First record of fall armyworm (*Spodoptera frugiperda*) in Indonesia and its occurrence in three provinces

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**Abstract.** The occurrence of a new invasive pest species of corn, the fall armyworms *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Indonesia is reported. Survey in 3 provinces in Indonesia: West Sumatera, Banten and West Java were conducted from March to June 2019. The first record of this pest was in 26 March 2019 in West Sumatera. Larvae were collected from each of those locations and field symptoms of damage on corn in the field were observed. Larvae were brought to and reared individually in Insect Biosystematics Laboratory of Plant Protection Department, Faculty of Agriculture, IPB University. Identification of fall armyworm employed morphological and molecular analysis. Identification of the larva based on morphological characters of larvae from 3 provinces confirmed the identity of the pest. The pest as fall armyworms, while the molecular analysis indicated that Banten samples belonged to the ‘rice strain’. Collected larvae from Banten were infected by *Metarhizium rileyi*. The infested plants in the field showed the typical damage symptoms caused by the fall armyworm.

1. **Introduction**

Fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is a native invasive pest of tropical and subtropical regions in America. The pest has been confirmed reported in many countries, including India [1], Thailand, Mynamar, China, Sri Lanka [2-4] and more than 43 African countries [5]. A validation by using Species Distribution Models (SDMs), Early *et al.* stated that fall armyworm has only invaded areas that have a climate similar to the native distribution, i.e very little forest cover, a minimum annual temperature of 18-26 °C and 500-700 mm rainfall in the three wettest months [6]. South and Southeast Asia and Australia are highly suitable for fall armyworm, given the climate conditions that would allow fall armyworm to invade [6]. Furthermore, arrival via imported commodities or passenger air travel is considerable potential for the global distribution of fall armyworm in these regions, including Indonesia.

Fall armyworm consists of 2 subpopulations that differ in host plant distribution and certain physiological features but are morphologically indistinguishable [7-10]. Larvae collected from rice and corn were found differ to molecular markers, and were designated as ‘rice strain’ or ‘corn strain’, respectively. Subsequent studies discovered that the ‘rice strain’ is most consistently found in millet and grass species associated with pasture habitats, while the ‘corn strain’ prefers corn and sorghum [11-13]. Strain differences have also been reported in female pheromone composition, mating behavior and physiology, though there appear to be substantial variability or plasticity in these
phenotypes [10, 14-17]. Recently, genetic polymorphisms are the most reliable method of identifying strains. Mitochondrial haplotypes are most commonly used, with those defined by polymorphisms in the Cytochrome oxidase subunit I gene (CO1) are the best character [18-20].

The ‘corn strain’ population based on the CO1 markers can be subdivided into 2 geographically distinct subgroups on the basis of differences in the frequency of certain haplotypes [21]. Overwinter ‘corn strain’ fall armyworm in southern Texas (TX) shares the same set of haplotypes in the mitochondrial CO1 gene as those found in overwinter populations in Florida (FL), although different in their relative frequency. This haplotype distribution difference is sufficiently reproducible to allow mapping of the migratory populations that emanate from the 2 overwinter locations [22]. Overall, the TX haplotype profile is found throughout most of the Western Hemisphere, with the FL profile limited to the eastern coast of the U.S. from Maryland to Florida and extending southward to Puerto Rico and the Lesser Antilles [23-25]. Analogous haplotype differences for the ‘rice strain’ have not yet been found, so it is unclear whether this group shows a similar geographical distribution.

Fall armyworm is polyphagous, primarily targeted corn, rice and sorghum and the pest may shift subsequently to other hosts in the absence of primer host [26]. Hence, the pest could survive and maintain the population throughout the year. Montezano et al. [27] in Brazil has reported a survey assessed from 76 plant families, principally 106 species of Poaceae, 31 of Asteraceae and 31 of Fabaceae are a host of fall armyworm. In Africa, the pest has caused about 20-50% of corn production losses [28,29] and estimated economic damage from US$ 2.5-6.2 billion/year in Africa and Europe combined [30]. There have been alleged reports of fall armyworm’s infestation in Indonesia, however further comprehensive reports are still slightly understood. Therefore, this study aimed to confirm the occurrence of fall armyworm in 3 provinces of Indonesia, as well as to describe the symptoms in the field.

