https://doi.org/10.17576/serangga-2026-3102-06

HEAD CAPSULE MORPHOMETRY FOR LARVAL INSTAR DETERMINATION OF *Spodoptera frugiperda* IN MAIZE ECOSYSTEMS OF BOGOR, INDONESIA

Maydiah Retno Palupi[1], Dewi Sartiami[1*], Ali Nurmansyah[1], Desi Septiani[1] & Ravindra Chandra Joshi[2, 3]
[1]Department of Plant Protection,
IPB University (Bogor Agricultural University),
Jl. Raya Dramaga Kampus IPB,
Dramaga Bogor 16680 West Java, Indonesia
[2]Department of Agriculture-Philippine Rice Research Institute,
Maligaya, Science City of Muñoz,
Nueva Ecija, 3119 Philippines
[3]Centre for Agriculture and Biosciences International– Malaysia,
Building A19, 43400 MARDI Serdang, Selangor, Malaysia
[*]Corresponding author email: *dsartiami@apps.ipb.ac.id*

Received: 6 January 2026; Accepted: 13 July 2026; Published: 23 August 2026

ABSTRACT

The fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), is a highly invasive insect pest capable of attacking numerous plant families, including key Gramineae crops such as maize, rice, wheat, sorghum, and sugarcane. *Spodoptera frugiperda* larvae cause severe damage, primarily by feeding on the shoot apical meristem (SAM), often resulting in shoot formation failure. In the field, larval morphology shows considerable variation, making accurate identification of larval instars challenging. Correct instar classification is essential for effective monitoring and management. This study aimed to classify the larval instars of *S. frugiperda* using head capsule width (HCW) as the diagnostic character. Larval samples were collected from predetermined sampling points within each location in Bogor Regency, Indonesia: Ciomas, Cikarawang, and Cibeureum. Morphological features were examined using a stereomicroscope. The HCW data were analyzed using boxplots, while variations in body color, head color and pattern, 'Y' letter color, dorsal line color, and lateral line color were analyzed descriptively. Measurements established six larval instars with definitive corresponding HCW ranges: 1st (0.38–0.44 mm), 2nd (0.63–0.67 mm), 3rd (0.82–0.89 mm), 4th (1.24–1.33 mm), 5th (2.00–2.08 mm), 6th (2.72–2.82 mm). Significant morphological variations were noted across the observed larval color characters. Instars 1–2 lacked distinct markings, while instars 3–6 showed variable head patterns. The inverted 'Y' suture line varied from brown to yellow/yellowish-brown across all stages. Furthermore, measurements of pupa length, pupa width, forewing length, hind wing length, and wingspan in adult *S. frugiperda* showed no significant difference in mean dimensions between sexes. These findings provide a reliable morphometric key for precision diagnostics and accurate larval instar determination of *S. frugiperda* in maize ecosystems, thus supporting improved monitoring and Integrated Pest Management (IPM) strategies.

Keywords: Fall Armyworm; head capsule width; morphometry; instar identification; corn; Southeast Asia

ABSTRAK

Ulat ratus, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), merupakan serangga perosak invasif yang sangat agresif dan berupaya menyerang pelbagai famili tumbuhan, termasuk tanaman utama daripada famili Gramineae seperti jagung, padi, gandum, sorgum, dan tebu. Larva *S. frugiperda* menyebabkan kerosakan yang teruk, terutamanya dengan memakan meristem apeks pucuk tanaman jagung, yang sering mengakibatkan kegagalan pembentukan pucuk. Di lapangan, morfologi larva menunjukkan variasi yang ketara, sekali gus menyukarkan pengenalpastian instar larva secara tepat. Pengelasan instar yang betul adalah penting bagi tujuan pemantauan dan pengurusan perosak yang berkesan. Kajian ini bertujuan untuk mengklasifikasikan instar larva *S. frugiperda* menggunakan lebar kapsul kepala (head capsule width, HCW) sebagai ciri diagnostik. Sampel dikumpulkan dari beberapa titik sampling yang telah ditetapkan di setiap lokasi di Kabupaten Bogor, Indonesia, iaitu Ciomas, Cikarawang, dan Cibeureum. Ciri-ciri morfologi diperhatikan menggunakan mikroskop stereoskop. Data HCW dianalisis menggunakan plot kotak (boxplot), manakala variasi warna badan, warna dan corak kepala, warna huruf "Y", warna garis dorsal, dan warna garis lateral dianalisis secara deskriptif. Hasil pengukuran mengenal pasti enam instar larva dengan julat HCW yang jelas, iaitu instar 1 (0.38–0.44 mm), instar 2 (0.63–0.67 mm), instar 3 (0.82–0.89 mm), instar 4 (1.24–1.33 mm), instar 5 (2.00–2.08 mm), dan instar 6 (2.72–2.82 mm). Variasi morfologi yang signifikan diperhatikan bagi semua ciri warna larva yang dikaji. Instar 1–2 tidak menunjukkan tanda yang jelas, manakala instar 3–6 memperlihatkan variasi corak pada bahagian kepala. Garisan sutur berbentuk huruf "Y" terbalik menunjukkan variasi warna daripada coklat hingga kuning atau kuning kecoklatan pada semua peringkat perkembangan. Selain itu, pengukuran panjang pupa, lebar pupa, panjang sayap hadapan, panjang sayap belakang, dan rentang sayap pada imago *S. frugiperda* menunjukkan tiada perbezaan yang ketara dalam nilai purata antara jantina. Dapatan kajian ini menyediakan kunci morfometrik yang boleh dipercayai untuk diagnosis yang tepat dan penentuan instar larva *S. frugiperda* secara berketepatan tinggi dalam ekosistem jagung, sekaligus menyokong penambahbaikan sistem pemantauan serta strategi Pengurusan Perosak Bersepadu (PPB).

Kata kunci: Ulat ratus; lebar kapsul kepala; morfometri; pengecaman instar; jagung; Asia Tenggara

INTRODUCTION

The fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae), is a highly invasive, polyphagous pest native to the Americas that infests over 80 plant species, including global staples like maize, rice, and sugarcane (Ginting et al. 2020; Goergen et al. 2016). Characterized by a remarkable migration capacity exceeding 500 km (Prasanna et al. 2018), FAW reached Asia in 2018 and was first reported in Indonesia in early 2019 (Nonci et al. 2019). This pest has also spread widely across Southeast Asia, including Malaysia, Thailand, Vietnam, Myanmar, and Cambodia (Kusano et al. 2025; Mohammed et al. 2026). The pest's devastating potential stems from a short life cycle, a voracious feeding capacity, and high fecundity, with a female laying up to 1,184 eggs, resulting in yield losses of 15% to 73% (Laminou et al. 2022; Marri et al. 2023; Subiono 2020). In Indonesia, infestation rates have reached 100%, with recorded losses up to 70% (Lestari et al. 2020).

Effective field management depends on the accurate identification of larval developmental stages. However, staging is complicated by highly variable morphological markers; larval body and head color can range unpredictably from green to blackish-brown or orange across instars (CABI 2019a; Deshmukh et al. 2018). Accurate instar determination is particularly important because the efficacy of management interventions, including biological agents and insecticides, often varies among developmental stages. The use of pheromone compounds in population monitoring and decision-making for pest control underscores the importance of an accurate number of pests in the fields. On the other hand, this method could obtain some adults as samples to be character-measured for accurate identification (Subagyo et al. 2026). Furthermore, molecular identification using the cytochrome oxidase I (COI) marker has been widely used to characterize populations and strains of *S. frugiperda* in South-East Asia (Mohammed et al. 2026), however, this approach requires laboratory facilities. Identification based on morphological characteristics offers a simpler, more practical approach, as it can be carried out directly in the field. One characteristic with potential for use is head capsule morphometry, which provides an objective basis for determining larval instar.

Head capsule width (HCW) morphometry is widely used for larva; instar determination because the dimensions of head capsule increase predictably between successive molts, consistent with Dyar's law (Dyar 1890). This approach has been successfully applied to determine larval instars in another *Spodoptera* species, including *Spodoptera exigua* and *Spodoptera litura* (Chen & Seybold 2013; Mo et al. 2013). The primary objective of this study is to establish a precise six-instar diagnostic key for *S. frugiperda* within the Bogor maize ecosystem based on HCW morphometry. Additionally, this research aims to comprehensively document the physical dimensions of pupal and adult stages and record morphological variability, such as the inverted Y-line patterns and body coloration.

While the larval identification key is essential for immediate field intervention, the inclusion of pupal and adult morphometry provides a holistic view of the pest's fitness within the Bogor ecosystem. Understanding the full life-cycle dimensions is critical for Integrated Pest Management (IPM) for two reasons: first, pupal size serves as a proxy for adult fecundity and reproductive potential; second, adult wingspan data aids in refining dispersal models. By documenting the entire developmental profile, this study provides the baseline data necessary for predictive population modeling and long-term monitoring of *S. frugiperda* adaptation in Indonesia.

