INTEGRATING GENOMIC, BEHAVIORAL, AND CHEMICAL EVIDENCE FOR INDONESIAN *Spodoptera frugiperda* STRAIN IDENTIFICATION AND PHEROMONE BLEND OPTIMIZATION

VANI NUR OKTAVIANY SUBAGYO

STUDY PROGRAM OF ENTOMOLOGY
FACULTY OF AGRICULTURE
IPB UNIVERSITY
BOGOR
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STATEMENT LETTER

I declare that this dissertation, entitled "Integrating Genomic, Behavioral, and Chemical Evidence for Indonesian *Spodoptera frugiperda* Strain Identification and Pheromone Blend Optimization," is my own original and authentic work conducted under the supervision of my dissertation committee. This dissertation has not been submitted to any university for the fulfillment of any degree requirements. All sources of information, whether published or unpublished, that were used or quoted in this dissertation have been properly cited and are listed in the Literature Cited chapter.

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Bogor, July 7, 2026

Vani Nur Oktaviany Subagyo
Student ID: A3601221001

SUMMARY

VANI NUR OKTAVIANY SUBAGYO. Integrating Genomic, Behavioral, and Chemical Evidence for Indonesian *Spodoptera frugiperda* Strain Identification and Pheromone Blend Optimization. Supervised by PURNAMA HIDAYAT, DADANG, and I MADE SAMUDRA

Maize (*Zea mays* L.) is a strategic commodity for the food, feed, and bioindustry sectors in Indonesia. The invasion of fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae), has increased pest pressure in maize production systems and may reduce productivity. In many regions, FAW control still relies heavily on synthetic insecticides, which can trigger resistance, resurgence, and increase residue risks. Therefore, more specific yet sustainable control approaches are needed. Sex pheromones are one of the most appropriate alternatives; however, their development requires strong local evidence because the composition and ratio of FAW sex pheromones may vary according to genetic background and geographic region.

This study aimed to build an integrated scientific basis for developing sex pheromone formulations based on the characterization of FAW populations in Indonesia through several stages: (i) measuring the percentage of attacked maize plants and damage intensity, and characterizing populations using the mitochondrial markers *cytochrome c oxidase subunit I* (COI) and *cytochrome c oxidase subunit II* (COII), and the nuclear gene *triose phosphate isomerase* (*Tpi*); (ii) analyzing population identity and structure based on whole-genome sequencing (WGS); (iii) observing various parameters related to mating behavior; (iv) quantifying pheromone components using gas chromatography–flame ionization detection (GC–FID) to determine the local pheromone blend ratio of Indonesian populations; and (v) preparing synthetic pheromone formulations based on the local pheromone blend, followed by laboratory testing and field evaluation.

Field surveys were conducted at 21 maize-growing locations in Banten, West Java, and Central Java Provinces. The results showed that the percentage of FAW attack varied widely, ranging from 11 to 94%. Meanwhile, damage intensity was generally within the range of 25–50%, corresponding to the moderate damage category, with three locations showing severe damage, namely Kuningan, Pemalang, and Brebes. These findings emphasize the importance of sex pheromone-based monitoring systems to provide early-warning signals for potential FAW population outbreaks under uneven attack distribution.

Population identity was analyzed using COI, COII, and *Tpi* from 41 FAW populations across Java, Sumatra, Sulawesi, and Nusa Tenggara. The analysis showed low haplotype and nucleotide diversity, consistent with the characteristics of invasive populations. However, discordance was detected between the mitochondrial markers and *Tpi*. Diagnostic *single-nucleotide polymorphism* (SNP) in *Tpi* showed that all analyzed individuals were aligned with the corn strain, whereas the mitochondrial markers still showed rice-strain signals in Indonesian FAW populations.

WGS-based genomic analysis of 19 FAW populations from Java, Sumatra, Sulawesi, and Nusa Tenggara strengthened the inference that Indonesian FAW populations are strongly aligned with the corn strain and belong to an invasive

cluster that is genetically close to populations from Africa and India. In addition, WGS analysis also revealed signals of local differentiation in certain genomic regions, particularly on chromosome 25.

