BIOLOGICAL EVIDENCES FOR THE ESTABLISHMENT OF ALIEN PECTINOPHORA GOSSYPIELLA SAUNDERS (LEPIDOPTERA: GELECHIIDAE) IN THE YANGTZE RIVER REGION OF CHINA

GUL ZAMIN KHAN*, KONGMING WU**, MUHAMMAD AMIN* and ZAFAR MEHMOOD***

* Nuclear Institute for Food and Agriculture (NIFA), Peshawar – Pakistan.
** Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing – China.
*** Department of Maths, Stats & Computer Science, Agricultural University, Peshawar – Pakistan.

ABSTRACT

Biological behaviors of Pakistani and Chinese populations of Pink bollworm, Pectinophora gossypiella (Saunders), were studied at State Key Laboratory for Biology of Plant Disease and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China during 2006 for seeking evidence of the hypothesis that this alien specie has been introduced indiscriminately into China from Pakistan and established successfully in the Yangtze River Region of China. The pink bollworm populations collected from the various cotton growing regions (Sindh and Punjab) of Pakistan (PP) and (Yangtze River) China (CC) were subjected to mating in no and free choice tests. The mating behaviors of the two different strains were compared and mating compatibility along with the age effect on mating between these strains was investigated. All the biological parameters between the two strains differed significantly except for the hatching period was found at par for the two populations. Results indicated successful mating compatibility between the strains with no adult’s sterility. Fecundity was significantly higher in the inter strains crosses PP×CC followed by CC×PP over intra strain PP×PP and CC×CC crosses. The sex ratio affected fecundity significantly and increased parallel with increasing sex number. The mating percentage of these strains was significantly reduced with increasing age. However, the fecundity of the young and old moth’s crosses up to age of 8 days was at par as compared with the same age crosses. The rituals, feeding and mating activities of these two different strains were studied by using harmless red dye on the wings for identity. Results on the mating behaviors showed no significant difference between the strains in all the tests including reciprocal crosses. In all the tests the male initiated fluttering and upwind movement for finding the female partner. The mating time was recorded from 01:30 to 7:00 after a series of visual and videocassette record (VCR) tests. The current studies highlighted the evolution, possible wide adoptability and invasive nature of this pest. Therefore, these findings can be helpful in redesigning the management strategy based on the random mating of the overlapping and migrating generations of the different invasive strains of pink bollworm in the cotton growing areas of the world.

Key Words: Biological behavior, Mating, Pink bollworm, compatibility, fecundity, invasive strain, videocassette record

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INTRODUCTION

Cotton is an important cash-generating crop. The pink bollworm, Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechiidae), is considered to be the major insect pest in all the cotton growing area of Pakistan and now in Yangtze River Region of China also. According to CAB International Institute of Entomology (1990), pink bollworm is one of the most serious lepidopteran pests of cotton worldwide. It has concealed mode of feeding and therefore, the use of insecticides is often futile, because the entire P. gossypiella larval development is completed in the internal part of the infested bolls (Naranjo et al., 2001). Its infestation become epidemic particularly in middle and late growing season of cotton under dry conditions (Ahmad et al 2003, Cao and Shu, 1986). In early season, larvae of pink bollworm (PBW) infest buds and flowers without significant damage, but later in the season, larvae feeding in bolls result in significant losses both in quantity and quality (Ahmad et al., 2003). The damage caused by PBW can be found from blooming of cotton to the end of the harvest period (Wene et al., 1961).

The pink bollworm is probably native to Indo-Pakistan, invaded China at the beginning of the 20th century. Chinese P. gossypiella have been assumed to be the result of indiscriminate introductions from Pakistan and America by transport of cotton seed (Liu et al., 2009). Biological invasions are recognized as part of the human induced global changes that have dramatically affected ecosystems worldwide and resulted in high environmental and economic costs (Vitousek et al., 1997; Pimentel et al., 2000). Pink bollworm has been recorded from most cotton-growing areas of the world (Ingram, 1994). P. gossypiella was first described by Saunders in 1843 as Depressaria gossypiella from specimens that were found to damage cotton in India (Naranjo et al., 2001). Its early distribution and spread to various countries in the Middle East, in Australia and on the Indonesian and Malaysian Islands has been traced (Ingram, 1994). The precise origin of the pink bollworm remained unknown. The preponderance of its parasites in Pakistan suggests it may be native to the
Indo-Pakistan area (Cheema et al. 1980). In China, the earliest cotton plants were Asian cotton brought from Pakistan about 2,000 years ago. In the 1860s, American cotton was introduced into China, and replaced Asian cotton step by step. The first record of *P. gossypiella* in China was in 1908. At present, with the exception of a few northwestern localities, *P. gossypiella* is found in many cotton growing areas, especially in the Yangtze River valley, which is one of the major cotton growing regions in China (Wan et al., 2004). The history of cotton planting and knowledge of the appearance of pink bollworm in China has led to the assumption that Pakistan and America were the sources of *P. gossypiella* in China resulting from cotton seed transport. In China the introduction of this pest in Yangtze River valley is believed to be from Indo-Pakistan. Invasion of this pest to China has been of great concern since it invaded in the early 20th century. Previous studies of pink bollworm showed that some adults disperse long distances (McDonald and Loftin 1935, Bariola et al. 1973), but most do not (Flint et al. 1975). Vernon (1979) reported the continuous dispersal of pink bollworm from one cotton growing area to another. Wu et al. (2006) measured the flight potential and propensity of *Pectinophora gossypiella* (Saunders) in the laboratory using a 32-channel, computer-monitored flight-mill system. According to their reports females flew significantly farther within a 72-h flight period than did males. The mean accumulated flight distance and flight duration of 1 day old female individual in a tethered flight test were 41.25±7.76 km and 23.87±2.55h respectively, whereas for male individuals the same parameters were 23.46±2.13 km and 14.12±1.12h. They also found that all the moths could fly normally at 16 to 36 degree celsius; however, the optimum temperature for flight ranged from 24 to 28 degree celsius, whereas the optimum relative humidity ranged from 75 to 90%. However, little work has been published on the migration and subsequent adaptations of *Pectinophora gossypiella* Saunders. Here, we report research results of the experiments conducted under technical cooperation project of cotton pest management, sponsored by International Atomic Energy Agency (IAEA). The present laboratory studies were conducted on biological characteristics, mating behaviours, compatibility of mating between the two strains and effect of age on mating of the Chinese and Pakistani strains of *Pectinophora gossypiella* for finding links to its distribution, adaptation and possible evolution and establishment in the Yangtze River Valley of China.