MATERIALS AND METHODS

Study Area and Duration

Larval samples of *S. frugiperda* from all stages (1st instar to 6th instar) were collected in tubes from farmer-owned maize fields within four distinct areas of Bogor Regency, Indonesia: Cikarawang Village 106°43'54"E – 6°33'0"S (Dramaga Subdistrict), Ciomas Village 106°46'08"E – 6°36'47"S, Cibeureum Village 106°56'38"E – 6°41'39"S (Cisarua Subdistrict), and Ciapus Village 106°44'54"E – 6°36'15"S (Ciomas Subdistrict) (Figure 1). Sampling sites represented a range of elevations from 192 to 789 m above sea level. Laboratory rearing and morphometric analyses were conducted at the Insect Biosystematics Laboratory, Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University (IPB University), between January and April 2020.

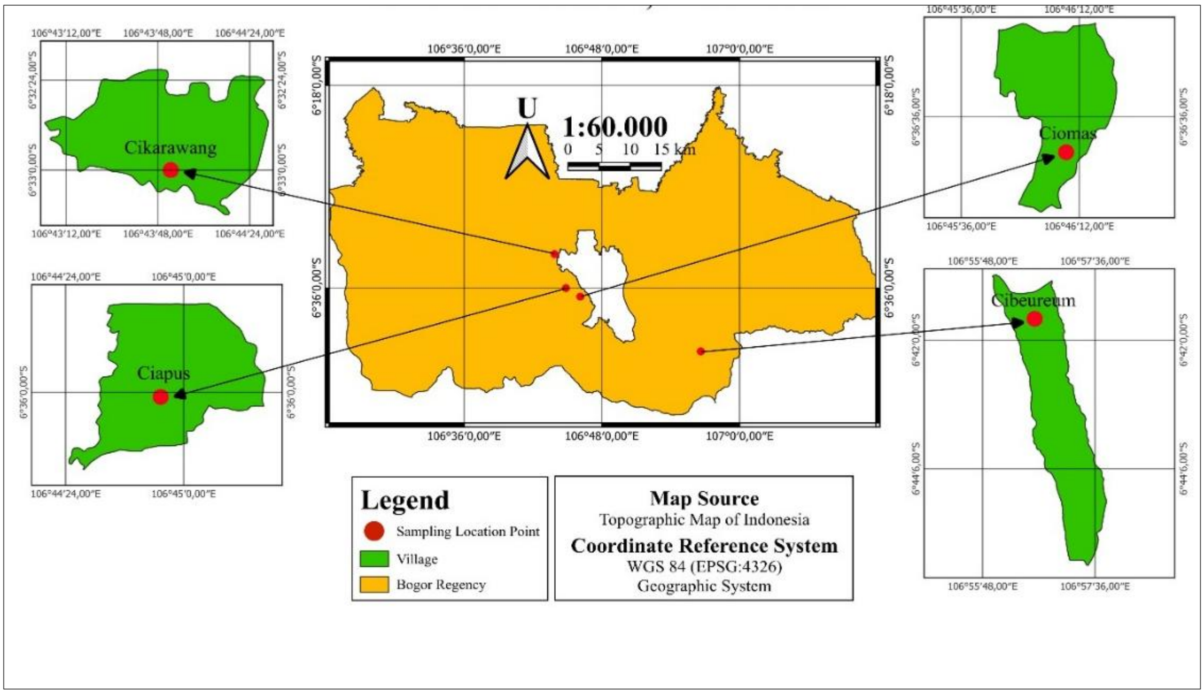

Figure 1. Larval sampling locations of *Spodoptera frugiperda* in maize fields of Bogor Regency, West Java, Indonesia

Materials and Equipment

Biological materials included *S. frugiperda* specimens, comprised of 330 field-collected larvae, 33 laboratory-reared pupae, and 42 laboratory-reared adults. In addition, fresh maize leaves were needed for nutrition, and 70% ethanol for immobilization to prevent movement of the object and facilitate accurate morphological observation, measurement, and capture of unique characters. Laboratory equipment consisted of a stereomicroscope with a calibrated ocular micrometer, modified ventilated plastic cups (6 x 3 x 4 cm^3), petri dishes, and standard entomological tools (tweezers, fine brushes, and dissecting needles).

Pest Sampling and Laboratory Rearing

A total of 330 larvae, which consisted of 10 first-instar larvae, 18 second-instar larvae, 22 third-instar larvae, 76 fourth-instar larvae, 78 fifth-instar larvae, and 112 final-instar larvae, were non-selectively collected from six maize plots, focusing on plants exhibiting symptomatic damage such as windowed leaves, feeding holes, and frass on leaves or tassels. The non-selective larva collection each instar varied according their natural abundance at the time of sampling. Specimens were individually reared in modified plastic cups at 24±1°C and RH 43-53%, and provided with fresh maize leaves daily. To facilitate natural pupation, late-stage larvae were provided with soil as a substrate to a depth of one-third cup. Pupae were maintained until adult emergence to allow for life-stage measurements.

Morphometric Analysis and Descriptive Observation

Species identification was confirmed using standard morphological characteristics cross-referenced with established literature (Capinera 2005; Deshmukh et al. 2018; EPPO 2015; Ganiger et al. 2018; King & Saunders 1984; Sartiami et al. 2020; Sisodiya et al. 2018; Trisyono et al. 2019). For larval morphometry, specimens were anesthetized with 70% ethanol and positioned to expose the head capsule. The head capsule width (HCW), defined as the maximum distance across the outer margins,was measured using a stereomicroscope with a calibrated ocular micrometer. Each measurement was replicated at least twice to establish the six-instar classification.

Pupal dimensions (length and width) and adult wingspan (tip-to-tip distance of forewings) were measured using a ruler. Sexual dimorphism in pupae is distinguished by the distance from the anal opening to the genitalia; in male pupae, this distance is shorter than in females (Sumaryati et al. 2023). Emerging adults were sexed, and qualitative morphological variations, including the inverted Y-line head pattern and dorsal and lateral body coloration, were recorded for each stage.

Data Analysis

Head capsule width (HCW) data were analyzed using boxplots and grouped to establish mean values for each instar following the framework of Ashley et al. (1983). Pupal and adult dimensions were summarized in Microsoft Excel. Differences in pupal and adult morphometry between sexes were evaluated using independent-samples *t*-tests (α = 0.05) after confirming normality and homogeneity of variances. Qualitative morphological variations were analyzed descriptively.

RESULTS

Field Infestation and Damage Assessment

Sampling sites represented a range of elevations from 192 to 789 m above sea level. Field observations indicated that *S. frugiperda* larvae were commonly encountered either resting on the leaf tips or feeding internally near the plant growing point. Distinct feeding damage patterns were observed in relation to larval development. Early instars produced windowed leaf symptoms, characterized by translucent leaf areas, whereas later instars caused perforated leaves and severe whorl damage, often accompanied by abundant larval frass (Figure 2).

Figure 2. Field damage symptoms caused by *Spodoptera frugiperda* on maize: (a) windowpanel feeding (instars 1–2), (b) perforated leaves (instars 3–4), and (c) severe damage to tassels with abundant frass (instars 5–6)

More severe damage was associated with the late instars (instars 4–6), which often exhibited a brownish body color and caused substantial harm to the reproductive parts of the maize plant. Symptoms included copious larval frass and feeding holes observed around and at the base of the male flowers (tassels) (Figure 2c). Extreme damage to the male flowers was evidenced by broken structures and a high frass density. These findings align with Sartiami et al. (2020), who reported that *S. frugiperda* larvae commonly target maize during its reproductive phase by feeding on both cobs and male flowers. Overall, the infestation in the Bogor maize ecosystems was found to span various growth stages, impacting plants during both the vegetative and reproductive phases.

Head Capsule Width Variation and Instar Classification

Head capsule width (HCW) increased progressively with larval development and showed clear separation among instars. Boxplot analysis revealed six discrete larval instars with non-overlapping HCW ranges (Figure 3). Mean HCW values consistently increased between successive instars, forming a distinct growth pattern. Based on HCW measurements, six larval instars were defined with the following ranges: instar 1 (0.38–0.44 mm), instar 2 (0.63–0.67 mm), instar 3 (0.82–0.89 mm), instar 4 (1.24–1.33 mm), instar 5 (2.00–2.08 mm), and instar 6 (2.72–2.82 mm) (Table 1). These measurements provided a quantitative basis for larval instar determination.