2. Methods

2.1. Field Survey and Species Identification
The survey was conducted in 3 provinces in Indonesia: West Sumatera, Banten and West Java, from March to June 2019. The coordinate location of the occurrence location of fall armyworm was recorded. Larvae were collected from each of those locations and field symptoms were observed. Larvae were brought to The Insect Biosystematics Laboratory of Plant Protection IPB and reared individually. The identification of fall armyworm involved morphological and molecular identifications. Morphological characteristics of larval and adult were established based on the study of fall armyworm biology by Sharanabasappa [31], while molecular analysis was carried out by using phylogenetic analysis.

2.2. Specimen collections and DNA preparation
Larval Specimens were obtained from corn plants at several corn plantations in Banten on 28 June 2019. Specimens were either air-dried or stored in ethanol at room temperature. DNA preparation was carried out using QIAGEN DNeasy Blood and Tissue Kit with the following protocol.

2.3. Characterization of the CO1
The CO1 markers are from the mitochondrial genome and so are maternally inherited. Two adjacent segments of CO1 were analyzed by DNA sequencing. The segment amplified by the CO1 primers 101F and 911R was used to identify species and fall armyworm host strain (CO1) corn-strain is designate CO1-CS, CO1 rice-strain as CO1-RS). All primers were obtained from Integrated DNA Technologies (Coralville, IA). Amplification of the CO1 barcode region was performed using primers 101F, 5'- TTCGAGCTGAAT TAGGGACTC -3' and COI-911R (5'- GATGTAAAATATGCTCGTG-3' to produce an 811 bp fragment. PCR reaction was carried out using Dream Taq PCR Mastermix (Thermoscientific), while sequencing of PCR products were conducted by a third-party company.
3. Results and discussion

The occurrence of fall armyworm invasion was found in 5 locations from the total 3 surveyed provinces in Indonesia (Table 1). Larvae and adult from those sites were confirmed as *S. frugiferda* based on morphological characteristics (Fig. 1). Mature larvae were characterized by an inverted Y-shape in yellow on the head (Fig. 1a), subdorsal and lateral lines along the body (Fig. 1b) and 4 black spots arranged in a square on the 8th abdominal segment (Fig. 1c). Larvae appeared light green to dark brown. Larvae would remain greenish or darken in color as they feed [32] and turn almost black if they were crowded by high population density and food shortage [30]. While, adult of *S. frugiferda* was characterized by the wing. The upper part of forewing was mottled (brown, light brown, dark grey) with a distinctive white patch at apex and the lower part was light gray. The hindwing was straw-colored with brown outer margin (Fig. 1d,e).

| Province       | Time         | Location                                      | Coordinate          |
|----------------|--------------|-----------------------------------------------|---------------------|
| West Sumatera  | 26 March 2019| Koto Baru, Luhak Nan Duo Sub-district, Pesaman District | 0°0’56.80”S 99°49’47.30”E |
|                | 26 March 2019| Koto Baru, Luhak Nan Duo Sub-district, Pesaman District | 0°0’2.49”S 99°52’52.80”E |
| Banten         | 27 June 2019 | Gunungkencana Village, Lebak District         | 6°33’30.96”S 106°4’2.04”E |
|                | 28 June 2019 | Cikoneng Village, Serang District             | 6°4’4.56”S 105°54’4.42”E |
| West Java      | 28 June 2019 | Kemang Sub-district, Bogor District           | 6°31’41.26”S 106°45’7.57”E |

It is still unclear whether ports as entry point introducing fall armyworm to Indonesia as both transportation links and their dispersal mechanism are highly possible. However, Early et al. [6] suggest that, if fall armyworm reaches one location in Asia or Australia, it could spread throughout the entire region using its own remarkable dispersal capacity. Adults of fall armyworm can travel several hundred kilometers in a single night by flying at an elevation of several hundred meters, which at such height winds can transport them in a directional manner [33]. In temperate states of North America, fall armyworm arrives seasonally and then dies out in cold winter months. But in much of Africa, continuous generations throughout the year have been reported due to tropical and subtropical climate [5]. As much of Indonesia falls under the tropical climate, the occurrence and establishment of fall armyworm are highly likely. Recently, the distribution of this invasive species has been recorded in most of provinces in Indonesia.

Further confirmation was established by molecular identification using phylogenetic analysis. The result showed that the species assembled from Banten was identified as *S. frugiferda* (Fig. 2). In addition, *S. frugiferda* obtained from Banten was assigned to the ‘rice strain’. This strain preferentially feeds on rice and various pasture grasses, while the other strain, ‘corn strain’, feed on corn, cotton and sorghum, although it may be geographically variable [10].
Fall armyworm larvae were found attacking corn both in generative and vegetative stages at the surveyed locations. In those locations, the plants exhibited the typical damage symptoms of fall armyworm caused by its feeding behavior (Fig. 3). Leaves showed skeletonization, windows on the lamina, margin feeding symptoms (Fig. 3a) and regular holes caused by feeding when leaves were folded (Fig. 3b). The central shoot was nibbled neatly by grown-up larva (Fig. 3c-e) and large quantities of faecal pellets or frass appeared like saw dust were seen in the whorl with mature larvae.
Figure 3. Fall armyworm symptoms damage on corn: (a) infected corn plantation area; (b) leaf damage; (c) regular holes on the leaf; (d-f) nibbled whorl by grown-up larva in different angles; (g) larva visible in the whorl; (h-i) dry frass on the outer older leaves; (j) larva feed on corn flower; (k-l) larva feed inside the corn cob.
either visible (Fig. 3f) or hidden (Fig. 3c-e). Patches of dry frass were also visible on the older outer leaves (Fig 3g,h). Some larvae were also visible fed on generative structeres of corn which were fed within the corn cob and on the corn flower.

This study also discovered that *S. frugiferda* from Banten was found infected by one of the important entomopathogenic fungi, *Metarhizium rileyi* (Fig. 4). *M. rileyi* is entomopathogenic fungi of *S. frugiferda*. Bosa *et al.* [34] reported *M. rileyi* can caused 95% mortality of the 3rd instar of *S. frugiperda*. In early stage of infection, mycelia of *M. rileyi* were velvety white to light grey (Fig. 4a), then gradually become pale green as further infection (Fig. 4b). The isolate of *M. rileyi* was obtained from infected larvae found in the corn field at Cikoneng, Banten and were brought to the laboratory for the next observation. *M. rileyi* was isolated and its growth was observed. The colonies were straw colored and slightly pale yellow as in the 3rd d of isolation. The colony growth rate in PDA were quite slow as it was also stated by Alvarez *et al.* [35]. Based on microscope observation, the vegetative and generative structures of isolated *M. rileyi* had several characteristic (Fig. 6), such as hyaline hyphae, conidiophores were straight and septate walls (Fig. 6b), while conidia were smooth, ellipsoidal (Fig. 6a) and in chains-like structure (Fig. 6b).

![Figure 4. *Metarhizium rileyi* infecting *S. frugiferda* larvae: (a) early infection on collected larvae found in the field; (b) 3 d since infection.](image)

![Figure 5. Colonies of isolated *M. rileyi*: (a) 2-d-old fungal colony culture; (b) 3-d-old fungal colony culture.](image)
Figure 6. Microscopic of *M. rileyi*: (a) conidia (400x); (b) conidiophores (400x) (scales bar: 10 µm).

4. Conclusion
Fall armyworm has invaded 3 provinces in Indonesia, e.i West Sumatera, Banten and West Java. Based on morphological identification, the species found in those location were confirmed as *S. frugiferda*. Further confirmation by molecular identification verified *S. frugiferda* from Banten is assigned to the ‘rice strain’. Larva obtained from Banten was also infected by *M. rileyi* The infested plants in the field exhibited the typical damage symptoms of fall armyworm.

