Hexapoda (*Insecta indica*) 10
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# Moth pheromone trapping system components in effective management of trans-boundary pests: Case study of Fall Army worm.

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**ABSTRACT**: Moth pheromones are most commonly used in agri-horticultural pest monitoring and mass trapping systems globally. Their pheromone blend constituents and ratios might vary with intra-species diversity associated with habitat-linked and/or geographical differences in populations. When moths migrate and invade new territories, there is an impending need to deploy trap-lure combinations which provide the most efficient trapping of the local target populations, so to more reliably map their distribution/invasion across wide areas, as well as effective tools for containing their local population build up and carry over to next season.

The complexity of the task of location-specific deployment of the appropriate pheromone blend composition/loading for moths could be best illustrated by case study of the Fall Armyworm-*Spodoptera frugiperda* (J. E. Smith), which has migrated all the way from North America via Africa to Australasia in the last few years. This mini-review seeks to illustrate aspects critical to overall understanding of main factors besides updating on more recent research outcomes.

The various R&D thrusts needed are country-wise characterization of local variation in blend composition/ratios of pheromones emitted by native female populations, besides assessing the relative response to pheromone blend ratios/loadings in candidate/commercial pheromone lures, along with molecular taxonomic mapping of intra-species genetic diversity.

The scope for developing local blend testing kits as user-friendly decision support backup system to implement this strategy is also indicated.

**Key words:** Invasive moths, pheromone blend, population variation, blend optimisation.

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***Lepidoptera-pheromones as management tool*:**

Lepidopteran pheromones have been used for insect monitoring, mass trapping, and mating disruption of a great diversity of insect pests (Wyatt 1998). Subsequent to the identification of the first moth pheromone, bombykol ((*E*10, *Z*12)-10, 12-hexadecadien-1-ol) from females of the silkworm moth-*Bombyx mori* (Butenandt *et al.,* 1959), we have the most extensive knowledge of the volatile sex pheromonal communication in Lepidoptera with sex pheromones having been identified from more than 600 species of Lepidoptera.

The hitherto published information on the pheromones of moths is voluminous, and the great majority of the female moth emitted attractants because of their economic importance (Ando 2012).Pheromone-baited traps have been used in a wide variety of ways for more sustainable pest management, including seasonal phenology, population estimation, and decision support, as well as early detection and delimitation of invasive species (Allison and Carde, 2016). For most of the invasive Lepidoptera, the mechanisms of assortative mating between species have been based on some specialisation at species level in the female-produced pheromone versus male response system - like change in pheromone blend composition and/or temporal partitioning of female pheromone release (calling) (Dopman *et al.,* 2004).

***Geographic variation in pheromonal response in Lepidoptera***

Geographic variation in sexual communication systems has been reported in several lepidopteran species and is of interest because changes in the sex pheromone signal and/or response to sex pheromones could result in reproductive isolation and subsequently may lead to speciation (Phelan 1992). Furthermore, geographically varying sexual communication is of interest for pest management, as many lepidopteran insects are pest species which are commonly monitored, disrupted or killed via pheromone mediated methods( Witzgall *et al.,* 2010),

***Invasive moth species-Fall Army worm as model:***

Among noctuid moths, the subfamily Heliothinae (also known as owlet moths) represent an excellent model system for examining divergence of traits associated with pheromone production, detection, and processing in closely related species, across 2528 genera and approximately 365 species (Cho *et al.,* 2008). Among Pyralid moths, the European corn borer (ECB), *Ostrinia nubilalis,* (Crambidae), represent a notorious migrant moth pest across North America, Europe and North Africa, whose sex pheromone polymorphism (Lassance, 2010) has also been a useful model for studying the initial stages of speciation.

As an invasive and polyphagous noctuid moth attacking a wide variety of crops throughout the Nearctic and Neotropical Western Hemisphere (Sparks 1979), the Fall Army worm (FAW), *Spodoptera frugiperda* (J.E. Smith), is known to occur in the same region as two strains that are defined by their host plant preferences (reviewed in Nagoshi and Meagher 2004),with corn strain feeding on corn and sorghum (corn strain) and the rice strain feeding on rice and forage grasses (Pashley 1986).,FAW has migrated into Europe in 1988 and recently Africa in 2016, whereafter it has invaded India in 2018 and reached China via Myanmar in 2019, finally reaching Japan in 2020,and can best illustrate a model system of intra-species diversity in pheromone responses in highly invasive moth species ( Nagoshi and Meagher 2004).