**MATERIALS AND METHODS**

**Insects Rearing**

Two different geographical strains of *Pectinophora gossypiella*, Pakistani (PP) and Chinese (CC) were collected from the different cotton growing areas of Pakistan (Sindh and Punjab) and Yangtze River of China. Both strains were reared under laboratory conditions of 29 ± 1 °C, LD 0: 24h photoperiod, and 60 ± 10% of relative humidity for larvae, 29 ± 1 °C, LD 15: 9h photoperiod, and 80 ± 5% of relative humidity for pupae and 24 ± 1 °C, LD 16: 8h photoperiod, and 60 ± 10% of relative humidity for eggs laying and hatching. Adults were supplied with 10% honey solution as food. Larvae of both strains were reared on an artificial diet composed mainly of casein, wheat germ and sucrose and the culture was maintained under controlled laboratory conditions as described above (Adkisson et al. 1960). Pupae were sexed, held individually, and checked daily for emergence. About 30 pairs of adult moths were reared at the standard conditions with a diet of 10% honey solution in an 850ml glass bottles (65cm×13cm) for oviposition. Individuals of pink bollworms from both the strains reared in this way were selected for studying biological related activities. Within 24 h of emergence, unmated adults were maintained singly in glass test tubes (3.4 cm² by 10 cm) with a piece of cotton moistened with 10% honey solution that was exchanged every other day until the biological tests for both the populations. Moths emerging during the 24 h from appearance of scotophase were designated as 1-d-old adults.

** Biology and Mating Behaviors of two Strains**

For recording the biology of the Pakistani (PP) and Chinese (CC) strains, replicated homogenous crosses were made under the same laboratory conditions as described above for insect culture. For studying intra strain rituals, feeding and mating behaviours of the virgin adults of these two strains, visual and videocassette record (VCR) based observations were recorded.

**Mating Compatibility (No Choice Tests)**

The laboratory culture of both the strains was selected for studying the mating compatibility. After emergence, each unmated adult was maintained in a test tube (3.4cm²×10cm) with a piece of cotton moistened with 10% honey solution that was replenished every 2nd day until putting into experiment. Moths emerged during 24 hours from appearance of scotophase were designated as 1-day-old adults. Different no choice intra and inter strains crosses were made individually in wire gauze plastic cage (40 cm x 20 cm x 20 cm) in insect rearing room at the same conditions as that of insect rearing. The data on fecundity of mated female by counting the number of laid eggs, fertility of female by counting ...... and longevity of ? were recorded daily.
Mating Compatibility (Free Choice Tests)

The free choice mating experiments were also done in insect rearing room at the same conditions as that of insect rearing. Two types of multiple-choice experiments were performed. Virgin adult female and male of both tested strains were simultaneously released into 500 ml glass jars for one type experiment and into the cage (40 cm × 20 cm × 20 cm) for another. Each one female and male of both strains was kept in the jar, while female and male group were placed into cage. Copulations were observed with 30-min intervals throughout the scotophase. Since the adults of these two strains were not distinguishable in appearance, the right wings were stained with ink in one and the left wings in the other strain. Each copulating pair was taken out for detection and put into a clear jar. They were put into the cage again from the jar when they separated. To measure the degree of randomness of mating, the mating percentage was recorded visually after every 30 minutes. The moths, which were still and continued mating for long time, were counted as one time mating.