Table 1. Larval head capsule width morphometry of *Spodoptera frugiperda* from the Bogor maize ecosystem compared with global historical data

Larvae Instar	N	Head Capsule Width Range (mm)	Mean (x̄±SD) (mm)	Ashley et al. (1983)	Pitre & Hogg (1983)	Santos et al. (2003)	Deshmukh et al. (2018)	Maharani et al. (2021)
1st instar	10	0.38–0.44	0.41±0.043	0.3–0.4	0.35	0.32	0.32–0.35	0.34
2nd instar	18	0.63–0.67	0.65±0.044	0.5–0.6	0.45	0.5	0.47–0.52	0.50
3rd instar	29	0.82–0.89	0.85±0.096	0.7–0.9	0.75	0.83	0.80–0.85	1.10
4th instar	82	1.24–1.33	1.29±0.199	1.0–1.6	1.3	1.42	1.17–1.30	1.69
5th instar	79	2.00–2.08	2.04±0.174	1.7–2.2	2.0	2.1	1.87–2.05	2.00
6th instar	112	2.72–2.82	2.77±0.265	2.3–3.0	2.6	–	–	2.84

Head capsule width (HCW) increased progressively with larval development and showed a clear, stepwise separation among instars. The established instar key is defined by the mean HCW, which increased from 0.41 mm in instar 1 to 2.77 mm in Instar 6, indicating a consistent growth pattern across larval stages. Instar 1 larvae (N = 10) showed a tightly constrained HCW range (0.38–0.44 mm) with a low level of variation, reflecting morphological uniformity at the earliest developmental stage. The second instar (N = 18) exhibited a distinct increase in mean HCW to 0.65 mm (range: 0.63–0.67 mm), with no overlap with the preceding instar, supporting clear stage differentiation.

This pattern continued through instar 3 (N = 29), which had a mean HCW of 0.85 mm (range: 0.82–0.89 mm), and instar 4 (N = 82), which showed a further increase to a mean of 1.29 mm (range: 1.24–1.33 mm). The complete separation of HCW ranges among these intermediate instars demonstrates that head capsule width provides a stable and reliable metric for instar discrimination under field conditions. The largest cohorts were observed in instar 5 (N = 79) and Instar 6 (N = 112), with mean HCW values of 2.04 mm (range: 2.00–2.08 mm) and 2.77 mm (range: 2.72–2.82 mm), respectively. Although sample size and variability increased in later instars, HCW ranges remained non-overlapping, indicating that head capsule width retains high diagnostic precision even at advanced larval stages.

Comparative analysis of head capsule width (HCW) from the Bogor population with four previous studies shows general agreement with historical data, particularly in the later instars. Mean HCW values for instar 4 (1.29 mm) and instar 5 (2.04 mm) closely align with those reported by Ashley et al. (1983), Santos et al. (2003), and Maharani et al. (2021) indicating consistency in larval growth patterns. Minor deviations observed in the early instars are likely influenced by differences in diet, local climatic conditions, and geographical strain variation, all of which can affect larval development. Analysis of data variance using box plots (Figure 3) demonstrates a clear relationship between instar stage, sample size, and HCW variability, with low variation in early instars and increasing diversity toward later stages, peaking in instar 6. The larger sample size in the final instar further contributes to the wider range of HCW values observed, confirming that HCW remains a reliable diagnostic parameter for instar determination, despite increasing variability in late-stage *S. frugiperda*.

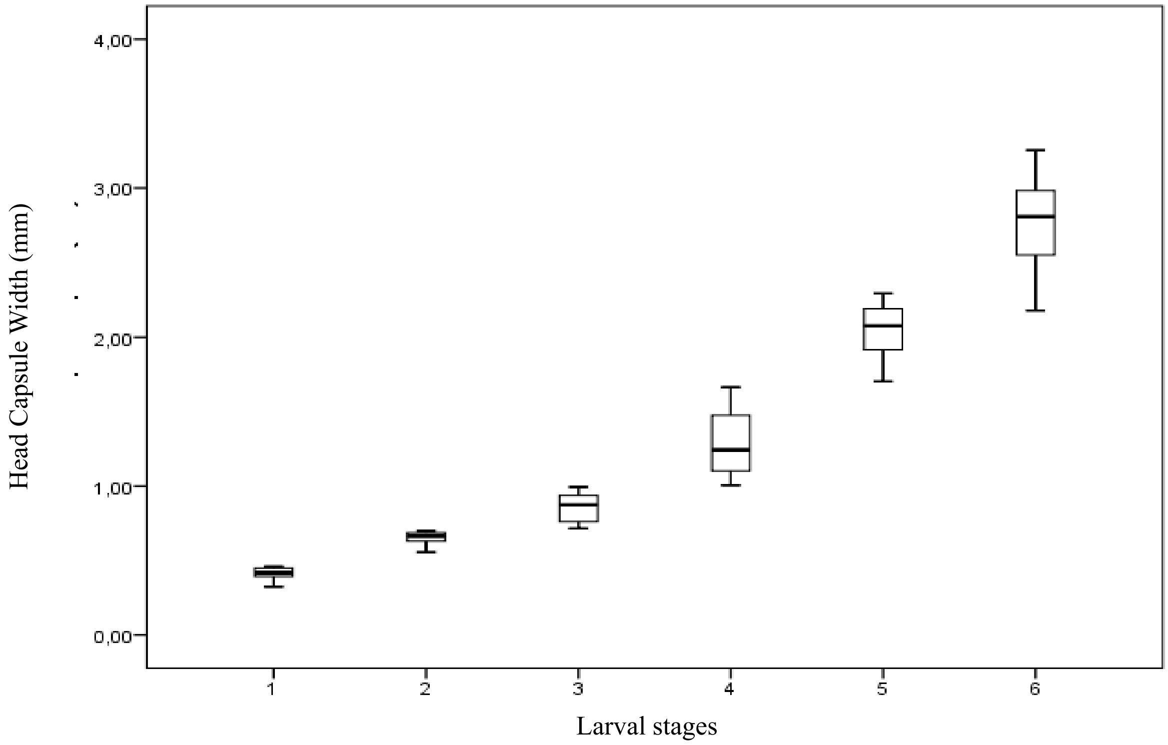

Figure 3. Boxplot of head capsule width (HCW) of *Spodoptera frugiperda* larvae across six instars

The analysis revealed that female pupae were marginally longer (1.63±0.10 cm) and wider (0.52±0.05 cm) than male pupae (1.62±0.10 cm in length; 0.49±0.04 cm in width). However, statistical analysis indicated that these differences were not significant (*P>0.05*) (Table 2). Overall, pupal size showed a narrow range of variation between sexes, indicating limited sexual dimorphism in pupal morphometry. The mean pupal lengths observed in both sexes were below the size commonly reported by Ajmal et al. (2024), which was that female and male pupae were approximately 1.7 cm in length.

Table 2. Sex-Specific Morphometric Analysis of Pupae of *Spodoptera frugiperda*

Sex	N	Length (x̄±SD, cm)†	Width (x̄±SD, cm)†
Male	20	1.62±0.10a	0.49±0.04a
Female	13	1.63±0.10a	0.52±0.05a

†Data represent the mean (x̄) and standard deviation (±SD) calculated from field-collected *S. frugiperda* pupae. The same letter following the mean values within the same column indicates no significant difference between the sexes at the 5% level (independent-samples *t*-test).

The mean wingspan of adult *S. frugiperda* was 3.12±0.18 cm in males and 2.98±0.28 cm in females. Mean forewing lengths were nearly identical between sexes (1.33±0.11 cm in males and 1.34±0.14 cm in females), while male hindwings (1.02±0.07 cm) were slightly longer than those of females (0.99±0.11 cm) (Table 3). Statistical analysis indicated that none of the measured adult wing parameters differed significantly between sexes ($P>0.05$). Overall, adult wing morphometry showed a narrow range of variation and limited sexual dimorphism, indicating that wingspan and wing length are not reliable characters for sex differentiation. The observed wing span and wing length were slightly shorter than those reported by Devi et al. (2024), who found that the average females had a 1.34 cm wing length and 3.08 cm wing span, while the males had 1.4 cm and 3.27 for wing length and wing span.

Table 3. Sex-Specific Forewing Length, Hindwing Length, and Wingspan Morphometry of Adult *Spodoptera frugiperda*

Sex	N	Forewing Length (x̄±SD, cm)†	Hindwing Length (x̄±SD,+cm)†	Wingspan (x̄±SD, cm)†
Male	15	1.33±0.11a	1.02±0.07a	3.12±0.18a
Female	27	1.34±0.14a	0.99±0.11a	2.98±0.28a

†Data represent the mean (x̄) and standard deviation (±SD) calculated from field-collected *S. frugiperda* adults. The same letter following the mean values within the same column indicates no significant difference between the sexes at the 5% level (independent-samples *t*-test).

Descriptive Morphological Variation in Larval Instars

Larval body color variation of *S. frugiperda* differed across instars, as summarized quantitatively in Figure 4 and illustrated morphologically in Figure 4a-f. Early instar (instars 1–2) were dominated by green-bodied larvae, accounting for 80% and 72% of individuals, respectively, while yellow-bodied larvae occurred at lower frequencies (Figures 4a–b), consistent with laboratory observations on maize-fed larvae (Deshmukh et al. 2018). This distribution indicates relatively low color variability during the earliest developmental stages. From the third instar onward, green, yellow, and brown body color phenotypes occurred simultaneously (Figures 4c–e), reflecting an increase in phenotypic diversity with advancing larval development. The proportion of brown-bodied larvae increased progressively from instar 3 to instar 5, although green and yellow phenotypes remained present across these stages.

