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Case study on the first immigration of fall armyworm, *Spodoptera frugiperda* invading into China



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Abstract

The fall armyworm (FAW), *Spodoptera frugiperda* is one of the most damaging crop pests, and it has become major threat to the food security of many countries. In order to monitor possible invasion of this pest into China, a searchlight trap was established in March 2018 in western Yunnan Province, China, where it has served as the “first station” for many pests that have migrated from Myanmar to China. A number of suspected FAW moths were captured and identified by DNA sequencing. The results showed that the FAW moth was first captured on December 11 and formed its first immigration peak in mid-December 2018. DNA detection revealed that the early invading FAW population was the “corn-strain”. The field survey indicated that the pest mainly colonized corn in Pu'er, Dehong and Baoshan areas. Migration trajectory simulation implied that the moths might have mainly come from the eastern area in the mid-latitude region of Myanmar (20–25°N, 94–100°E). This case study confirmed the first immigration of FAW into China, and will be helpful for guiding monitoring and management work to control this pest.

Keywords: *Spodoptera frugiperda*, Yunnan Province, China, invasion, accumulated temperature, migration trajectory

1. Introduction

The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) is native to tropical and subtropical regions of the Americas (Sparks 1979), where it is recognized as one of the most damaging crop pests. FAW breeds continuously in tropical and subtropical regions of the Americas, but also migrates into temperate North America for summer-breeding. The caterpillars of FAW can damage more than 350 species of plants, including many major crops, such as corn, sorghum, rice, wheat, barley, buckwheat,

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oat, millet, ryegrass, soybean, tobacco, tomato, potato, peanut, cotton, sugar beet, alfalfa and onion (Montezano *et al.* 2018).

As a notorious pest, FAW can cause severe yield losses in many economically important crops. The preferred host of FAW is corn, and it generally causes yield losses between 15–73% (Day *et al.* 2017). The newly-invasive FAW populations damaged corn with reported average losses of 26.6% in Ghana and 35% in Zambia (Day *et al.* 2017).

FAW consists of two strains, the “corn-strain” and the “rice-strain”. The former strain prefers corn, cotton, and sorghum, while the latter prefers rice and pasture grasses (Dumas *et al.* 2015). The two strains are identical in morphology but differ in host range, mating behavior and pheromone compositions (Pashley 1986; Groot *et al.* 2008), and can be clearly distinguished by molecular techniques (Lu *et al.* 1994; Lu and Adang 1996).

In January 2016, FAW was first discovered in West Africa (Nigeria and Ghana) (Goergen *et al.* 2016), and since then it has spread to almost all countries in sub-Saharan Africa (Stokstad 2017). In May 2018, FAW was discovered in Karnataka in southwest India (Sharanabasappa *et al.* 2018), and by late 2018 FAW outbreaks had been found in additional southeastern Asia countries, such as Bangladesh, Myanmar and Thailand (Guo *et al.* 2018).

To get a head-start on the possible invasion of FAW to China, we established a monitoring schedule since March 2018 in Yunnan, a province neighboring Southeast Asia. Yunnan is the “first station” for many important pests migrating from southeastern Asia into China and thus is an important location for monitoring and forecasting migratory pests. For example, in early-mid April every year, the important migratory rice pests *Nilaparvata lugens* (Bao *et al.* 2018), *Sogatella furcifera* (Shen *et al.* 2011; Wu *et al.* 2017) and *Cnaphalocrocis medinalis* (Bao *et al.* 2015) start migrating into Yunnan Province by southwestern flow from Southeast Asia, and subsequently northward into Central and northern China.

Although the larval damage in Yunnan Province (Yang *et al.* 2019) and the subsequent northward expansion over other provinces (autonomous regions, municipalities) in China have been widely reported (Jing *et al.* 2020), the first immigration of adult *S. frugiperda* and possible source was still obscure. Here we reported the evidence of light-trapping, molecular analysis, and field survey for the first invasion of adult *S. frugiperda* and analyzed the possible source area of immigrants. The case study confirmed the exact date of the first invasion of FAW and its possible source, which is useful for making better decision in monitoring and controlling this pest in the future.