***Early studies on FAW pheromone constituents*:**

The first sex pheromone component of *S. frugiperda* identified was (Z)-9-tetradecenyl acetate (Z9-14:Ac) (Sekul and Sparks, 1967), while later studies have reported several additional components ( Tumlinson *et al.,* 1986; Descoins *et al.,* 1988), wherein. Tumlinson *et al.,* (1986) provided a baseline on analyses of extracts of pheromone glands and of volatiles from calling female fall armyworm moths, which revealed the presence of seven compounds: dodecan-l-ol acetate, (Z)- 7-dodecen-l-ol acetate, ll-dodecen-l-ol acetate, (Z)-9-tetradecenal, (Z)-9- tetradecen- 1-ol acetate, (Z)- 1 l-hexadecenal, and (Z)- 11-hexadecen- 1-ol acetate. They also demonstrated that *volatiles emitted by calling females differed from the gland extract 4*in that the two aldehydes were absent. Field tests were conducted with sticky traps baited with rubber septa formulated to simulate blend with the same component ratios as those emitted by calling females demonstrated that both (Z)-7-dodecen-1-ol acetate and (Z)-9-tetradecen-1-ol acetate are required for optimum activity.

***Early studies with FAW commercial lures:***

Mitchell et.al.(1985) studied a four component pheromone blend that consisted of (percentage by weight) (Z)-7-dodecen-l-ol acetate (Z7-12:AC), (0.45%), (Z)-9-dodecen-l-ol acetate (0.25%), (Z)-9-tetradecen-l-ol acetate (Z9-14:AC) (81.61%), and (Z)-ll-hexadecen-l-ol acetate (17.69%) was highly effective when tested as a lure for the FAW when formulated at 2 mg total pheromone blend in rubber septa, polyvials, or microtubules. The IP moth trap baited with the four-component pheromone blend (2 mg) on rubber septa dispensers was thereafter used to capture FAW moths over a wide geographic area covering French Guiana to Canada, so confirming their usefulness for surveys.

There are also reports of commercial blends blends that proved successful in trapping fall army worm males in North America and Europe, but performed poorly when tested in Brazil (Cruz *et al.,* unpublished), Costa Rica (Andrade *et al.,* 2000), and Mexico (Malo *et al.,* 2001). The lack of response among Brazilian fall armyworm males to European and North American lures may have been caused by geographic variability in the sex pheromone of *S. frugiperda*, among two morphologically indistinguishable hostplant-specific strains (Pashley *et al.,* 1985, 1992; Pashley, 1986; Levy *et al.,* 2002) reported to be present in Brazil (Busato *et al.,* 2002).

***Studies in early 2000s***

Batista-Pereira *et al.,* (2006) reported several studies which have shown intraspecific geographical variation in the composition of sex pheromones. It was based on the experience that pheromone lures from North America and Europe were not effective against FAW and so they examined the composition of the sex pheromone produced by females from Brazilian populations. Virgin female gland extracts showed the presence of nine acetate constituents-namely,(Z)-7-dodecenyl acetate (Z7-12:Ac), (E)-7-dodecenyl acetate(E7-12:Ac), dodecyl acetate, (Z)-9-dodecenyl acetate, (Z)-9-tetradecenyl acetate (Z9-14:Ac), (Z)-10-tetradecenyl acetate, tetradecyl acetate/(Z)-11-tetradecenylacetate (Z11-16:Ac), and (Z)-11-hexadecenyl acetate, in relative proportions of 0.8:1.2:0.6:traces:82.8:0.3:1.5:12.9, respectively. This is the first report of E7-12:Ac from the pheromone gland of *S. frugiperda*. Only three compounds, Z9-14:Ac, Z7-12:Ac, and E7-12:Ac, elicited antennal responses, and there were no differences in catch between traps baited with either binary blend-Z7-12:Ac + Z9-14:Ac or trinary blend- Z7-12:Ac + Z9-14:Ac + Z11-16:Ac blends. However, the Z7-12:Ac + Z9-14:Ac + E7-12:Ac blend was significantly better than Z7-12:Ac + Z9-14:Ac, indicating that E7-12:Ac is an active component in the sex pheromone of the Brazilian populations..