In the sixth instar, larvae were predominantly brown and green (Figure 4f) in agreement with previous studies reporting wide color variability in late instars (CABI 2019b), indicating that body coloration becomes more heterogeneous rather than convergent in the final larval stage. Overall, body color showed a clear instar-related shift from relatively uniform coloration in early instars to high variability in later instars, demonstrating that body color alone does not provide a consistent basis for precise instar determination under field conditions.

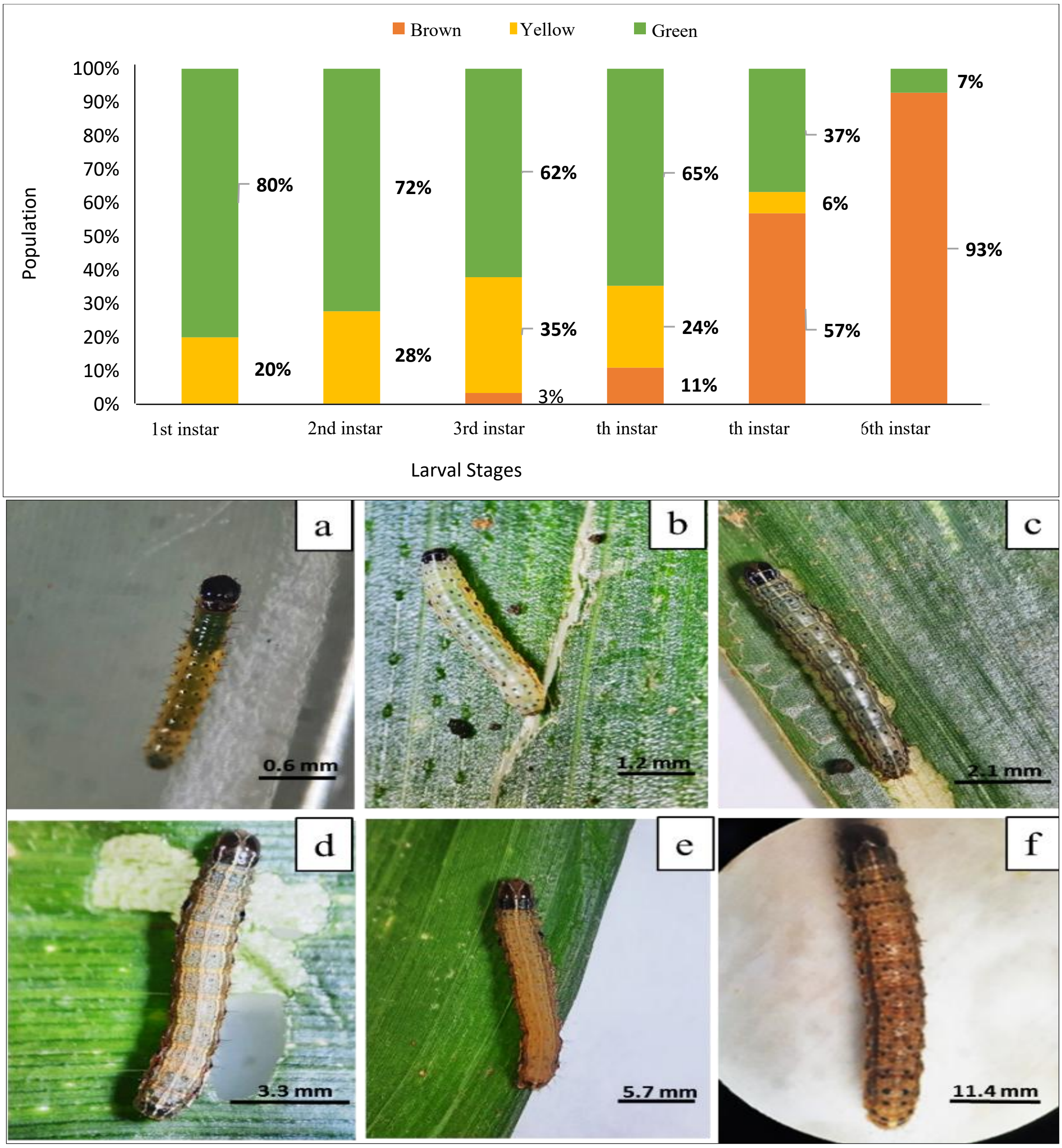

Figure 4. Body color variation and morphological progression of *Spodoptera frugiperda* larval instars collected from the Bogor maize ecosystem. The stacked bar chart shows the proportion of brown, yellow, and green body color phenotypes observed in each larval instar based on field samples. Photographs illustrate representative larval morphology and coloration for each instar: (a) yellow first instar, (b) green second instar, (c) green third instar, (d) brown fourth instar, (e) brown fifth instar, and (f) brown sixth instar. Millimeter measurements indicate approximate larval body length for visual reference and do not represent head capsule width

Head capsule color of *S. frugiperda* larvae varied across instars, as shown in Figure 5. First- and second-instar larvae exhibited more than one head color category, with brown and brownish-black heads occurring alongside darker phenotypes (Figures 6a and 6b). This indicates that head coloration is not uniform in the earliest larval stages. From the third instar onward, larvae displayed a broader range of head capsule colors, including black, brownish-black, and brown. A clear dominance of the black head phenotype was observed in intermediate instars, accounting for 64% of individuals in instar 3 and reaching a maximum of 73% in instar 4 (Figures 6c and 6f). In later instars, the proportion of black-headed larvae declined, with black phenotypes comprising 53% of instar 5 and 43% of instar 6 samples (Figures 6i and 6l). This shift reflects an increase in color heterogeneity in the final larval stages, with brown and brownish-black phenotypes occurring more frequently. Overall, head capsule coloration showed an instar-related change in both frequency and dominance patterns, indicating that while certain color phenotypes are common at specific developmental stages, head color alone does not provide a consistent or exclusive basis for larval instar determination under field conditions.

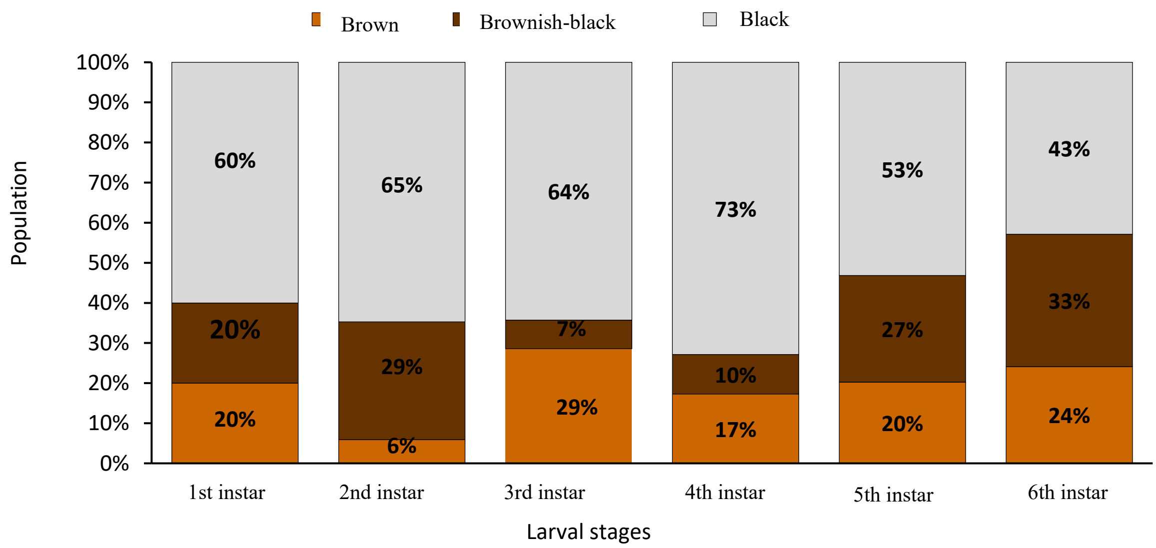

Figure 5. Variation in head capsule color of *Spodoptera frugiperda* larvae across instars. The number of dominant head colors (Black, Brownish-black, Brown) in each instar of *Spodoptera frugiperda*

Head capsule patterning of *S. frugiperda* larvae showed a clear instar-dependent progression, as summarized in Figure 6. No head capsule patterns were observed in first- and second-instar larvae, indicating a uniform and unpatterned head morphology at early developmental stages (Figures 6a–d). The onset of head pattern variation was first detected in the third instar, where patterned individuals accounted for approximately 3% of the observed samples. These early patterns were generally irregular and localized, most frequently appearing along the lateral regions of the head capsule. The proportion of larvae exhibiting head capsule patterns increased markedly with advancing instar. Patterned individuals comprised 21% of fourth-instar larvae, indicating an intermediate stage of pattern development. This trend continued in later instars, with 58% of fifth-instar larvae exhibiting distinct head patterns (Figures 6g and 6i). Head capsule patterning became nearly ubiquitous in the final larval stage, with 96% of sixth-instar larvae displaying well-defined and clearly visible patterns (Figures 6k and 6l). Overall, these results demonstrate a progressive and cumulative increase in head capsule pattern expression across larval development, indicating that head patterning is strongly associated with instar advancement but is absent or unreliable as a diagnostic character in early instars.