Mating behavior observations were conducted on 9 FAW populations from Lampung and East Java Provinces. The results showed that dominant copulation activity occurred in the early dark phase or scotophase, especially 3–6 h after the onset of scotophase. This period was established as the operational window for pheromone-gland sampling in female FAW. In addition to copulation timing, this study recorded other mating behavior parameters, including copulation success of 53.05%, copulation frequency of 0–3 times per pair, precopulatory period of 1.30–2.78 days, copulation duration of 1.20–2.05 h, and male and female age at copulation, averaging 1.9 and 2.4 days, respectively. This information is important as a basis for understanding the reproductive behavior of FAW as an invasive pest in Indonesia.

The chemical basis of FAW sex pheromones in Indonesian populations was established from 9 populations originating from Lampung, West Java, and East Java Provinces. Pheromone gland extracts from unmated female adults (virgin) were analyzed using GC–FID for three main acetate components, namely (Z)-7-dodecenyl acetate (Z7–12:OAc), (Z)-9-tetradecenyl acetate (Z9–14:OAc), and (Z)-11-hexadecenyl acetate (Z11–16:OAc). The analysis showed that total pheromone amount varied according to female age and timing within the scotophase. To ensure comparability across populations, the reference extraction window was established using 3-day-old virgin females sampled at the 5th hour of scotophase. The main finding of this study was the establishment of a local pheromone blend reference for FAW sex pheromones in Indonesian populations with a ratio of 80:16:4 (Z9–14:OAc:Z11–16:OAc:Z7–12:OAc).

Candidate synthetic blends were then designed based on the local pheromone reference and prepared as formulations for laboratory and field testing. Wind tunnel and field evaluations showed that male FAW moths responded to several candidate pheromone formulations, with the most stable cross-season performance shown by formulation C (80:15:5). This result positions formulation C as a relevant operational starting blend for further development as a pheromone lure for FAW monitoring and control in Indonesia.

Overall, this study establishes an Indonesia-specific translational pipeline for FAW pheromone development by integrating field attack ecology, sequence-based genetic characterization, genome-wide population analysis, nocturnal reproductive behavior, and pheromone chemistry. This framework links invasion biology, strain interpretation, behavioral standardization, local pheromone profiling, and candidate blend evaluation to support pheromone-based monitoring and future mass trapping strategies within integrated pest management programs for maize in Indonesia.

Keywords: genomic analysis, pheromone blend, pheromone monitoring, pheromone profiling, reproductive behavior

RINGKASAN

VANI NUR OKTAVIANY SUBAGYO. Integrasi Bukti Genomik, Perilaku, dan Kimia untuk Identifikasi Strain dan Optimasi *Blend* Feromon *Spodoptera frugiperda* di Indonesia. Dibimbing oleh PURNAMA HIDAYAT, DADANG, dan I MADE SAMUDRA

Jagung (*Zea mays* L.) merupakan komoditas strategis bagi sektor pangan, pakan, dan bioindustri di Indonesia. Invasi ulat grayak jagung (UGJ), *Spodoptera frugiperda* (Lepidoptera: Noctuidae), telah meningkatkan tekanan hama pada sistem produksi dan berpotensi menurunkan produktivitas. Di banyak wilayah, pengendalian UGJ masih didominasi oleh insektisida sintetis, yang dapat memicu resistensi, resurjensi, dan meningkatkan risiko residu. Oleh karena itu, diperlukan pendekatan pengendalian yang lebih spesifik namun berkelanjutan. Feromon seks merupakan salah satu alternatif yang tepat, namun dalam upaya pengembangannya diperlukan bukti lokal yang kuat, karena komposisi dan rasio feromon seks UGJ dapat bervariasi menurut latar belakang genetik dan wilayah geografisnya.

Penelitian ini bertujuan membangun dasar ilmiah terintegrasi untuk pengembangan formulasi feromon seks berdasarkan karakterisasi populasi UGJ di Indonesia melalui beberapa tahapan: (i) pengukuran persentase tanaman jagung terserang dan intensitas kerusakannya, dan karakterisasi populasi menggunakan penanda mitokondria *cytochrome c oxidase subunit I* (COI) dan *cytochrome c oxidase subunit II* (COII), dan gen inti *triose phosphate isomerase* (*Tpi*); (ii) analisis identitas dan struktur populasi berbasis *whole-genome sequencing* (WGS); (iii) pengamatan berbagai parameter terkait perilaku reproduksi; (iv) kuantifikasi komponen feromon dengan *gas chromatography–flame ionization detection* (GC–FID) untuk menetapkan rasio *blend* lokal populasi Indonesia (*local pheromone blend*); dan (v) formulasi feromon sintetis berbasis *local pheromone blend* yang dilanjutkan dengan pengujian di laboratorium dan evaluasi lapangan.