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References
[1] Chormule A, Shejawal N, Sharanabasappa, Kalleshwaraswamy C M, Asokan R and Mahadeva H M 2019 *J of Ento And Zoo Stud*. 7(1) 114-117
[2] IPPC 2018 *First detection of Fall Army Worm on the border of Thailand* Accessed on https://www.ippc.int/en/countries/thailand/pestreports/2018/12/first-detection-of-fall-armyworm-on-the-border-of-thailand/
[3] IPPC 2019 *First Detection Report of the Fall Armyworm Spodoptera frugiperda (Lepidoptra: Noctuidae) on Maize in Myanmar* Accessed on https://www.ippc.int/en/countries/myanmar/pestreports/2019/01/first-detection-report-of-the-fall-armyworm-spodoptera-frugiperda-lepidoptera-noctuidae-on-maize-in-myanma/
[4] FAO 2019 *Fall Armyworm* Accessed on http://www.fao.org/fall-armyworm/background/overview/en/
[5] Prasanna B M, Joseph E, Huesing, Eddy R and Virginia M P 2018 *Fall Armyworm in Africa: A Guide for International Pest Management* (Mexico: First Edition)
[6] Early R, Moreno P G, Murphy S T and Roger D 2018 *NeoBiota* 40 25-40
[7] Pashley D P 1986 *Annals. of The Entomol. Socie. of America* 79 898-904
[8] Pashley D P and Martin J A 1987 *Annals of the Entomological Society of America* 80 731-733
[9] Pashley D P 1988 *Evolution* 42(1) 93-102
[10] Pashley D P, Hammond A M and Hardy T N 1992 *Annuals of the Entomological Society of America* 85(4) 400-405
[11] Nagoshi R N and Meagher R L 2004 *Environ. Entomol.* 33(4) 881-889
[12] Nagoshi R N and Meagher R L 2004 *Florida Entomologist* 87(4) 440-449
[13] Meagher R L and Nagoshi R N 2004 *Ecol. Entomol.* 29(5) 614-620
[14] Groot A T, Marr M, Schofl G, Lorenzs S, Svatos A and Heckel D G 2008 *Frontiers in Zoology* 5 20
[15] Kost S, Heckel D G, Yoshido A, Marec F and Groot A T 2016 *Evolution* 70(6) 1418-1427
[16] Lima E R and McNeil J N 2009 *Chemoecology* 19(1) 29-36
[17] Pashley D P, Sparks T C, Quisenberry S S, Jamjanya T and Dowd P F 1987 *Louisiana Agriculture Magazine* 30 8-9
[18] Nagoshi R N, Meagher R L, Adamczyk J J, Braman S K, Brandenburg R L and Nuessly G 2006 *J. Econ. Entomol.* 99(3) 671-677
[19] Pashley D P 1989 Host-associated differentiation in armyworms (Lepidoptera: Noctuidae): an allozymic and mitochondrial DNA perspective *Electrophoretic Studies on Agricultural Pests* ed Loxdale H D and der Hollander J (Oxford: Oxford University Press)
[20] Lu Y J and Adang M J 1996 *Florida Entomologist* 79 48-55
[21] Nagoshi R N, Silvie P and Meagher R L 2007 *J. Econ. Entomol.* 100(3) 954-961
[22] Nagoshi et al. 2008 *J. Econ. Entomol.* 101(3) 742-749
[23] Nagoshi et al. 2015 *J. Econ. Entomol.* 108(1) 135-44
[24] Nagoshi R N, Meagher R L and Hay-Roe M 2012 *Ecology and Evolution* 2(7) 1458-1467
[25] Nagoshi R N, Meagher R L and Jenkins D A 2010 *J. Econ. Entomol.* 103(2) 360-367
[26] Sharanabasappa et al. 2018 *Pest Manag. in Hort. Eco.* 24(1) 21-29
[27] Montezano et al. 2018 *Afr. Intornol.* 26(2) 286-301
[28] Day et al. 2017 *Outlooks Pest Manag.* 28(5) 196-201
[29] Feldmann F, Rieckmann U and Winter S 2019 *J. Plant Dis. Prot.* 126(5) 97-101
[30] CABI 2019 *Centre agriculture bioscience international*. Available at http://www.cabi.org/isc/fallarmyworm (Accessed on 2020)
[31] Sharanabasappa et al. 2018 *Indian J. Entomol.* 80(3) 540-543
[32] Hebert, Cywinska P D, Ball S L and DeWaard J R 2003 *Biological Science* 270 313-321
[33] Westbrook J K, Nagoshi R N, Meagher R L, Fleischer S J and Jairam S 2016 *Int. J. of Biomet.* 60 255-267
[34] Bosa et al. 2004 *Revis Colom. de Entomol.* 30 93-97
[35] Alvarez et al. 2018 *Indian J. Microbiol.* 58(2) 222-226