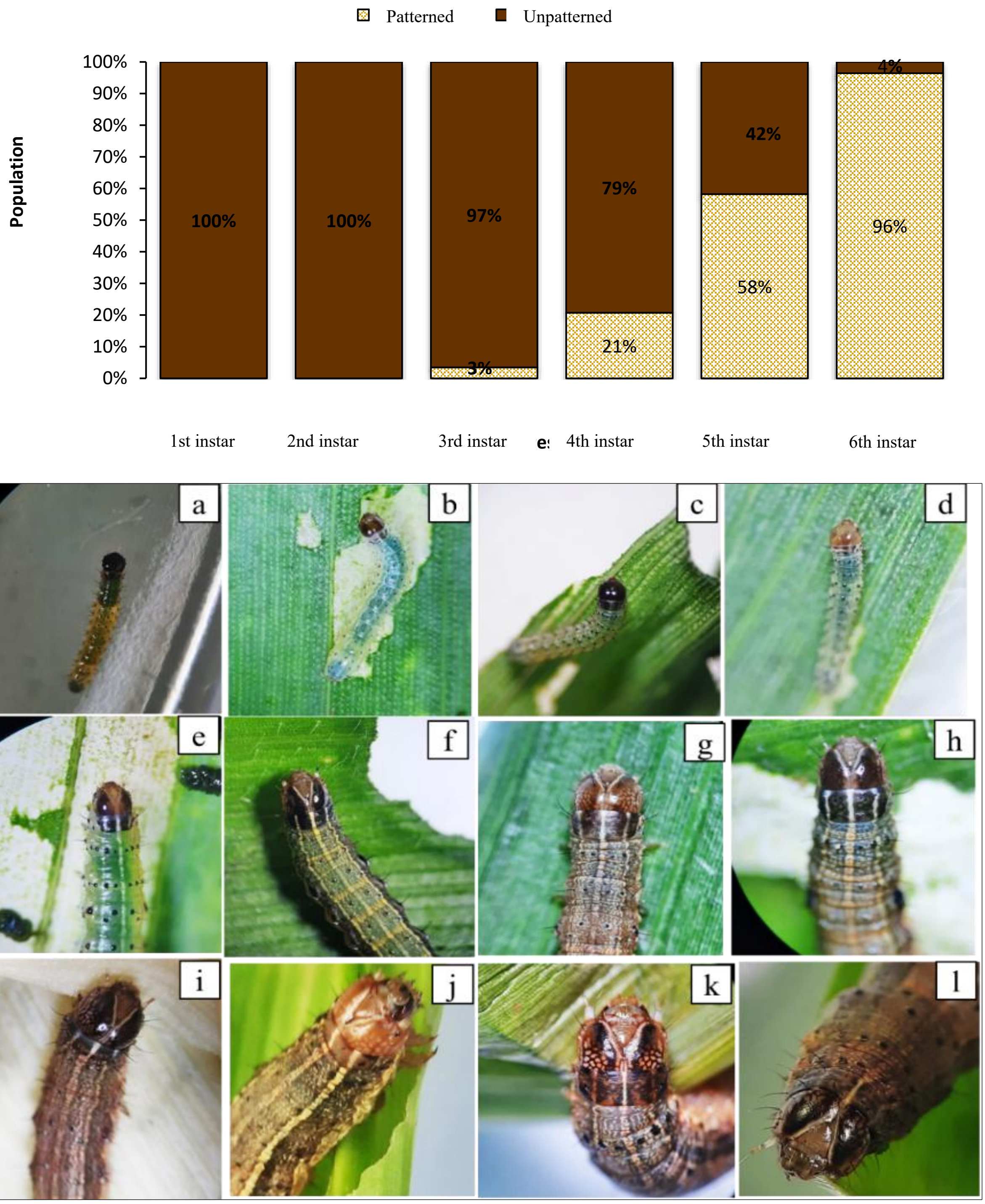

Figure 6. Presence and variation of head capsule coloration and patterning in larval instars of *Spodoptera frugiperda*. The stacked bar chart shows the proportion of patterned and unpatterned head capsules across larval instars based on field-collected samples. Photographs illustrate representative head capsule color and pattern variation across instars: (a) unpatterned black first instar; (b) unpatterned dark brown second instar; (c) unpatterned black third instar; (d) unpatterned brown second instar; (e) unpatterned black third instar; (f) patterned black fourth instar; (g) patterned brown fifth instar; (h) patterned dark brown fifth instar; (i) patterned black fifth instar; (j) patterned brown sixth instar; (k) patterned dark brown sixth instar; and (l) patterned black sixth instar

The inverted Y-shaped frontal suture of *S. frugiperda* larvae exhibited a clear instar-related progression in both visibility and coloration, as shown in Figure 7. In the first instar, the Y-line lacked distinct coloration and closely matched the dark head capsule, rendering the suture shape only weakly discernible (Figure 7a). By the second instar, color differentiation became more apparent, with yellowish-brown representing the dominant Y-line coloration, accompanied by less frequent brown and yellow variants (Figure 7b). In the third and fourth instar, the Y-line was consistently distinguishable from the surrounding head capsule, remaining predominantly yellowish-brown, while the relative frequency of yellow increased notably in the fourth instar (Figures 7c–d). In the later instars (instars 5 and 6), the Y-line became highly conspicuous and strongly contrasting, with pale yellow and yellowish-brown phenotypes predominating (Figures 7e–f). Overall, Y-line expression showed a progressive increase in visibility and contrast with advancing larval development, indicating a strong association between Y-line morphology and instar stage under field conditions.

Dorsal stripe development in *S. frugiperda* larvae appeared earlier and was more variable in field-collected populations than previously reported from laboratory studies (Figure 8). In early instars, most first-instar larvae lacked visible dorsal stripes (90%; Figure 8a), although faint greenish-white stripes were already present in a small proportion, and stripe visibility increased substantially in the second instar, where most larvae exhibited greenish-white or yellowish-white stripes (Figure 8b). By intermediate instars, stripe presence became consistent and diagnostically reliable, with third instars dominated by whitish green and whitish yellow stripes (Figure 8c), and fourth instars showing the highest color diversity, though greenish-white remained predominant (Figure 8d). In late instars, the dorsal stripe was clearly paler than the body and highly conspicuous, shifting mainly toward brownish-white in the fifth instar (Figure 8e), and becoming strongly dominant in the sixth instar (Figure 8f). These patterns indicate marked morphological plasticity under natural field conditions and support the dorsal stripe as a useful, albeit qualitatively variable, secondary diagnostic character across larval stages.

Lateral stripe development in *S. frugiperda* larvae was detected earlier and showed greater color variability in field-collected individuals than previously reported in laboratory-based studies (Figure 9). In the early instars, most first-instar larvae lacked visible lateral stripes, although a small proportion already exhibited yellowish-white lines (Figure 9a). Stripe expression increased in the second instar, with multiple color phenotypes observed, including yellowish-white, white, and yellow, while a subset of larvae still lacked visible stripes (Figure 9b). From the third instar onward, the lateral stripe became a consistent diagnostic feature, dominated by white and yellowish-white in the third instar (Figure 9c). In the fourth instar, stripe coloration became more variable, with yellowish-white, yellow, and white occurring at comparable frequencies (Figure 9d). In the late instars, lateral stripes were highly contrasting and clearly visible. The fifth instar showed predominance of yellowish-white with substantial color diversity (Figure 9e), whereas the final instar exhibited a strong shift toward yellow, making this coloration a reliable, high-contrast field marker for identifying late-stage *S. frugiperda* in the Bogor maize ecosystem (Figure 9f).

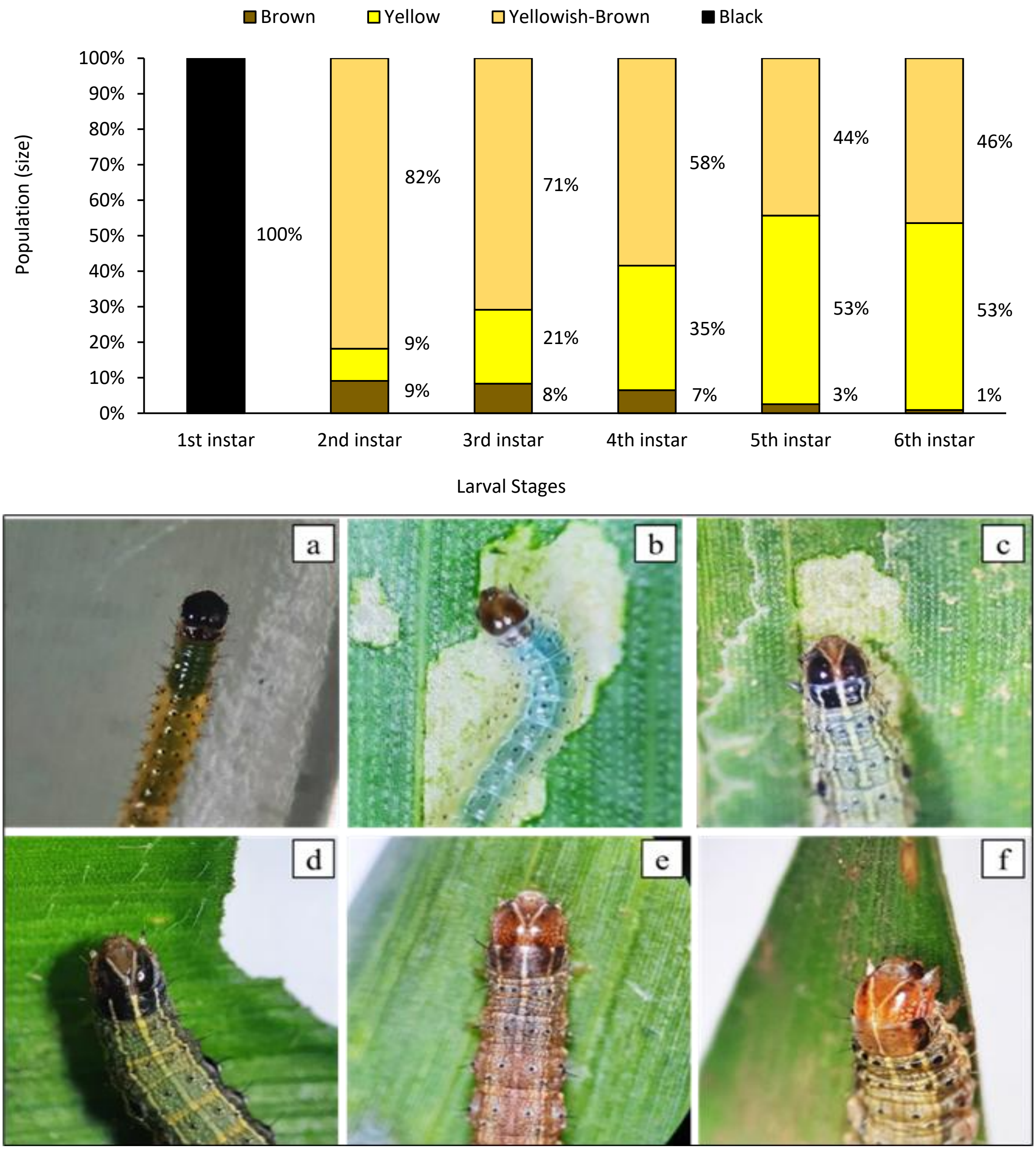

Figure 7. Color variation and morphological progression of the inverted Y-shaped suture on the head capsule of *Spodoptera frugiperda* larval instars. The stacked bar chart shows the proportion of inverted Y-suture color categories (black, brown, yellowish-brown, and yellow) observed at each instar based on field-collected samples. Photographs illustrate representative changes in visibility and coloration of the inverted Y suture across instars: (a) black and indistinguishable in the first instar; (b) faint brown line on a dark head capsule in the second instar; (c) yellowish-brown as the head cuticle lightens in the third instar; (d) distinctly yellow and clearly diagnostic in the fourth instar; (e) yellowish-brown in the fifth instar; and (f) fully visible and typically yellow in the sixth instar

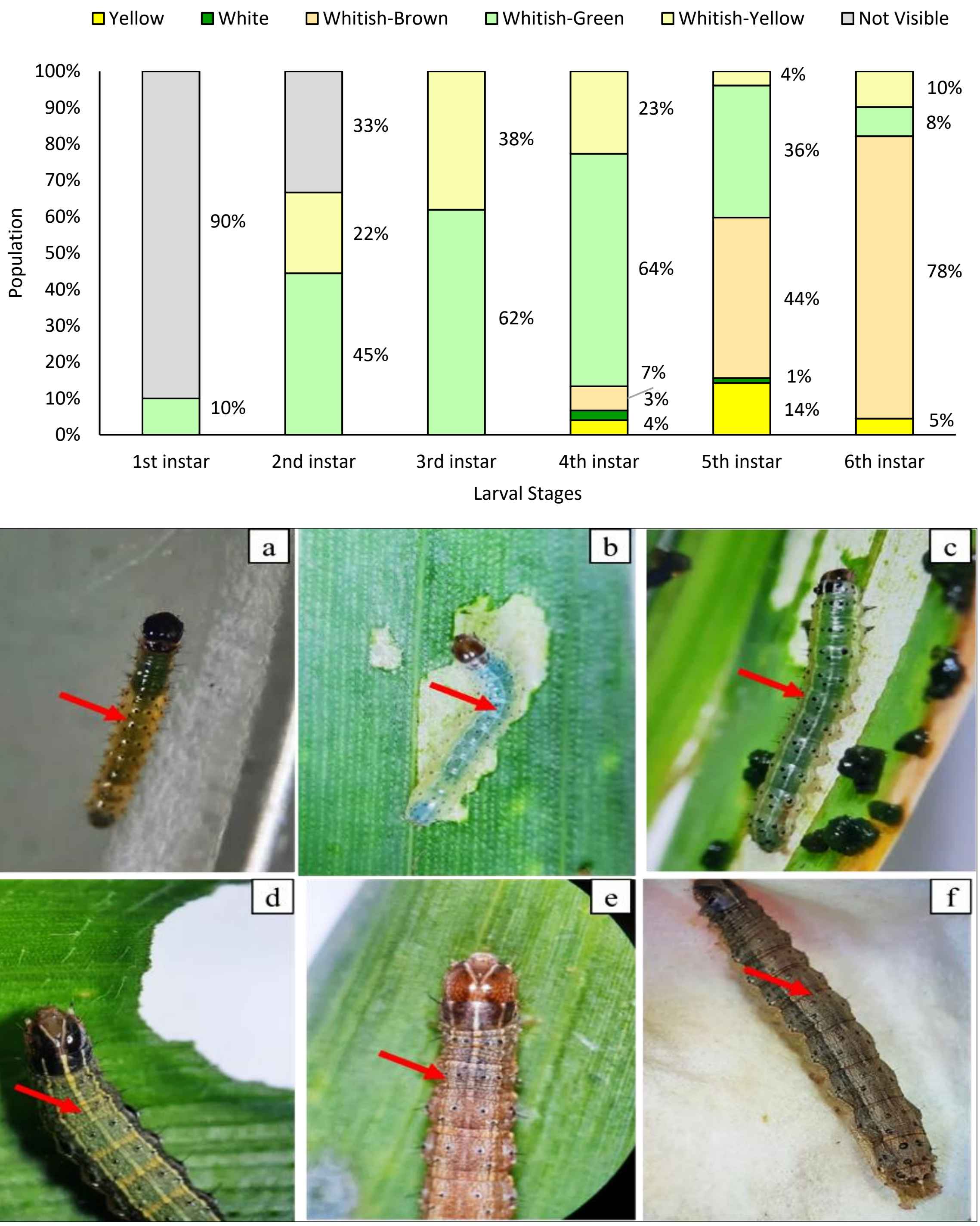

Figure 8. Color variation and developmental progression of the dorsal stripe in *Spodoptera frugiperda* larval instars. The stacked bar chart shows the proportion of dorsal stripe color categories (not visible, greenish-white, yellowish-white, yellow, white, and brownish-white) observed at each instar based on field-collected samples. Photographs illustrate representative dorsal stripe appearance across instars (indicated by arrows): (a) not visible in the first instar; (b) not visible in the second instar; (c) white in the third instar; (d) yellow in the fourth instar; (e) brownish-white in the fifth instar; and (f) brownish-white in the sixth instar