***Findings in recent decade***

Meagher *et al.,* (2013) studied traps baited with 4 different commercial sex pheromone lures that contained different numbers of components- from Scentry(4) , Trécé(2), and Suterra(3)- sold to capture male fall armyworm in rubber septa lures ,tested in Alabama, Georgia, Florida, and Texas during 2006-2009. While each lure released the number of components expected, Trécé lure was found to release relatively higher amounts of the minor component Z7-12:Ac and at a higher percentage of its blend, than the other lures. The 4 lures attracted similar numbers of moths in Alabama, Georgia, and Texas, but in Florida, the lures caught significantly different number os moths in one season .but not significantly different in another season.

Studies by Meagher and Nagoshi (2013) on the attraction of wild male fall army worm moths in Florida with traps baited either with a commercial sex pheromone lure or corn and rice strain females obtained from laboratory colonies showed that commercial pheromone lure attracted over four times more males than virgin corn or rice strain females. Both corn and rice strain females attracted a higher percentage of rice strain males, providing evidence that the commercial lure was biased to attract corn strain males and underestimated rice strain relative to corn strain numbers. It was also shown that corn and rice strain males were attracted more to corn strain females than rice strain females, although there was variation in relative response according to location and season.

Unebehend *et al.,* (2013) observed some consistent strain-specific differences in the sexual communication system of *S. frugiperda*, wherein laboratory and field females showed strain-specific pheromone differences in their relative amount of Z7-12:OAc and Z9-12:OAc. While males were not attracted to females of their own strain in wind tunnel assays, apparently due to differential calling times of the females, differential attraction of males was found in the field. In both corn field and grass fields, both corn- and rice-strain males were more attracted to synthetic corn-strain blend than synthetic rice-strain blend, with males of both strains showing strain-specific responses to Z7-12:OAc, clarifying that strain-specific differences in sexual communication may be marginal and not be sufficient to cause assortative attraction in FAW.

Recent studies by Canas Hoyas *et al.,* (2017) showed that FAW in Colombia has diverged into strains in maize and rice as their most frequent hosts and reproductive isolation was manifest with females of the corn strain rarely mating with males of the rice strain, while females of the rice strain mated with both strains. They examined the volatile compounds for both strains, considering the time of extraction and male stimulation in production of metabolites and found that the most relevant were the pheromones (*Z*)-9-tetradecenyl acetate and (*Z*)-7-dodecenyl acetate, while 11 constituents were exclusive for the rice strain and eight for the corn strain, nine were common to both strains. A detrended correspondence analysis associated a group of compounds with each strain, while no significant differences were found in the abundance of compounds in common.

***Recent field studies on invasion and blend ratio in India***

The occurrence of FAW in India was first reported from Devanagere, Shivamogga and Chitradurga districts of Karnataka in 2018 (Ganiger *et al.,* 2018; Mahadeva samy *et al.,* 2018;]. The pest was reported mainly on maize in Maharashtra, Tamil Nadu, Andhra Pradesh and Telangana states of India during 2018.,while their occurrence on other crops like sugarcane sweet sorghum and sorghum were also observed later(Chromule *et al.,* 2019a, b). Continuous generations of FAW, throughout the year, which was reported in Africa, apparently could have been favoured by the tropical and subtropical climates in India, leading possibly to inter-strain overlaps in distribution between and within host range, besides the spatio-temporal diversity in elative dispersal/colonisation/carry over attributes of the invasive FAW populations in India.

More recent initiatives by Bhanu *et al.,* (2020) to identify the optimum local blend ratio for FAW populations in South India included field and laboratory experiments. The field studies showed that pheromone blend with Z-9-tetradecenyl acetate, Z-11-hexadecenyl acetate and Z-7-dodecenyl acetate in 87: 12.5 : 0.5 ratio attracted and trapped significantly highest number of male moths compared to other five blends tested in funnel traps, although the same six different pheromone blends compared for Electroantennogram (EAG) responses with male antenna elicited statistically on par responses among them. Tests of different dosages in the rubber septa lures using the optimum attracting blend showed that 2 mg loading trapped highest number of moths, but was statistically on par with 3 mg pheromone loading. Further field studies showed that lures with 2 mg loading of pheromone blend remained effective in attracting and trapping male moths up to 60 days.

***Recent studies in China.***

Following the invasion of FAW to China via Myanmar in 2019 and subsequent rapid spread in a dozen provinces in China, Jiang and Wang (2019) undertook identifying the sex pheromone of the invaded populations of FAW and using the sex pheromone for its population monitoring and control. While both the strains were morphologically indistinguishable and shared the same gland extract components, but had strain-specific ratios of pheromone components. They prioritised identifying the sex pheromone of the invaded populations of FAW and study of olfactory coding mechanisms of males of FAW to pheromone blends between the two stains of FAW and outcomes of the hybrids.

***Most recent studies in Japan***

Since FAW has recently invaded Japan via Africa, South Asia and China, studies by Wakamura *et al.,* (2021) with gas chromatography/mass spectrometry analyses of hexane extracts of abdominal glands of Okinawan females revealed six candidate compounds for sex pheromone, (*Z*)-9-tetradecenyl acetate (Z9-14:Ac, ca. 6 ng/female), (*Z*)-11-hexadecenyl acetate, (*Z*)-11-tetradecenyl acetate, (*Z*)-7-dodecenyl acetate (Z7-12:Ac), (*E*)-9-dodecenyl acetate, and (*Z*)-9-tetradecen-1-ol at the ratio of 100:10:1.3:0.90:0.13:1.8, respectively. While only small numbers of males were captured with the original blend of these compounds in Okinawa, much more males were attracted to a 100:3 blend of Z9-

14:Ac and Z7-12:Ac which had been shown to be the most effective blend in a Florida population. In another test in Okinawa, even more males were captured with a 100:1 blend of these compounds than with a 100:3 blend, showing that the more powerful and convenient sex pheromone formulation to monitor *S. frugiperda* populations in Okinawa was a 100:1 blend of Z9-14:Ac and Z7-12:Ac.

**Table.1.Major Fall Army Worm pheromonal constituents reported in Pherobase data**

| Country (location) | Acetates | Aldehydes | Total constituents | Blend ratio observed | Reference |
|---|---|---|---|---|---|
| Brazil | 7 | Nil | 7 | 82.8:12.9:1.5:1.2:0.8:0.6:0.3 | Batista-Pereira *et al.*, 2006 |
| Mexico | 1 | 1 | 4 | NA | Malo *et al.*, 2002 |
| NM | Nil | 1 | 1 | NA | Meagher *et al.*, 2001 |
| Central America | 2 | Nil | 2 | 99.6: 0.4 | Andrade *et al.*, 2000 |
| NM | 3 | 1 | 4 | 80.3:19.2:10.0:0.5 | Meagher *et al.*,1998b |
| North America | 9 | Nil | 9 | 73.6:16.6;3.6:1.2:1.1:0.53: 0.50:0.43: 0.21 | Descosins *et al.*,1988 |
| North America | 4 | 2 | 6 | 69:13:9:4:3:2 | Tumlinson *et al.*,1986 |
| North America | 4 | Nil | 4 | 81.61:17.69:0.45:0.25 | Mitchell*et al.*,1985 |
| North America | 2 | Nil | 2 | 10:1 | Jones & Sparks,1979 |
| North America | 2 | Nil | 2 | NA | Mitchell & Doolittle,1976 |
| NM | 1 | Nil | 1 | NA | Sekul & Sparks,1967 |
| NM | 2 | Nil | 2 | NA | Warthen & Jacobson,1967 |

