distribution curves. This probably explains why only five peaks could be detected by the fitted model for the laboratory sample, although 66 % of the larvae passed through six or seven instars.

Using head-capsule widths, it is possible to separate instars 1–4 in banana weevil with a satisfactory degree of accuracy. However, separation of the fifth and higher instars was not possible for field-collected larvae. Therefore, a series of point estimates of age distribution (*i.e.* egg, first, second, third, fourth, ≥5th instars, pupae), weighted by duration of each instar might be used for lifetable work such as estimation of cohort stages. Such age distributions could be obtained for different cultivars or plants at different stages of development.

Food quality appeared to influence larval size, including head-capsule widths. Larger larvae were collected from the field than were reared in the three laboratory trials and separation points for field-collected instars were greater than for corresponding instars of laboratory-reared larvae (Figs 1, 2; Table 4). Additionally, larval head-capsule widths in Trial 2 were smaller than in other trials for corresponding instars. It is also possible that variability in food quality between cultivars or plant stages may influence larval size (Mesquita *et al.* 1984; Mesquita & Caldas 1986). This suggests that studies requiring separation of larval stages on the basis of head capsules should also include development of histograms of head-capsule widths to test for conformity to the field results presented here.

The relative duration of each stage was determined for laboratory reared banana weevil larvae. Under ambient temperatures of 22–27 °C, the mean egg-development times in three laboratory trials in Uganda was 6.9 days. In Benin (mean temperature 27 °C), Traore *et al.* (1993), using constant temperatures, found a thermal requirement of 89 degree-days over 12 °C with optimum development (*i.e.* shortest stage duration) at 30 °C. Uganda populations, under fluctuating temperatures, had mean thermal requirements of 93–96 degree-days above 12 °C. This suggests only a modest difference in thermal requirements for East and West African banana weevils. In the Benin study, however, the empirical data suggested thermal requirements of only 75.9 and 79.5 degree-days at 25 °C and 27 °C, respectively. Further work will consequently be necessary to determine the possibility of East and West African biotypes having different thresholds and developmental rates.

In addition to larval size, both instar number and duration of the larval period (*i.e.* days) appeared to be affected by rearing methods. For example, the larval period was prolonged in Trial 2 (6.75 instars, 32.2 days), where larvae were unable to penetrate into corm sections, compared to Trial 1 (5.7 instars, 23.1 days) or Trial 3 (6.0 instars, 25.9 days), where they could penetrate. These results are similar to those of Mesquita *et al.* (1984) and Mesquita & Caldas (1986), who found a similar range of instars, and that prolongation of larval stage duration was accompanied by a greater number of ecdyses. The data in the present study suggest that the developmental period from egg to adult is 6–8 weeks under ambient temperatures of 22–27 °C.

The significance of a prolonged larval period and supernumerary moults in banana weevil in Trial 2 is not easy to explain at present. These observations are in agreement with Roder (1953) and Wigglesworth (1965), who reported that under unfavorable conditions such as poor nutrition, larval development may be delayed with supernumerary instars. Slowed larval growth may also be an evolved phenomenon, because the poor food source may preclude higher rates of food consumption, metabolism and growth (Slansky & Rodriguez 1987). Furthermore, the trigger mechanism for moulting may be related to an accumulation of sufficient lipids required to ensure survival during a moult (Slansky & Rodriguez 1987). In Trial 2, weevils pupating after six or seven instars were smaller than corresponding polymorphic groups in the other two trials. The effects of size on fecundity are well known for insects (Slansky & Rodriguez 1987; Honek 1993), and have also been demonstrated for banana weevil (M. Griesbach & C.S. Gold, unpubl.). Weevil larvae thus face the consequences of fitness in undergoing prolonged development, with its associated risks, as against those involved in producing a sub-optimal individual in the next stage.

In each trial, however, banana weevil larvae passing through more instars had larger head-capsule widths, suggesting greater body weights and related fitness. These results contrast with

those of Mesquita *et al.* (1984). These authors found the larval stage reared on 17 different cultivars ranged from 25–32 days but the data presented in their tables suggest a moderate negative relationship between larval period and pupal weight. Their results suggest that prolongation of the larval period may have a modest effect on adult fitness. Additionally, Mesquita & Caldas (1986) found that the food quality of crop residues and older plants reduced weevil fitness (*i.e.* increasing larval mortality, developmental period and/or number of moults, while reducing pupal weight).

The cryptic lifestyle of banana weevil in the field precludes multiple observations of individuals during their development. Therefore, the distribution of larvae into the different instar groups under natural conditions remains unknown. Presumably the field samples could be a mixture of all four instar groups, perhaps fewer or even more, with varying proportions of each category that may be present. It is unclear whether the larger size of field-collected larvae simply reflects better food quality or indicates a higher proportion of individuals entering more than six instars. However, the estimated mean values for head-capsule widths of earlier instars for laboratory and field populations were similar although the latter showed higher levels of variability.

Lifetable analysis for banana weevil will require destructive sampling and separation of larvae into instars. To estimate the proportions of larvae recruited into each stage, it will be necessary to weight each stage on the basis of its relative duration (*i.e.* dividing the number of insects in a stage by the mean number of days or proportion of time spent in that stage).

With the exception of the last instar, most larvae spent a relatively uniform length of time (*i.e.* 3.1–4.7 days) in each instar. In Trials 1 and 3, for example, 74 % of the larvae passed through six instars. These spent an average of 15.4, 12.6, 15.0, 16.8, 18.3 and 22.1 % of the total larval stage in instars 1–6, respectively. These percentages (combining the time in the fifth and sixth instars) might be used as weights in determining age structures in lifetable studies.

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