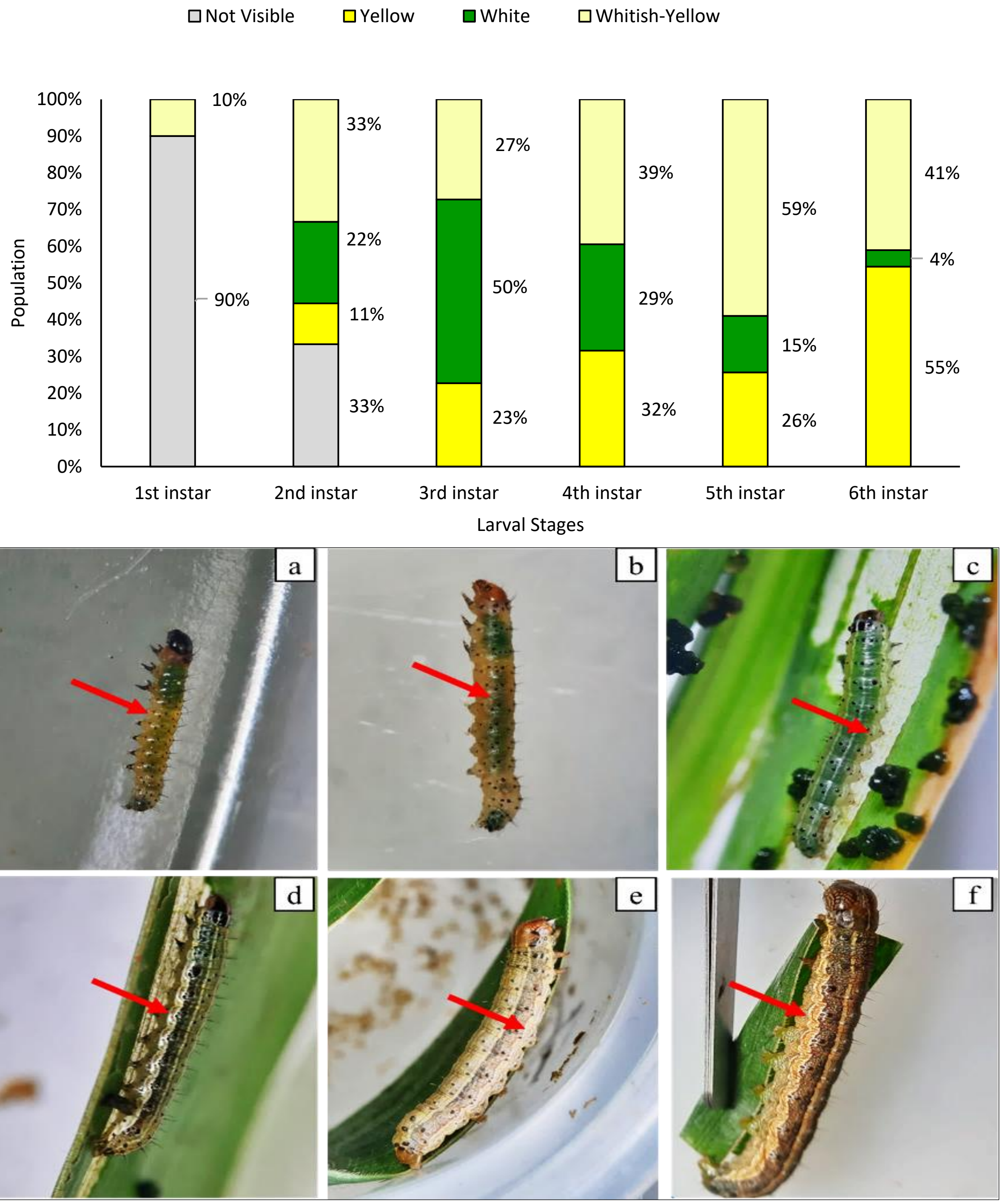

Figure 9. Color variation and developmental progression of the lateral stripes (subdorsal and lateral) in *Spodoptera frugiperda* larval instars based on field-collected samples. The stacked bar chart shows the proportion of lateral stripe color categories (not visible, yellow, white, and yellowish-white) observed at each instar, illustrating the early appearance and subsequent dominance of the lateral stripe feature. Photographs illustrate representative lateral stripe development across instars (indicated by arrows): (a) not visible or distinguishable in the first instar; (b) still not visible in the second instar; (c) clearly defined and white in the third instar; (d) distinct and white along the lateral abdomen in the fourth instar; (e) transitioning to yellow in the fifth instar; and (f) prominent yellow in the sixth instar

DISCUSSION

The present study demonstrates that HCW is a reliable and precise morphometric character for determining larval instars of *S. frugiperda* under field conditions. The clear separation of HCW ranges across six instars, along with the progressive increase in mean HCW values, strongly supports the applicability of Dyar's Law to field-collected larvae populations, which predicts a geometric increase in HCW successive larval instars (Dyar 1890). This finding is consistent with previous studies on *S. frugiperda*, which had shown that head capsule growth follows a predictable geometric progression and provides a reliable morphometric basis for instar classification (Ashley et al. 1983; Deshmukh et al. 2018; Pitre & Hogg 1983; Santos et al. 2003). In other *Spodoptera* species, including *Spodoptera exigua* and *Spodoptera* litura, that conducted which uses a similar instar separation based on HCW also reported that the width of the head capsule increases gradually between molts and in accordance with Dyar's Law (Chen & Seybold 2013; Mo et al. 2013)

This study indicates that the smaller head capsule dimensions observed in the Bogor population compared to laboratory-reared populations reported from other regions may be attributed to differences in environmental conditions, host plant quality, or rearing conditions. The larvae examined in this study (instars 1–6) were collected directly from field populations in Bogor. These individuals developed under natural conditions characterized by fluctuating temperatures and variable host plant quality, in contrast to laboratory-reared populations reported in the literature, which are more controlled in terms of environmental conditions and diet. Previous studies reported that those factors can influence FAW's biological performance (Chen et al. 2022; Nurkomar et al. 2023). Furthermore, the quality of the host plants that were fed by insects can directly affect larval and pupal size and weight (Nandhini et al. 2023). In addition, nutrient availability, including proteins, carbohydrates, and water, plays an important role in insect growth and development (Awmack & Leather 2002). Therefore, variation in host plant characteristics within the Bogor maize ecosystem may contribute to the morphological variability observed among field-collected *S. frugiperda* larvae.

Establishing a six-instar diagnostic key based on HCW is particularly important, given the high morphological variability observed in larval color and pattern traits. In this study, early instars lacked distinct markings, whereas later instars exhibited pronounced variation in head capsule coloration, inverted Y-shaped suture lines, and dorsal and lateral stripes. Such qualitative characters have often been used for rapid field identification; however, their high plasticity, as observed here, limits their reliability as standalone diagnostic markers. Environmental factors such as temperature, host plant quality, and nutritional availability are known to influence larval pigmentation and pattern expression, which may explain the wide range of phenotypes observed across sampling sites and elevations (Chen et al. 2022).

The absence of pronounced sexual dimorphism in pupal and adult morphometric traits observed in this study aligns with earlier reports indicating limited size differentiation between male and female *S. frugiperda*. The absence of significant sexual dimorphism in pupal and adult morphometry is further supported by the statistical results presented in Table 3, where independent-samples *t*-tests detected no significant differences ($P>0.05$) between male and female individuals across all measured parameters. These results indicate that sex-based size differentiation is minimal in field populations, suggesting that pupal and adult morphometric traits are of limited value for sex discrimination under natural conditions.

The combined documentation of the six-instar larval diagnostic key with the morphometric measurements of pupal and adult stages provides a baseline developmental data for the Bogor population. From an IPM perspective, this allows practitioners to move beyond simple identification. For instance, if field-collected 6th instar larvae consistently produce pupae below the established mean (14.65 mm), that may indicate suboptimal developmental conditions during the larval stage, which is similar to the findings from previous studies reported by Ashley et al. (1983), Santos et al. (2003), and Maharani et al. (2021). In other Lepidoptera species, Rostaee et al. (2021) conducted tests on the species *Leucinodes orbonalis* and stated that head capsule width is a more accurate indicator than body length for determining larval instar, as changes in head capsule size occur directly as a result of molting or ecdysis. This rigid exoskeleton is shed and replaced, increasing the size of the head capsule (Esperk et al. 2007). Thus, these morphometric data presented establish baseline measurements for the Bogor population and provide a reference for future monitoring of *Spodoptera frugiperda* populations.

Despite this variability, descriptive morphological traits still provide useful complementary information when interpreted alongside HCW measurements. The consistent appearance of the inverted Y-shaped suture line in later instars, for example, can assist preliminary field identification, while HCW measurements offer definitive confirmation. This combined approach enhances diagnostic accuracy, especially in maize ecosystems where multiple lepidopteran pests may coexist and visual misidentification can lead to inappropriate management decisions.

CONCLUSION

This study established a reliable six-instar diagnostic key for *S. frugiperda* based on head capsule width (HCW), confirming conformity with Dyar's Law under field conditions. The clear separation of HCW ranges across instars provides a robust quantitative basis for accurate instar determination, which is essential for population monitoring and optimal timing of pest management, particularly in late instars. No pronounced sexual dimorphism was detected in pupal or adult morphometric traits, indicating limited usefulness for sex discrimination in field populations and highlighting the influence of environmental and nutritional factors. Although larval color and pattern traits showed high variability, certain descriptive features, such as the contrasting inverted Y-shaped frontal suture in later instars, remain useful as complementary indicators. Overall, the integration of HCW-based diagnostics with localized morphological observations offers a practical and precise framework for managing *S. frugiperda* in Indonesian maize agroecosystems.

AUTHORS DECLARATIONS

Funding Statement
The authors did not receive any specific funding for this research.

Conflict of Interest
The authors declare that they have no competing interests.

Ethics Declarations
No ethical issue is required for this research.

Data Availability Statement
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author's Contributions
Maydiah Retno Palupi contributed to observations, data collection and curation. Dewi Sartiami contributed to the conceptualization, methodology, wrote the manuscript, reviewed, and edited. Ali Nurmansyah designed the methodology, contributed to formal analysis, wrote the manuscript, and reviewed it. Desi Septiani contributed to data analysis and manuscript editing. Ravindra Chandra Joshi reviewed the initial and final drafts of the manuscripts.