**NA=Not available NM=not mentioned**

**Table2. Individual FAW pheromone constituents (and associated ones) Phero base data**

| INDIVIDUAL CONSTITUENTS | OTHER COSTITUENTS ASSOCIATED |
|---|---|
| Z-11-16Ac | +6Ac, +2Ac&1Ald, +8Ac, +3Ac&2Ald, +3Ac |
| Z-11-16-Ald | +4Ac&1Ald |
| Z-11-14Ac | +8Ac |
| Delta11-12Ac | +3Ac&2Ald |
| Z-10-14Ac | +6Ac |
| Z-9-14Ac | +6Ac,+1Ald(?),+1Ac**,+2Ac&1Ald,+8Ac,+3Ac&2Ald,+3Ac, |
| Z-9-14Ald | +4Ac+1Ald |
| E-9-14Ac | +8Ac |
| Z-9-12Ac | +7Ac, +8Ac, +3Ac, +1Ac, |
| Z-9-E12-14Ac | +1Ac |
| Z-7-12Ac | +7Ac,+1Ac,+2Ac&1Ald,+8Ac,+3Ac&2Ald,+3Ac, |
| E-7-12Ac | +6Ac |

**=Three occasions

**Table: 3. Variation in FAW pheromone blend component ratios: 1976-2013 literature**

| Pheromone component | (Z)-9-tetradecen-1-yl acetate | (Z)-7-dodecen-1-yl acetate | (Z)-9-dodecen-1-yl acetate | (Z)-11-hexadecen-1-yl acetate | (E)-7-dodecen-1-yl acetate | (Z)-11-hexadecen-1-yl aldehyde |
|---|---|---|---|---|---|---|
| Code* | Z9-14:Ac | Z7-12:Ac | Z9-12:Ac | Z11-16:Ac | E7-12:Ac | Z11-16:Al |
| FAA 1 | 81.6 | 0.5 | 0.3 | 18.0 | - | - |
| FAA 2 | 79.0 | 5.0 | 2.0 | 10.0 | - | 3.0 |
| FAA 3.1 | 96.6 | 3.4 | - | - | - | - |
| FAA 3.2 | 92.9 | 3.3 | - | 3.8 | - | - |
| FAA 3.3 | 91.9 | 3.0 | 1.7 | 3.4 | - | - |
| FAA 3.4 | 88.3 | 3.2 | - | 3.6 | - | - |
| FAA 3.5 | 86.9 | 3.1 | 1.7 | 3.5 | - | - |
| FAA 4.1 | 92.9 | 3.3 | - | 3.8 | - | - |
| FAA 4.2 | 90.1 | 3.2 | - | 2.6 | - | - |
| FAA 4.3 | 91.9 | 3.2 | - | 2.7 | - | - |
| FAA 4.4 | 96.6 | 3.4 | - | - | - | - |
| FAA 4.5 | 91.9 | 3.0 | 1.7 | 3.4 | - | - |
| FAA 4.6 | 94.4 | 3.3 | - | - | - | - |
| FAA 4.7 | 94.4 | 3.3 | 2.3 | - | - | - |
| FAA 4.8 | 90.1 | 3.2 | 2.2 | 2.6 | 2.6 | - |
| FAA 5.1 | 1.00g | 0.01g | - | - | - | - |
| FAA 5.2 | 1.00g | - | - | - | 0.01g | - |
| FAA 5.3 | 1.00g | 0.01g | - | - | 0.01g | - |
| FAA 6 | - | - | 100 | - | - | - |
| FAA 7 | 2.0 | - | 98.00 | - | - | - |

*Sources:

FAA 1- Tumlinson *et al.,* (1986) cited fby Malo *et al.,* (2013),
FAA 2- Table 2 of Tumlinson *et al.,* (1986).
FAA 3.1-3.5- Table 3 of Tumlinson *et al.,* (1986.)
FAA 4.1-4.8 - Table 4 of Tumlinson *et al.,* (1986)
FAA 5.1-5.3 - From Fig.9-Batista-Pereira *et al.,* (2006)
FAA 6- Fig-1- Test- 2- Mitchell (1976)
FAA 7- Fig-3- Mitchell (1983)

***In conclusion:***

The present mini-review illustrates the complexity of pheromone blend composition in FAW as a case study for future R&D thrusts towards maximising the reliability of pheromone lures used in tracking the spread of the migrant populations within a country and in preventive quarantine monitoring. The scope for developing and availing local blend ratio response testing kit with promising alternative blend ratios/loadings is evidently great for not only such invasive moth species , but even in non-invasive moths which evolve into geographically distinct populations towards maximising their survival with intra-species level biodiversity.

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