Survei lapangan dilakukan di 21 lokasi pertanaman jagung di Provinsi Banten, Jawa Barat, dan Jawa Tengah. Hasil survei menunjukkan bahwa persentase serangan UGJ sangat bervariasi (11–94%). Sementara itu, intensitas kerusakan umumnya berada pada kisaran 25–50% (kategori sedang), dengan tiga lokasi mengalami kerusakan berat, yaitu Kuningan, Pemalang, dan Brebes. Temuan ini menekankan pentingnya sistem pemantauan berbasis feromon seks untuk memberikan sinyal peringatan dini terhadap potensi ledakan populasi UGJ yang sebaran serangannya tidak merata.

Identitas populasi dianalisis menggunakan COI, COII, dan *Tpi*, dari 41 populasi UGJ di Pulau Jawa, Sumatra, Sulawesi, dan Nusa Tenggara. Hasil analisis menunjukkan keragaman haplotipe dan nukleotida yang rendah, konsisten dengan karakteristik populasi invasif. Namun, terdapat ketidaksesuaian antara hasil penanda mitokondria dan *Tpi*. Polimorfisme nukleotida tunggal (*single-nucleotide polymorphism*, SNP) diagnostik pada *Tpi* menunjukkan bahwa seluruh individu yang dianalisis berafiliasi dengan strain jagung, sedangkan penanda mitokondria masih menunjukkan sinyal strain padi pada populasi UGJ di Indonesia.

Analisis genom berbasis WGS terhadap 19 populasi UGJ dari Pulau Jawa, Sumatra, Sulawesi, dan Nusa Tenggara memperkuat inferensi bahwa populasi UGJ Indonesia berafiliasi kuat dengan strain jagung dan berada dalam klaster invasif

yang berkerabat dekat secara genetik dengan populasi dari Afrika dan India. Selain itu, analisis WGS juga menunjukkan sinyal diferensiasi lokal pada wilayah genom tertentu, terutama pada kromosom 25.

Pengamatan perilaku reproduksi UGJ dilakukan pada 9 populasi dari Provinsi Lampung dan Jawa Timur. Hasil pengamatan menunjukkan bahwa aktivitas kopulasi dominan terjadi pada awal fase gelap atau skotofase, terutama 3–6 jam setelah awal skotofase. Waktu tersebut ditetapkan sebagai jendela operasional pengambilan sampel kelenjar feromon pada betina UGJ. Selain waktu kopulasi, penelitian ini juga mencatat parameter perilaku reproduksi lainnya, yaitu keberhasilan kopulasi sebesar 53,05%, frekuensi kopulasi 0–3 kali per pasangan, periode prakopulasi 1,30–2,78 hari, durasi kopulasi 1,20–2,05 jam, serta umur jantan dan betina saat kopulasi, masing-masing rata-rata 1,9 dan 2,4 hari. Informasi ini penting sebagai dasar pemahaman perilaku reproduksi UGJ sebagai hama invasif di Indonesia.

Dasar kimia feromon seks UGJ populasi Indonesia ditetapkan dari 9 populasi yang berasal dari Provinsi Lampung, Jawa Barat, dan Jawa Timur. Hasil ekstraksi kelenjar feromon imago betina yang belum kopulasi (*virgin*) dianalisis menggunakan GC–FID untuk 3 komponen asetat utama, yaitu (Z)-7-dodecenyl acetate (Z7–12:OAc), (Z)-9-tetradecenyl acetate (Z9–14:OAc), dan (Z)-11-hexadecenyl acetate (Z11–16:OAc). Hasil analisis menunjukkan bahwa jumlah total feromon bervariasi menurut umur betina dan waktu pada periode skotofase. Untuk menjamin keterbandingan lintas populasi, ditetapkan jendela rujukan ekstraksi pada betina *virgin* berumur 3 hari dengan pengambilan kelenjar pada jam ke-5 skotofase. Temuan utama penelitian ini adalah penetapan rujukan *local pheromone blend* UGJ populasi Indonesia dengan perbandingan 80:16:4 (Z9–14:OAc:Z11–16:OAc:Z7–12:OAc).