REFERENCES

Ajmal, M.S., Ali, S., Jamal, A., Saeed, M.F., Radicetti, E. & Civolani, S. 2024. Feeding and growth response of fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) towards different host plants. *Insects* 15(10): 789.

Ashley, T.R., Barfield, C.S., Waddill, V.H. & Mitchell, E.R. 1983. Parasitization of fall armyworm larvae on volunteer corn, bermudagrass, and paragrass. *Florida Entomologist* 66(2): 267-271.

Awmack, C.S. & Leather, S.R. 2002. Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology* 47(1): 817-844.

CABI. 2019a. *Community Based Fall Armyworm (Spodoptera frugiperda) Monitoring, Early Warning and Management. Training of Trainers Manual*. First Edition. Rome, Italy: FOA and Wallingford, UK: CAB International.

CABI. 2019b. *Spodoptera frugiperda* (*Fall Armyworm*). CABI Digital Library: CABI Compendium.

Capinera, J.L. 2005. *Fall Armyworm, Spodoptera frugiperda (J.E. Smith) (Insecta: Lepidoptera: Noctuidae)*. IFAS Extension (EENY098). Florida: University of Florida.

Chen, Y.C., Chen, D.F., Yang, M.F. & Liu, J.F. 2022. The effect of temperatures and hosts on the life cycle of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Insects* 13(2): 211.

Chen, Y. & Seybold, S.J. 2013. Application of a frequency distribution method for determining instars of the beet armyworm (Lepidoptera: Noctuidae) from widths of cast head capsules. *Journal of Economic Entomology* 106(2): 800–806.

Deshmukh, S., Kalleshwaraswamy, C.M., Maruthi, M.S. & Pavithra, H.B. 2018. Biology of invasive fall army worm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) on maize. *Indian Journal of Entomology* 80(3): 540-543.

Devi, R., Chandra, U., Rajak, R.K., Kumar, P., Singh, S.K. & Veer, R. 2024. Biology of fall armyworm *Spodoptera frugiperda* (JE Smith) on maize under laboratory conditions. *International Journal of Environment and Climate Change* 14(2): 463-469.

Dyar, H.G. 1890. The number of molts of lepidopterous larvae. *Psyche* 5(175–176): 420–422.

EPPO. 2015. *Spodoptera littoralis, Spodoptera litura, Spodoptera frugiperda, Spodoptera eridania:* Diagnostic Protocol, PM7/124 (1). *European and Mediterranean Plant Protection Organization* 45(3): 410–444.

Esperk, T., Tammaru, T. & Nylin, S. 2007. Intraspecific variability in number of larval instars in insects. *Journal of Economic Entomology* 100(3): 627-645.

Ganiger, P.C., Yeshwanth, H.M., Muralimohan, K., Vinaya, N., Kumar, A.R.V. & Chandrashekara, K. 2018. Occurrence of the new invasive pest, Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), in the maize fields of Karnataka, India. *Current Science* 115(4): 621-623.

Ginting, S., Zarkani, A., Wibowo, R.H. & Sipriyadi. 2020. New invasive pest, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) attacking corn in Bengkulu, Indonesia. *Serangga* 25(1): 105-117.

Goergen, G., Kumar, P.L., Sankung, S.B., Togola, A. & Tamò, M. 2016. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. *PLoS One* 11: 1–9.

King, A.B.S. & Saunders, J.L. 1984. *The Invertebrate Pests of Annual Food Crops in Central America: A Guide to Their Recognition and Control.* London, UK: Overseas Development Administration.

Kusano, E., Poapongsakorn, N., Jantarasiri, U., Pantakua, K., Tran, C.H., Tar, K.A., Kong, Phimphachanhvongsod, V. & Kobori, Y. 2025. Fall armyworm outbreak in mainland Southeast Asia: Spatial mapping and impact on regional maize production. *CABI Agriculture and Bioscience* 6(1): 0030.

Laminou, S., Moussa, O.Z., Amadou, L. & Baoua, I. 2022. Review of research main results on the fall armyworm *Spodoptera frugiperda* J.E. Smith in Africa. *International Journal of Innovation and Applied Studies* 38(2): 243-261.

Lestari, P., Budiarti, A., Fitriana, Y., Susilo, F., Swibawa, I.G., Sudarsono, H., Suharjo, R., Hariri, A.M., Purnomo & Nuryasin. 2020. Identification and genetic diversity of *Spodoptera frugiperda* in Lampung province, Indonesia. *Biodiversitas* 21(4): 1670–1677.

Maharani, Y., Puspitaningrum, D., Istifadah, N. & Ismail. 2021. Biology and life table of fall armyworm, *Spodoptera frugiperda*. (J.E. Smith) (Lepidoptera: Noctuidae) on maize and rice. *Serangga* 26(4): 161-174.

Marri, D., Mensah, S.A., Kotey, D.A., Abraham, J., Billah, M.K. & Osae, M. 2023. Basic developmental characteristics of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), reared under laboratory conditions. *Psyche: A Journal of Entomology* (1): 6917316.

Mo, H.H., Jang, K.B., Park, J.J., Lee, S.E., Shin, K.I., Lee, J.H. & Cho, K. 2013. Interactive effect of diet and temperature on instar numbers in *Spodoptera litura*, with reference to head capsule width and weight. *Journal of Asia-Pacific Entomology* 16(4): 521-525.

Mohammed, M.A., Buang, M.G., Remil, V.G., Harry, A., Adzmuri, N., Jien, F.J., Mei, N.J., Aman-Zuki, A. & Yaakop, S. 2026. COI haplotype analysis reveals predominance of corn-associated lineages of *Spodoptera frugiperda* in Sarawak, East Malaysia. *Serangga* 31(1): 191-204.

Nandhini, D., Deshmukh, S. S., Kalleshwaraswamy, C.M., Satish, K.M. & Sannathimmappa, H.G. 2023. Effect of host plants on the biology and nutritional indices of fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). *Animal Biology* 73(2): 153-170.

Nonci, N., Kalqutny, S.H., Mirsam, H., Muis, A., Azrai, M. & Aqil, M. 2019. *Pengenalan Fall Armyworm (Spodoptera frigiperda J.E. Smith): Hama Baru pada Tanaman Jagung di Indonesia*. Maros: Balai Penelitian Tanaman Serelia.

Nurkomar, I., Trisnawati, D.W., Fahmi, F. & Buchori, D. 2023. Survival, development, and fecundity of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) on various host plant species and their implication for pest management. *Insects* 14(7): 629.

Prasanna, B.M., Huesing, J.E., Eddy, R. & Peschke, V.M. 2018. *Fall Armyworm in Africa: A Guide for Integrated Pest Management*. First Edition. Mexico, CDMX: CIMMYT.

Pitre, H.N. & Hogg, D.B. 1983. Development of the fall armyworm on cotton, soybean, and corn. *Journal of the Georgia Entomological Society* 18(2): 182-187.

Rostaee, A.B, Adam, N.A & Jamian, S. 2021. Life cycle and determination of larval instars of *Leucinodes orbonalis* Guenee (Lepidoptera: Crambidae). *Serangga* 26(2): 166-182.

Santos, L.M., Redaelli, L.R., Diefenbach, L.M.G. & Efrom, C.F.S. 2003. Larval and pupal stage of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) in sweet and field corn genotypes. *Brazilian Journal of Biology* 63(4): 627-633.

Sartiami, D, Dadang, Harahap, I.S., Kusumah, Y.M. & Anwar, R. 2020. First record of fall armyworm (*Spodoptera frugiperda*) in Indonesia and its occurrence in three provinces. *IOP Conference Series: Earth and Environmental Science* 468: 012021.

Sisodiya, D.B., Ranghunandan, B.L., Bhatt, N.A., Verma, H.S., Shewale, C.P., Timbadiya, B.G. & Borad, P.K. 2018. The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae); First report of new invasive pest in maize fields of Gujarat, India. *Journal of Entomology and Zoology* 6(5): 2089-2091.

Subagyo, V.N.O., Samudera, I.M., Dadang & Hidayat, P. 2026. Temporal mating patterns of the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) populations in Indonesia: Implications for strain inference and pheromone collection. *Serangga* 31(1): 287-317.

Subiono, T. 2020. Preferensi *Spodoptera frugiperda* (Lepidoptera: Noctuidae) pada beberapa sumber pakan. *Journal Agroekoteknologi Tropika dan Lembab* 2(2): 130-134.

Sumaryati, B., Sartiami, D. & Santoso, S. 2023. Biologi dan neraca kehidupan ulat grayak jagung, *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) pada tongkol jagung muda (*Zea mays* Linn.) sebagai pakan alternatif. *Jurnal Entomologi Indonesia* 20(2): 188-188.

Trisyono, Y.A., Suputa, S., Aryuwandari, V.E.F., Hartaman, M. & Jumari, J. 2019. Occurrence of heavy infestation by the fall Armyworm *Spodoptera frugiperda,* a new alien invasive pest, in corn, Lampung, Indonesia. *Jurnal Perlindungan Tanaman Indonesia* 23(1): 156-160.