2. Materials and methods

2.1. Searchlight trapping

In order to detect the FAW invasion as early as possible, in Lancang (22°30′26″N, 99°53′13″E), Yunnan Province, one searchlight trap was set up to monitor possible FAW invasion from South and Southeast Asia since March 2018 (Fig. 1). The searchlight trap, equipped with a 1-kW metal-halide lamp, can attract insects flying up to or above 500 m above the ground level (Feng *et al.* 2009). The searchlight trap was turned on at sunset and turned off at sunrise every day. The trapped insects were funneled into a nylon net bag (60 mesh), killed by freezing at –20°C, and then further identified.

2.2. Species identification of FAW

In addition to the morphological identification, the suspected FAW moths captured in November (5 moths) and December (12 moths) of 2018 were identified by DNA analysis (Nagoshi *et al.* 2017). For this, we used the mitochondrial cytochrome c oxidase subunit 1 (COI) and the sex-linked triosephosphate isomerase (*Tpi*) genes to identify the moth species and the corresponding strain type. For DNA analysis, total genomic DNA of individual suspected FAW was isolated with the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer’s instructions. Partial fragments of the COI and the sex-linked *Tpi* genes were amplified to confirm FAW identification. The COI barcode region sequencing was performed using primers 101F (5′-TTCGAGCTGAATTAGGGACTC-3′) and 911R (5′-GATGTAAATATGCTCGTGT-3′) (811 bp). The *Tpi* gene segment used the primers 282F (5′-GGTGAATCTCCCCTGCTATG-3′) and 850R (5′-AATTTTATTACCTGCTGTGG-3′) (500 bp) (Nagoshi *et al.* 2017). PCR runs were performed with each specific primer pair using 2× Goldstar *Taq* Master Mix in a 25-μL PCR reaction (CWBIO, Beijing, China). PCR parameters were as follows: an initial denaturation step (95°C for 10 min); followed by 35 cycles of 95°C for 1 min, 55°C for 40 s, 72°C for 1 min; and a final extension of 10 min at 72°C. The PCR products were examined by 2% agarose gel electrophoresis, and the positive fragments were sent to the Beijing Genomic Institute (Beijing, China) for sequencing in both directions. All the assembled sequences were subjected to molecular identification using the online NCBI BLAST (<https://www.ncbi.nlm.nih.gov/>). Multiple sequence alignments of the *Tpi* gene sequences of FAW from the “corn-strain” and “rice-strain”, and the strain captured in Lancang, were generated using MEGA 6.0 Program with the default gap penalty parameters.

2.3. Field survey

Various crops that might be damaged by FAW caterpillars are planted in Yunnan Province. In order to elucidate the host preference of FAW under low population density in Yunnan, we surveyed several crops (corn, rice, sugar cane, sorghum, rape, Chinese cabbage, garden cabbage, artemisia, leek, sweet potato, strawberry and banana) and weeds for any damage due to FAW from the 16th to 23rd January in Ruili (within the Dehong area), and on the 18th and 22nd January in Jiangcheng (within the Pu'er area), which is about 200 km away from the searchlight trapping site (Lancang) (within the Pu'er area) (Fig. 1). The surveys were accomplished according to the five-point sampling method and the larval instar stages and corresponding numbers of caterpillars were recorded.

After the first identification of FAW in Yunnan, all plant protection stations of Yunnan launched wide-range field surveys in January 2019, under instructions from the National Agro-Tech Extension and Service Center of China. The survey procedure, implemented on the main crops (such

as corn, rice, sugar cane and sorghum), was the same as that described above.

2.4. Inferring immigrating dates

It is very possible that the first batch of immigrating FAW moths could not be captured by the searchlight trap due to the scarcity of immigrant FAW moths in the early stages. Thus, the possible dates of the earliest invading FAW moths were inferred based on the ages of field caterpillars and accumulated temperatures. For this, we assumed that the FAW moth would oviposit immediately after landing and the oldest age of larvae found in the field was used to start the calculation. For this calculation, the development threshold of FAW was set as 12.57°C (Schlemmer 2018), and the accumulated temperatures were set as 35.73, 47.14, 26.86, 21.58, 24.78, 28.53, and 32.57 degree days for egg, 1st, 2nd, 3rd, 4th, 5th, and 6th instars, respectively (Schlemmer 2018).

The accumulated temperature was calculated by tools contributed by Agricultural and Natural Resources, University



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Fig. 1 Location of Yunnan Province, China and sites of light trapping (Lancang) and field surveys (Ruili and Jiangcheng). Colored areas (Dehong, Pu'er and Baoshan) are where the *Spodoptera frugiperda* larvae were found in January 2019. The first reported dates of *S. frugiperda* caterpillars found in Bangladesh, Myanmar and Thailand are given below the country names.

of California, USA (<http://ipm.ucanr.edu/WEATHER/ddretrievetext.html>). Meteorological data (daily max and min temperatures) of the station nearest to the searchlight and survey sites that were used to calculate accumulated degree days, were downloaded from National Climatic Data Center, National Oceanic and Atmospheric Administration (<https://gis.ncdc.noaa.gov/maps/ncei/cdo/daily>).

2.5. Source area analysis

Source areas of the FAW that invaded Yunnan were determined by backward trajectory simulation. The inferred oviposition dates or dates with FAW moths captured by searchlight trapping were set as trajectory simulation dates. In the backward trajectory simulation, FAW flight altitudes were set as 200, 500, 800, 1 200, 1 500 and 2 000 m above the ground level. As FAW is a nocturnal pest, the valid takeoff times were set as 1900, 2000, and 2100, and the landing times 0500, 0600 and 0700. Local time used was Beijing Time (UTC+8 h). The location of the survey field or searchlight trap was set as the end location. The flight speed of FAW was set the same as the wind speed. The max flight duration of FAW was limited to 13 h.

The simulation of backward migration trajectories was analyzed by the TrajStat module in Meteoinfo (version 1.3.3) (Wang 2014). The TrajStat module calculates trajectories using the method employed in the Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) (Stein et al. 2015). HYSPLIT was developed by the National Oceanic and Atmospheric Administration Air Resources Laboratory of the United States, and has been used to simulate migratory trajectories of many

insect species (e.g., Qi et al. 2014; Westbrook et al. 2016; Hu et al. 2017). In trajectory simulation, the meteorological data sets of Global Data Assimilation System (GDAS) with a global resolution of 1° latitude× 1° longitude, were obtained from the National Center for Environmental Prediction, National Oceanic and Atmospheric Administration of the United States.

3. Results

3.1. Molecular identification and analyses

FAW moths identified by morphological criteria were further verified by sequence analysis of segments of the mitochondrial *COI* gene and the sex-linked *Tpi* gene. The expected fragments of all suspected specimens captured in November 2018 were failed to amplify. Of the 12 suspected specimens trapped in December 2018, the expected fragments of eight of them were amplified and sequenced successfully. Based on the BLAST analyses of the obtained sequences in the NCBI GenBank Database, the eight samples were identified as FAW, supported by 99–100% similarity in sequence data and coverage. The *Tpi* region can also be used to distinguish the two FAW host strains (“corn-strain” and “rice-strain”). Based on polymorphism characteristics in the *Tpi* gene, all specimens were from “corn-strain” (Fig. 2).

3.2. Immigration dynamics of FAW

By morphological identification and DNA detection, all suspected specimens captured before December 2018

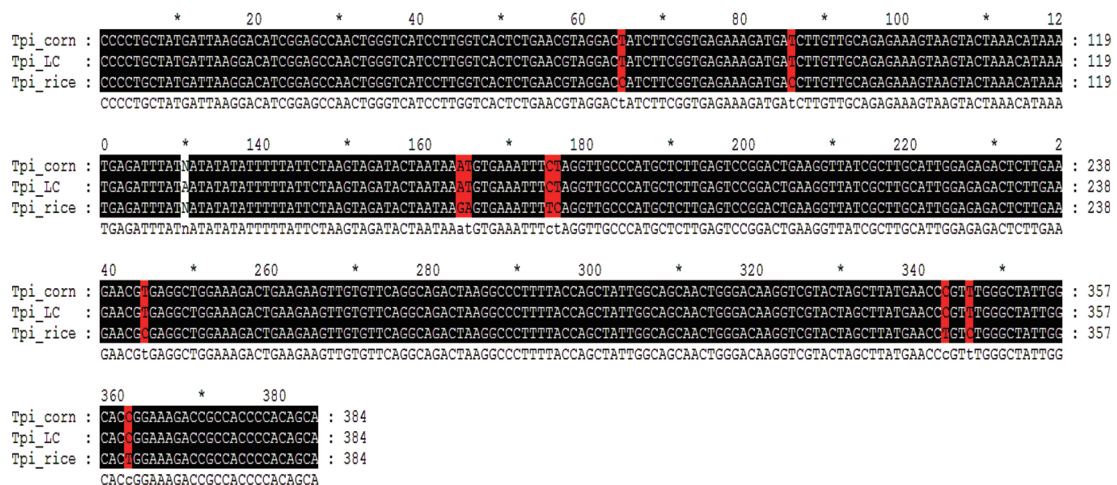


Fig. 2 Strain identification of *Spodoptera frugiperda* moths trapped in Lancang based on haplotype analysis of *Tpi* gene fragments. Tpi_corn, Tpi_rice and Tpi_LC represent the *Tpi* gene sequences of “corn-strain”, “rice-strain” and the strain captured in Lancang, respectively. “Corn-strain” or “rice-strain”-specific polymorphic loci are marked in red.

were excluded as FAW moths. The earliest date of a clearly confirmed FAW moth was 11th December 2018 (Fig. 3). The numbers of captured FAW moths were very low and irregular in December 2018 and February 2019. However, after February 2019, the number of trapped moths was much higher than that in the previous two months (Fig. 3). The uptrend of moths under searchlight trapping, and the first reported dates of FAW in Bangladesh (mid-November 2018), Myanmar (mid-December 2018) and Thailand (mid-December 2018), together indicate that the moths trapped in mid-December 2018 represented the peak of the first batch of FAW invading into Yunnan. In January 2019, FAW caterpillars were discovered in west and south Yunnan counties by local plant protection stations (Fig. 1), which confirms the successful invasion of FAW into China.

On the day with the first captured FAW moth (11th December 2018), there were only one male and one female moth caught in total. In the early invasion period (from 11th to 18th December 2018), the sex ratio of moths was generally equal to 1 (with six males and four females). In the next 64 days (from 19th December 2018 to 20th February 2019), only eight moths were caught (four males and four females). In the subsequent 55 days (from 21st February

to 16th April 2019) when the number of moths caught was much higher than previously, the number of females was obviously greater than the number of males, with a sex ratio of 0.69 (53 males and 77 females).

3.3. Inferred invasion dates

Based on the development threshold, larval ages, and accumulated temperatures, we inferred the probable invasion period of FAW into Ruili and Lancang. The last generation of FAW moths producing the caterpillars in the corn field, generally invaded Yunnan during the 14th–23rd of December in Ruili, and during the 12th–15th of December in Jiancheng (Table 1). The inferred invasion dates of FAW moths for both Ruili and Jiancheng are generally in accordance with dates of moths trapped by the searchlight in Lancang (Fig. 3). The inferred invasion dates of FAW indirectly confirmed the accuracy of the results of searchlight trapping.

3.4. Source areas of FAW immigrating into Yunnan

For FAW moths landing at Ruili during 14th–23rd December,

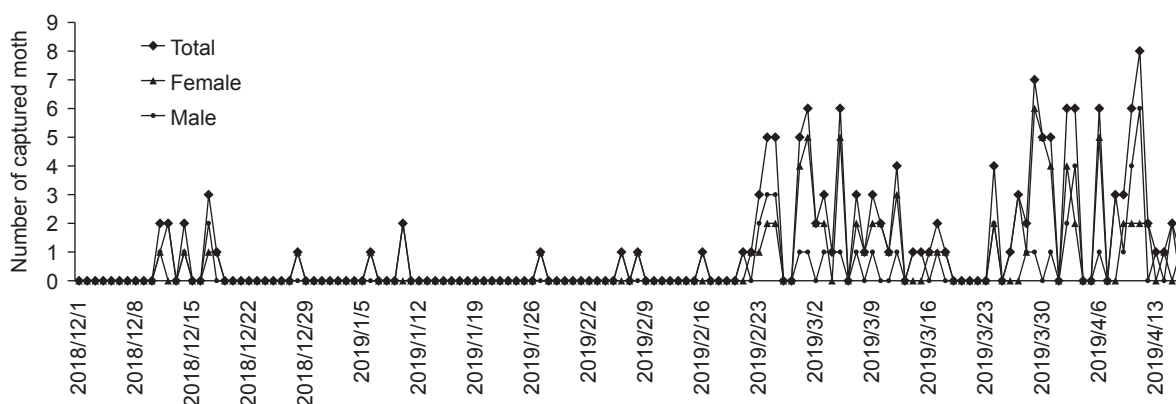


Fig. 3 Population dynamics of immigrating *Spodoptera frugiperda* moths (males and females) under searchlight trap in Lancang, Yunan Province, China.

Table 1 Inferred oviposition dates of invading fall armyworm (FAW), *Spodoptera frugiperda* moths in Ruili and Lancang

Location	Survey date in 2019	Instar with most caterpillars	Max. instar stage	Inferred oviposition date of FAW moths in 2018
Ruili	16th Jan.	2nd	4th	15th Dec.
	17th Jan.	2nd	4th	15th Dec.
	18th Jan.	2nd	2nd	23rd Dec.
	19th Jan.	2nd	3rd	20th Dec.
	20th Jan.	2nd	4th	17th Dec.
	21st Jan.	3rd	5th	14th Dec.
	22nd Jan.	1st	4th	18th Dec.
	23rd Jan.	2nd	5th	15th Dec.
Jiangcheng (near Lancang)	18th Jan.	3rd	5th	12th Dec.
	22nd Jan.	2nd	5th	15th Dec.

the majority of endpoints for the backward trajectories were distributed in the eastern area in the mid-latitude region of Myanmar (22–25°N, 95–98°E), which neighbors Yunnan Province, with a few endpoints located in part of the Indian region between Myanmar and Bangladesh (Fig. 4). The average distance of the backward trajectory routes is about 100 km away Ruili, with the farthest being about 550 km. FAW caterpillars were first reported in late November 2018 in Bangladesh, and in mid-December 2018 in Myanmar (Fig. 1), which means that the moths invading Yunnan might have come directly from Myanmar or indirectly from Bangladesh by multiple re-emigrations.

Combining the inferred dates from field larval stages (in Jiangcheng) and dates of captured moths from the searchlight trap (in Lancang), we simulated the backward trajectory of the FAW invasion from 11th to 18th December 2018 in Lancang. The endpoints of the backward trajectory were also scattered in the eastern area in the mid-latitude region of Myanmar (20–23°N, 96–100°E) (Fig. 4), while these endpoints were located in a much more southern region than that for Ruili. The endpoints of backward trajectory were, on average, about 300 km from Lancang,

with the farthest distance being about 600 km.

3.5. Host preference of early invading FAW

After field surveying, the local plant protection stations of Yunnan Province found that FAW caterpillars were detected only in Pu'er (13th January), Dehong (14th January) and Baoshan (18th January) areas in January 2019. To elucidate the host preference of FAW when invading into a new region, our field survey found that FAW caterpillars damaged no crops or weeds other than corn, which means that the FAW caterpillars preferred corn under the low population density conditions. During the survey period, the corns were at growing stages of 7th to 12th leaf, which is suitable for FAW caterpillar development. The larval stage with the greatest number in the corn fields was generally the 2nd instar (Table 1). The oldest age of FAW caterpillars surveyed in Ruili and Lancang ranged from 2nd to 5th instars (Table 1).

4. Discussion

The invasion of FAW into China threatens the food



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Fig. 4 Endpoints of backward trajectories of invading *Spodoptera frugiperda* moths in Ruili (14th–23rd December) and Lancang (11th to 18th December), Yunnan Province, China.

production of China and eastern Asia, and should be dealt with seriously. In this study, we monitored the invasion process of FAW into Yunnan, China. Our results showed that the first batch of FAW immigrating into China landed in west and south of Yunnan by air transport in the middle of December 2018. The invading FAW mainly came from the eastern side of Central Myanmar, which neighbors Yunnan Province. In addition to Myanmar, all other countries in Southeast Asia are also all-year reproductive regions for FAW, and can provide continuous streams of FAW moths migrating into China. Thus, the international cooperation on FAW control among all eastern Asian countries is both necessary and urgent.

FAW may invade China from Southeast Asia by two main migratory routes: the western pathway, mainly from Myanmar; and the eastern pathway, mainly from Indochina (Li *et al.* 2020). Yunnan Province is primarily on the western pathway. Immediately after discovering FAW in Yunnan, a wide ranging field survey was initiated in other southern provinces of China (Guangxi, Guangdong, Hainan) that are generally on the eastern migratory route (Fig. 1). By 11th March 2019, Guangxi Province was reported as the second province with FAW invasion, which supports the idea that FAW first invaded China by the western pathway rather than the eastern pathway (NATESC 2019). FAW was found earlier on the western migratory pathway than on the eastern one, which might be because the direction of FAW expansion was from west to east in Asia (from Africa to Asia) during its first invasion. When FAW successfully breeds in the whole range of southern Asian countries in the future, the main route or the earliest route by which FAW migrate into China should be unveiled. According to spatiotemporal characteristics of the East Asian monsoon, FAW might mainly migrate into China by the western route in March, April and May, and by both routes in June, July and August in the future (Wu *et al.* 2019). Influenced by many factors (such as numbers and distribution of FAW populations in source areas, and meteorological conditions), the direction and pace of FAW migration from the Indochina Peninsula might be complex, and this needs to be further investigated in the future.

The main sources of the first batch of invading FAW moths were inferred to be near Yunnan Province and at the eastern area in the mid-latitude region of Myanmar. In the Mandalay region (Fig. 4), which is at the center of Myanmar, FAW caterpillars were first found on 19th–20th December 2018 (Khin *et al.* 2019). The Mandalay region is the main corn growing region of Myanmar (Li *et al.* 2019), which could directly supply emigrating moths for the invasion of China. Due to the lack of investigation data, whether the source regions near Yunnan Province are the areas of direct

emigration could not be ascertained.

Many species of insects carry out similar seasonal long-distance migrations in East Asia, including many of the most serious crop pests, such as the oriental armyworm (*Mythimna separata*) (Ruilo and Ziangshi 1987), beet armyworm (*Spodoptera exigua*) (Feng *et al.* 2003), rice leaf roller (*C. medinalis*) (Riley *et al.* 1995) and brown planthopper (*Nilaparvata lugens*) (Riley *et al.* 1994). Thus, for comprehensive forecasting of all the important migratory pests, the pace and direction of migration patterns of these pests need to be compared in the future.

In this study, we used field caterpillar age and accumulated temperatures to infer oviposition dates and the source region of a migratory pest. From the results of the trajectory simulation, we found that this method is reasonable and acceptable for inferring the immigration dates when direct landing dates were not available, especially when the pest migrates into a new region. When applying accumulated temperatures for inferring immigration dates, the accumulated temperatures of pests in different regions may vary widely. However, for migratory insects, the accumulated temperatures can be used for a broad region within the same migration arena, such as in the East Asian migration arena. In this study, the accumulated temperatures of FAW were measured on Africa FAW populations, but not on local populations. Since FAW has recently invaded from Africa into Asia within the past three years, the accumulated temperature of the invaded FAW population probably changed very little. In the future, the accumulated temperature for Asian FAW populations should be determined when the recently-colonized FAW have adapted to the Asia environment.

The acceptable accuracy of results in the present study demonstrated that the methods used here are reliable for monitoring the early invasion of a migratory insect. In the future, the invasion events of migratory insects may increase greatly with the increased intercontinental trade. The various methods used here can be used to monitor other potential migratory insects and discover their presence in early stages of invasion.

Due to the consecutive hosts (corn, sugarcane, rice and etc.), suitable climate conditions and the East Asian summer monsoon, Southeast Asia would appear to be an ideal region for providing numerous FAW populations that could migrate into China. Meanwhile, the numbers of FAW moths captured by the searchlight trap obviously increased in March and April in Yunnan (Fig. 3), indicating that FAW may have successfully colonized the countries in Southeast Asia or South China. At present, FAW mainly damage to corn, sugarcane and sorghum in China, and the potential damage to other crops, needs to be determined.

5. Conclusion

Here we clarified that the first batch of the FAW population migrating into China peaked in the middle of December 2018, and may have mainly come from the eastern area in the mid-latitude region of Myanmar (20–25°N, 94–100°E). The early invading FAW population was the “corn-strain” and it successfully colonized the Pu'er, Dehong and Baoshan areas. Our results can improve our understanding of the migration system of FAW in Asia, and provide knowledge for developing a management strategy to control this pest in China.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

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