Kandidat *blend* feromon sintetis kemudian dirancang berdasarkan rujukan feromon lokal dan disiapkan sebagai formulasi untuk pengujian laboratorium dan lapangan. Hasil pengujian *wind tunnel* dan lapangan menunjukkan bahwa ngengat jantan UGJ merespons sejumlah kandidat formulasi feromon, dengan kinerja paling stabil lintas musim ditunjukkan oleh formulasi C (80:15:5). Hasil ini menempatkan formulasi C sebagai *blend* awal operasional yang relevan untuk dikembangkan lebih lanjut sebagai umpan feromon bagi pemantauan dan pengendalian UGJ di Indonesia.

Secara keseluruhan, penelitian ini membangun *pipeline* translasi spesifik Indonesia untuk pengembangan feromon UGJ dengan mengintegrasikan ekologi serangan lapangan, karakterisasi genetik berbasis sekuens, analisis genomik, perilaku reproduksi nokturnal, dan kimia feromon. Kerangka ini menghubungkan biologi invasi, interpretasi strain, standardisasi perilaku, profil feromon lokal, dan evaluasi kandidat *blend* feromon untuk mendukung pemantauan berbasis feromon serta strategi perangkap massal dalam Pengendalian Hama Terpadu (PHT) jagung di Indonesia.

Kata kunci: analisis genomik, *blend* feromon, pemantauan feromon, perilaku reproduksi, profil feromon

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VANI NUR OKTAVIANY SUBAGYO

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Non-Committee Examiner in the Closed Examination:
1. Dr. Sri Utami, S.P., M.Si.
2. Dra. Dewi Sartiami, M.Si., Ph.D.

Non-Committee Examiner in the Doctoral Promotion:
1. Dr. Delicia Yunita Rahman, M.Si.
2. Dr. Ir. Ruly Anwar, M.Si.

Title of Dissertation : Integrating Genomic, Behavioral, and Chemical Evidence for Indonesian *Spodoptera frugiperda* Strain Identification and Pheromone Blend Optimization

Name : Vani Nur Oktaviany Subagyo

Student ID : A3601221001

Endorsed by
Advisory Committee

Principal Supervisor:
Prof. Dr. Ir. Purnama Hidayat, M.Sc.

Co-Supervisor:
Prof. Dr. Ir. Dadang, M.Sc.

Co-Supervisor:
Dr. Ir. I Made Samudra, M.Sc.

Acknowledged by

Head of Study Program of Entomology:
Prof. Dr. Ir. Dadang, M.Sc.
NIP. 196402041990021002

Dean of Faculty of Agriculture:
Prof. Dr. Ir. Suryo Wiyono, M.Sc.Agr.
NIP. 196902121992031003

Closed Examination Date: April 29, 2026

Conferral Date: 07 JUL 2026

PREFACE

This dissertation, entitled "Integrating Genomic, Behavioral, and Chemical Evidence for Indonesian *Spodoptera frugiperda* Strain Identification and Pheromone Blend Optimization," addresses important scientific and practical needs in managing the invasive fall armyworm in Indonesian maize production systems. This work integrates field ecology, genetics and genomics, behavioral observations, and chemical ecology to support strain identification and the development of sex pheromone-based tools within an integrated pest management (IPM) framework. The dissertation research was conducted from 2022 to 2025. Field activities were carried out in maize-growing areas across Sumatra, Java, Sulawesi, and East Nusa Tenggara, while field trials were conducted at the Taman Bogo Experimental Farm, Indonesian Soil and Fertilizer Engineering and Testing Institute (ISFETI). Laboratory and experimental activities, including molecular and genomic analyses, behavioral assays, pheromone extraction and chemical analysis, as well as insect rearing were conducted at the Genomics Laboratory, National Research and Innovation Agency (BRIN), and at the Cikeumeuh laboratory, Center for Agricultural Biotechnology and Genetic Resources Assembly and Modernization (BRMP Biogen).

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Bogor, July 7, 2026

Vani Nur Oktaviany Subagyo

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES