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Delineation of larval instars in field populations of rice yellow stem borer, *Scirpophaga incertulas* (Walk.)

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ABSTRACT

Delineation of larval instars of an insect is important in morphological and physiological studies. We applied Dyar's rule to morphometric measurements of larval instars from field populations of the yellow stem borer (YSB), Scirpophaga incertulas (Walk.) collected on two paddy cultivars over three seasons. The conventional head capsule width (HCW) measurement was compared with mandibular width (MW) of larvae from both field and laboratory populations. Frequency distribution of HCW showed seven peaks with size overlaps indicating seven instars in field populations. Distinct size classes were observed in MW with means of 0.042, 0.083, 0.125, 0.166, 0.208, 0.250 and 0.291 mm for 1st, 2nd, 3rd, 4th, 5th, 6th and 7th instars, respectively, in the field population. However, size classes fell into five instars from HCW and MW measurements of larvae reared on cut paddy stems under constant temperature (25 ± 1°C) and humidity (60± 5%) indicating two additional, late larval instars under variable field conditions. For the same instar, mean HCW size slightly varied and was overlapping while mean MW size was identical between field and laboratory populations for each of the first five common larval instars.

Key words: *Dyar's rule, mandibular width, rice, yellow stem borer, Scirpophaga incertulas*

Rice is one of the most important staple food crops in the world, with China and India being the lead producing countries. Large numbers of insect pests are reported to ravage the rice fields in the tropics The yellow stem borer (YSB), *Scirpophaga incertulas* (Walker) is the most destructive and widely occurring insect pest of rice that attacks all stages of the crop (Bandong and Litsinger, 2005). Light trap collections across locations over the years indicated that YSB is spatially and temporally the dominant species across seasons in India (DRR, 2000-09). Feeding by YSB larvae causes death of affected tiller (dead heart symptom) in the vegetative, and chaffy, unfilled panicles (white ear symptom) in the reproductive crop growth phases. Yield loss estimates across India varied from 11.2 to 40.1% due to dead hearts and 27.6 to 71.7% due to white ears, respectively (Krishnaiah and Varma, 2012). A 1% incidence level of dead heart or white ear or both resulted a grain loss of 108, 174 and 278 kg ha^{-1}, respectively (Muralidharan and Pasalu, 2006).

YSB larvae bore into paddy stems and stay hidden for most part of their development. Most stem borer larval instars can only be distinguished with morphometric measurements (Guglielmino *et al.,* 2006). In most arthropod species at least some parts of the exoskeleton are strongly sclerotized and unable to expand (Sehnal, 1985). This rigidity prevents continuous growth, and such structures therefore grow in a stepwise manner. This results from moulting, *i.e.* replacement of the old cuticle by a new one formed during the intermoult cycle prior to ecdysis (Sehnal, 1985).

Heavily sclerotized structures, such as head capsules and mandibles, remain approximately unchanged within an instar. Dyar was the first to suggest frequency distributions of head capsule width for instar determination (Dyar, 1890). This is a simple and easy method for identification of larval instars in field population. Instars are indirectly determined through a plot of the number of individuals per class size, where each distinct peak in the plot corresponds to one instar (Fink, 1984). Several mathematical models have been used to describe linear measurements of sclerotized parts in successive instars. The linear progression, $y = a + bx$, and the geometric progression $y = a\ b^x$ which is termed as Dyar's rule were often used by Entomologists to ascertain the actual number of instars (Klingenberg and Zimmermann, 1992). In contrast, Dyar's rule assumes a geometric progression of size measures, where succeeding growth ratios (i.e. post moult size/premoult size; also termed Dyar's coefficient) or percentage increments are constant, but not the absolute increments (Dyar, 1890; Sehnal, 1985). Therefore, a plot of log transformed size measurements against instar number reveals a straight-line relation. Several studies substantiated Dyar's rule to larval growth (Mohammed Shahow, 2011). In most of the studies, head capsule width (HCW) was taken as a measure of larval size. However, HCW can increase slightly, but significantly, within stadia (Bliss and Beard, 1954). In identifying stem borer instars, some workers considered mandibular width (MW) a better distinguishing criterion because mandibles are contiguous in different instars (Pathak and Khan, 1994). Nguyen van Huynh (1993) opined that larval HCW and mandibular length were the most reliable criteria for differentiating YSB populations in a study comprising four populations of *S. incertulas*. Precise identification of larval instar is very crucial in physiological, ecological and molecular studies and also for implementing control measures. The present study is aimed at determining variability in number of larval instars of YSB field populations based on HCW and MW measurements and application of Dyar's rule.

MATERIALS AND METHODS

Rice seedlings (20 days old) of cultivar Pusa Basmati 1 (PB1) were transplanted into plastic pots (8 cm diameter, 5 seedlings pot^{-1}) containing puddled clay soil. Field collected adult moths of YSB were released on potted plants covered with a cylindrical mylar cage (45 cm. height and 14 cm diameter) for egg laying. Leaf portions with egg masses were clipped and incubated in plastic boxes (5 cm diameter) lined with moist filter paper for hatching. Egg masses of similar age at black head stage were used for field infestation. Cohorts of neonate larvae were used for laboratory experiments.

Field experiments (1000 m^2) were conducted at research farm, Directorate of Rice Research, Hyderabad, Andhra Pradesh for three seasons *viz.*, wet season 2010, wet and dry seasons of 2011 with two paddy cultivars, BPT 5204 and Pusa Basmati 1 (PB 1). At maximum tillering stage of the transplanted crop, 30 polythene enclosures (1 m^2) comprising 36 plants each were erected randomly in the field every season for each variety and one egg mass (Viajante and Heinrichs, 1987) of known size was pinned to the central hill within the caged arena. Each cage was covered with a plastic sheet to prevent natural YSB infestation throughout the study. Egg masses in black-head stage were pinned to ensure hatching on the same day. After hatching of egg masses, every alternate day one cage was randomly selected, all 36 plants uprooted, carefully dissected and examined for YSB larvae. Sampling was done every alternate day up to 50 days from day of hatching of egg mass and later at weekly intervals upto harvest. At each sampling date variation in larval numbers and sizes was observed. Therefore, a sample of 5-10 larvae representing different larval sizes was selected for measurements on each day of sampling. Thus in all, 1200 larvae were studied for their morphometrics from the field population. The sixth and seventh instar were identified when moulting larvae were intercepted in the stems at the time of dissection of the plant samples. Larval length, HCW and MW were measured using a calibrated ocular micrometer in a binocular dissecting microscope (Olympus SZ-CTV, Japan, at 1.8 X magnification) and data were used for determining instars according to Dyar's rule (Gullan and Cranston, 2005).

Laboratory experiments were carried out in environmental chambers (MLR 350H, SANYO electric Co.Ltd., Japan) set at constant temperature (25 ± 1°C), relative humidity (60 ± 5 %) and photoperiod (14L:10D). Neonate larvae (n=200) were individually released on cut stems of rice (cultivar PB 1) in glass tubes and plugged with cotton. Larvae were examined every alternate day to record moulting and stage of development by opening stems through destructive sampling and then supplied with fresh cut stems.

Table 1. Morphometrics of field populations of *S.incertulas* larvae collected from two rice cultivars

Larval stage	Larval length (mm)		Head capsule width (mm.)				Mandibular width (mm.)			
			PB 1		BPT 5204		PB 1		BPT 5204	
	PB 1 Mean ± SE (Range)	BPT 5204 Mean ± SE (Range)	Mean ± SE; n1; (Range)	Rate of growth	Mean ±SE; n2; (Range)	Rate of growth	Mean (mm.)	Rate of growth	Mean (mm.)	Rate of growth
I instar	5.25 ± 0.11 (4-6)	5.32 ± 0.12 (4-6)	0.241 ± 0.002; 58 (0.208 - 0.250)	1.469	0.239 ± 0.002; 58 (0.208 - 0.250)	1.407	0.042	2.000	0.042	2.000
II instar	9.21 ± 0.30 (7-13)	9.50 ± 0.34 (7-14)	0.354 ± 0.009; 63 (0.208 - 0.416)	1.416	0.336 ± 0.011; 53 (0.208 - 0.416)	1.464	0.083	1.500	0.083	1.500
III instar	12.21 ± 0.14 (10-14)	12.26 ± 0.14 (11-14)	0.501 ± 0.009; 46 (0.374 - 0.624)	1.304	0.492 ± 0.006; 45 (0.374 - 0.582)	1.354	0.125	1.333	0.125	1.333
IV instar	12.80 ± 0.35 (9-14)	13.20 ± 0.47 (12-20)	0.653 ± 0.007; 93 (0.499 - 0.832)	1.282	0.666 ± 0.007; 76 (0.499 - 0.832)	1.257	0.166	1.250	0.166	1.250
V instar	15.78 ± 0.21 (7-21)	16.34 ± 0.25 (10-23)	0.837 ± 0.003; 160 (0.707 - 0.915)	1.168	0.837 ± 0.004; 132 (0.624 -1.040)	1.200	0.208	1.200	0.208	1.200
VI instar	16.91 ± 0.23 (12-24)	17.18 ± 0.21 (13-24)	0.977 ± 0.004; 119 (0.874 - 1.082)	1.197	1.004 ± 0.004; 176 (0.874 - 1.082)	1.141	0.250	1.167	0.250	1.167
VII instar	17.38 ± 0.33 (13-23)	17.09 ± 0.34 (12-23)	1.170 ± 0.016; 61 (0.957 - 1.456)		1.145 ± 0.018; 60 (0.957 - 1.456)		0.291		0.291	

Values in parentheses represent the range of measurement for each instar, n1 & n2 -are the number of larvae observed,
Rate of growth= measurement after moult / measurement before moult

Simultaneously, HCW of moulted larva was measured. At each moult, 10 larvae were selected at random keeping in view the variation in size and MW measured by separating the mandibles under the stereomicroscope using a dissecting needle.

Data on log HCW was regressed with larval instars with the help of curve expert 1.4 (copyright ©1995-2009 Daniel Hyams). Mean HCW of larvae in each instar from the two cultivars was compared by t test (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Frequency distribution plot of larval numbers sampled from two paddy cultivars across seasons against HCWs indicated seven overlapping clusters (Fig. 1). Plotting of frequency distribution of MWs of the same larvae also indicated seven distinct groups (Fig. 2). From both these parameters it can be inferred that there were seven larval instars for YSB under field conditions. The figures also indicated that the number of larvae in L6 and L7 were fewer in number and were found at the end of the crop season.

Mean values of larval length, HCW and MW of *S. incertulas* field populations from two cultivars are presented in table 1. Mean larval lengths across larval instars ranged between 5.25-17.38 mm on PB1 and 5.32 -17.18 mm on BPT5204 and there was overlapping of measurements in larval length across the instars. Mean HCWs across the seven instars ranged from 0.241 to 1.170 mm on PB 1 and 0.239 to 1.145 mm on BPT5204. Growth ratio (Dyar's ratio) for the HCW decreased with increase in instar number and varied from 1.469 to 1.197 on PB1 and from 1.464 to 1.141 on BPT 5204 except for the ratio (1.407) observed when larvae moulted from 1st to 2nd instar. There was no significant difference in the mean HCWs for each larval instar sampled on the two cultivars in t-test except in 6th instar (t value 4.4323, 293 df, at 5% L). MW was 0.042, 0.083, 0.125, 0.166, 0.208, 0.250 and 0.291 mm. for L1 to L7 larval instars, respectively in both the varieties. Growth ratio for MW decreased with increase in instar number from 2.0 to 1.167.

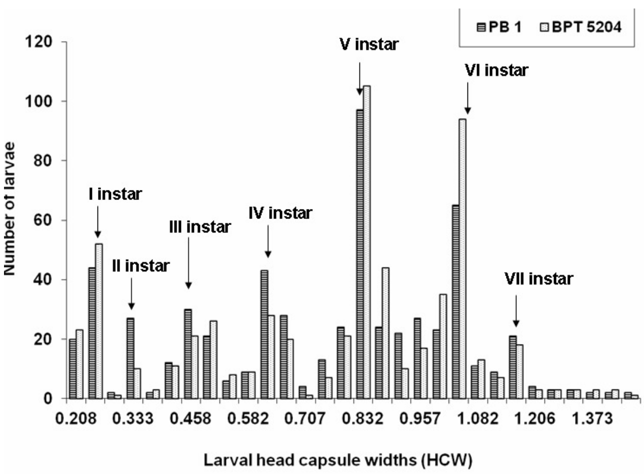


Fig. 1. Frequency distribution of larval head capsule width in field population of *S. incertulas*

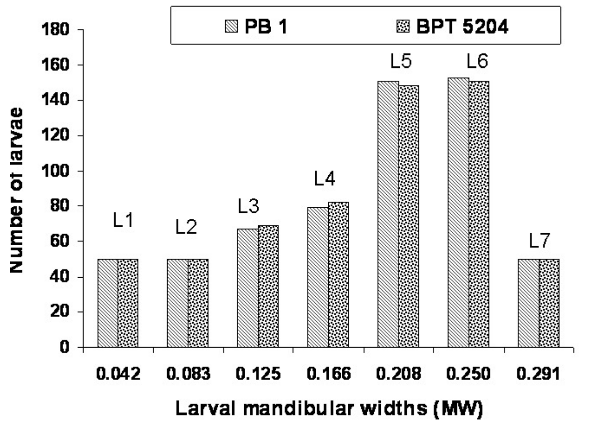
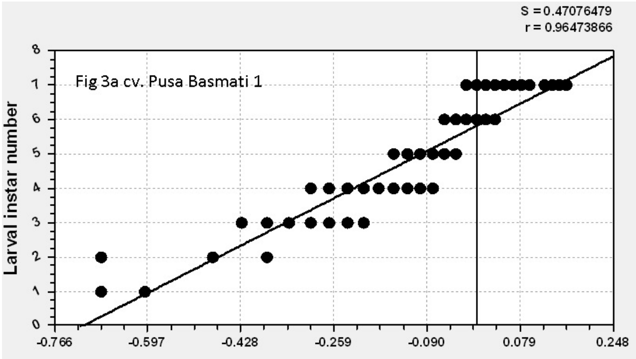


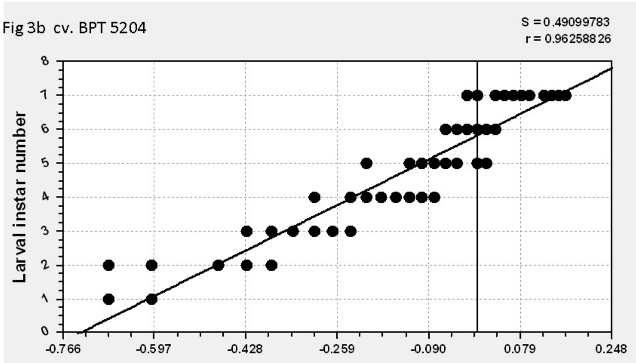
Fig. 2. Frequency distribution of larval mandible widths in field populations of *S. incertulas*

Regression of log values of HCW with instar number revealed a linear relationship with overlapping values in each instar, correlation coefficient, r = 0.97 in PB1 (Fig. 3a) and r =0.96 in BPT5204 (Fig. 3b). Also the plot indicated that there were no missing instars in the present field study. Regression of instar number with MW was linear and highly significant (Y=0.0416x; correlation coefficient (r) = 0.999) (Fig. 4). Earlier studies on YSB reported either five larval instars (Bora *et al.,* 1994; Hugar *et al.,* 2009) or six larval instars (Puttarudriah, 1945; Rothschild, 1971; Islam and Catling, 1991; Panigrahi and Rajamani, 2008; Khan *et al.* 1991 and Pathak and Khan (1994) reported that *S. incertulas* larvae have 4-7 instars prior to pupation. but under field conditions with variable weather factors, seven instars were recorded. Both larval development and adult emergence of YSB are affected by temperatures below



Each dot is a value from 'n' larvae
S-Standard error; r- Correlation coefficient

Fig. 3a. Log values of larval head capsule width (HCW)



Each dot is a value from 'n' larvae
S-Standard error; r- Correlation coefficient

Fig. 3b. Log values of larval head capsule width (HCW)

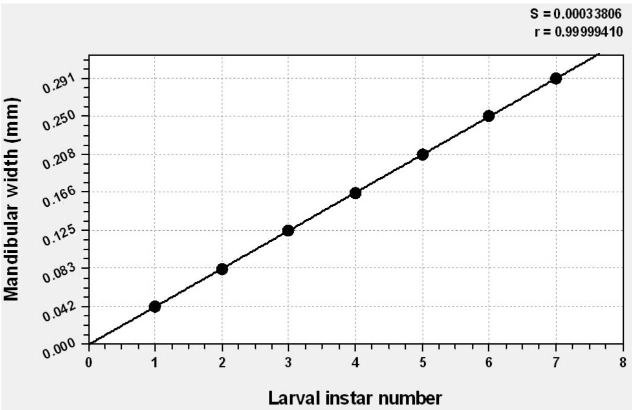


Fig. 4. Relationship between mandibular widths and instars in larval field populations of *S. incertulas*

16°C and above 35°C (Pathak and Khan, 1994). Temperatures of 29–30°C were the most favourable for the YSB development (Varma *et al.,* 2000). Variation in instar number in response to rearing temperature and nutrition has been documented in many insects (Wigglesworth, 1972). The variation in temperature, photoperiod, food quality and quantity, humidity, rearing density, physical condition, inheritance and sex are the most common factors influencing number of instars (Esperk *et al.*, 2007; Hai-Jun Xiao *et al* 2010).

Studies on biology of the insect under laboratory conditions from the observation of exuviae after moulting revealed that there were five distinct larval instars. The results in this study indicated that though there was variation in the length of the larvae and HCW within each instar, MW was constant with reference to an instar (Table 2). The results are in consonance with field observations except that the insect had pupated after five instars. Mean larval length ranged from 6.01 to 14.50 mm across five larval instars on PB1. HCWs were 0.241, 0.374, 0.526, 0.672 and 0.826 mm for L1 to L5, respectively. Growth ratios for HCW in successive instars were 1.552, 1.406, 1.277 and 1.229 indicating that greatest proportionate growth was

Table 2. Morphometrics of *S.incertulas* larvae on PB 1 under laboratory conditions

Larval stage	Larval length (mm.)	Head capsule width (mm.)		Mandible width (mm.)	
	Mean ± SE	Mean ± SE; Range	Rate of growth	Mean	Rate of growth
I instar	6.01 ± 0.19 (5 -6)	0.241 ± 0.004 (0.208 - 0.250)	1.552	0.042	2.000
II instar	7.95 ± 0.22 (6 -8)	0.374 ± 0.010 (0.333 - 0.416)	1.406	0.083	1.500
III instar	10.17 ± 0.81 (7 -14)	0.526 ± 0.014 (0.458 - 0.624)	1.277	0.125	1.333
IV instar	11.89 ± 0.26 (9-12)	0.672 ± 0.018 (0.499 - 0.832)	1.229	0.166	1.250
V instar	14.50 ± 0.41 (12 - 17)	0.826 ± 0.007 (0.749 - 0.874)		0.208	

Values in parentheses depict the range for the respective parameters between L1 to L2 and L2 to L3. Growth ratios for MW in successive instars were 2.00, 1.50, 1.33 and 1.25. Most larvae had five instars when reared at 23-29°C. Our results also agree with Pathak and Khan (1994), wherein under laboratory conditions at 25 ± 1°C we could observe only five larval instars.

The larval length varied from 5.25 to 17.38 mm in various instars under both field and laboratory conditions which is different from that reported by Puttarudriah (1945) where the length of larvae were 1.5, 3.0, 3.5-4.0, 5.0, 8.0-9.0 and 12.0 mm for L1 to L6 instars, respectively. Though the larvae hatched at the same time from an egg mass and entered into the plants, a clear variation in size was observed when they were dissected out from within the stems. Overlapping measurement of larval length observed across the instars made it difficult to identify larval instars based on size. The variation could be attributed to abiotic factors and experimental conditions. Variations in the larval length across instars recovered from the same egg mass suggested that larval size cannot be considered as a criterion for determining the instar. The widths of the head capsule recorded in our study also differed from those reported by earlier workers (Puttarudriah, 1945; Hugar *et al.* 2009).

Regression analysis of the data showed a significant relationship between larval instars and logarithms of HCW by producing straight line which indicated that there is no missing instar, fitting in with Dyar's rule both under field and laboratory study. From our results, it is pertinent to note that the widths of head capsule in a particular instar are continuous and overlapping. Width of the head capsule was considered as criteria for differentiating larval instars of YSB (Puttarudriah 1945; Rothscild, 1971; Hugar *et al.,* 2009) but this may not be reliable in all instances due to overlapping frequency distributions of larval head capsule widths of successive instars and variable number of instars (Gaines and Campbell, 1935; Goettel and Philogene, 1979). It is well known that larvae of YSB undergo facultative hibernation /diapause and the reasons attributed are age of host plant, temperature, photoperiod and humidity (Khan *et al.,* 1991). This raises the possibility that developmental plasticity in instar number may represent an adaptive response to slow growth conditions at some temperatures. Additional instars in short day length conditions often seem to be an adaptation enabling individuals to stay in the larval stage in those species that hibernate as larva. These additional instars may be inserted under the diapause-inducing conditions before the actual diapause (Shintani and Ishikawa, 1998) or even during the diapause (Chippendale and Yin, 1973; Honek, 1979; Kfir, 1991). Temperature can influence the number of instars by changing the quality of environment. It also can have an indirect influence to number of instars serving as a cue for the duration of the season (Adachi, 1994).

Typically, instar number tends to increase under adverse rather than favourable conditions. This conclusion is consistent with the compensation scenario, according to which additional instars are inserted in poor conditions when larvae fail to reach a species specific threshold size with the normal instar number. The rate at which insects complete their life cycles depend mainly on weather factors, particularly temperature (Pandey *et al.,* 2001). In many cases, plasticity in instar number is also used as a mechanism ensuring that the developmental stage capable of surviving the unfavourable season is reached at the right time of the year. Dejiu (1988) reported the relationship between photoperiod, instar number and induction of diapause in *S.incertulas* larvae. For YSB, rice stubbles are the niche for survival of the hibernating larvae and therefore serve as an inoculum source for infestation in the next season (Padmakumari *et al.,* 2012).

Though the peaks in the frequency distribution of HCW (Fig. 1) indicated the presence of seven instars, it cannot be considered as a parameter for delineating the instars as the measurements are overlapping. Ghent (1956) and Richards (1949) reported that the linear progression model is appropriate if there's a straight line relation between untransformed size measures and instar number i.e if absolute growth increments are same in all moults. Our results indicate a straight-line relation between MW and instar number and the absolute growth increments are the same in all moults. Hence, larval MW can be used for determination of the larval instars of *S. incertulas* as they are quite distinct for each of the instars across both varieties and populations. This could form the basis for determining larval instars of *S. incertulas* in field and laboratory populations.

In the present study, a decrease in the ratio of the means of the successive instars with respect to widths of the head capsule and mandible widths was observed and this is in accordance with Abdel Malek and Goulding (1948). The greatest proportionate growth tended to fall between the first and second instars and the least between the penultimate and the last which is similar to that reported by Gaines and Campbell (1935).

A high level of plasticity in life history parameters such as developmental time and size, provided by the plastic instar number, might contribute to a species tendency to become an opportunistic pest. Information about life history of insects is important for understanding population dynamics, life table analysis, key factor analysis and other important ecological investigations, and also to determine their community structure. The study assumes significance because it's the first of its kind which address the study of field populations of *S.incertulas* and identifying mandible width as an important parameter for appropriate identification of the larval instars under field conditions which would help in understanding the various frontier areas of insect science like physiological processes, insect plant interactions and aid in developing prediction models for forecasting.

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