Age Effect on Mating

For age effect on the mating efficiency between the two strains, moths of different age starting from day 1 to day 10 were reared separately and subjected in reciprocal crosses under the recommended laboratory conditions. Data on the mating percentage and fecundity were recorded for all the combinations. The mating percentage was recorded following the same method of using harmless dye on the right and left wings of moths for demarcation and careful observations. The mating percentage was recorded visually after every 30 minutes for all the reciprocal of young and old moths under study. The fecundity of each cross was recorded daily by counting the number of eggs laid on the substrate.

Statistical Analysis

Student $t$-test (one-way ANOVA should also be used for the statistical analysis) was performed for all the biological parameters of the different geographical strains and all the proportions of mating percentage ($\chi^2$ should be used for data of mating percentage), fecundity, age effect on mating using SAS (SAS institute, 1998). Statistical significance was determined at the $P = 0.05$ level.

RESULTS AND DISCUSSION

Biology and Mating Behaviors of two Strains

Results showed that the larval (29.00 days, Please indicate the results of ANOVA analysis) and pupal periods (7.33 days) of Chinese strain (CC) were significantly higher than Pakistani strain (PP) where, these parameters were 22.33, 6.33 days respectively. On the other hand fecundity (80.00) and male, female longevity (15.66, 26.33) of PP strain was significantly higher than CC strain having the fecundity of 68.00 and male, female longevity of (12.33, 22.00 days) respectively. However, the hatching period was at par between the two strains (Table I).

<table>
<thead>
<tr>
<th>Strains</th>
<th>Eggs Hatching (No. of days)</th>
<th>Larval period (No. of days)</th>
<th>Pupal period (No. of days)</th>
<th>Longevity (No. of days)</th>
<th>Fecundity</th>
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<td></td>
<td></td>
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<td></td>
<td>Male</td>
<td>Female</td>
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<tr>
<td>Chinese</td>
<td>6.33 a</td>
<td>29.00 a</td>
<td>7.33 a</td>
<td>12.33 b</td>
<td>22.00 b</td>
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<tr>
<td>Pak</td>
<td>5.67 a</td>
<td>22.33 b</td>
<td>6.33 b</td>
<td>15.66 a</td>
<td>26.33 a</td>
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</table>

Mean Visual plus Video Cassette Record (VCR) based observations of feeding, rituals and mating time showed slight variations and all the parameters under consideration were found at par for the two strains of pink bollworm. The percentage of activities under studies at the specific time showed no significant difference between the strains. The activities like the onset of feeding on honey solution, rituals, mating were slightly more in the PP strain. However, the range of maximum mating time (05:00 to 07:00) was found similar in the two respective strains (Fig.1).
Mating Compatibility (Free and No Choice Tests)

The mating compatibility tests of inter and intra strains crosses showed that successful mating between the two strains in free choice tests. The Intra-strain mating percentage was significantly higher in PP×PP (73.67) as compared to CC×CC (62.54). On the other hand the inter-strain mating percentage of PP×CC (68.46) was found significantly higher than CC×PP (51.25). In no choice experiments inter and intra strains crosses resulted into different fecundity, fertility and male, female longevity. The fecundity was found positively correlated with increasing number of sexes in all the intra and inter strains no choice crosses. This parameter was found significantly higher in the inter strain crosses as compared to intra crosses. However, higher in the intra strain as compared to the inter strain crosses. The longevity was not affected by any reciprocal crosses and was found at par in all the resultant crosses (Fig 2-4).
Age Effect on Mating

The age affected the mating percentage in all the intra and inter strains crosses. The mean percent mating decreased with the increasing age and reached to zero after the moths age of 8 days in all the intra and inter strains crosses under observation. However, the mean resulted fecundity found at par between old and young age moths in all the tests (Fig. 5 - 7) Subtitle needed for the remained.
Fig. 5. Effect of age on mating percentage of inter and intra strains crosses. The sex ratio of female to male in the crosses of 1x2, 1x3, … 1x8 is various? Please specify.

The hypothesis of indiscriminate introduction of pink bollworm from Pakistan into China raises deductions like: pink bollworm must be a strong candidate for dispersal and subsequent establishment in the new environment. Many authors have tested and supported the former deduction and demonstrated the potential for long and short-range dispersal of pink bollworm. Whereas our study is an attempt to seek evidence for the later deduction. McDonald and Loftin (1935), Bariola et al. (1973) recorded dispersal range of 56 to 105 km in an isolated cotton fields.

Fig. 6. Effect of age on fecundity of inter and intra strains crosses (Female x male). Please indicate the title of Y-axis. the sex ratio of female to male in the crosses of 1x2, 1x3, … 1x8 is various? Please specify.
They also reported the adult moth catches of pink bollworm up to altitudes of 1,000m. On the other hand Flint et al. (1975) marked pink bollworm males by feeding them with a sugar solution containing phosphoric acid labeled with $^{32}$P. Out of 30 wild males and 30 sterile males released at dusk in a cotton field, 12 wild males and 14 sterile males were recovered in the following dawn by searching in 1.4 ha of the cotton field with a Geiger counter. The mean distance from the release point was 61m for wild males and 8m for sterile males. Similarly Van Steenwyk et al. (1978) marked wild pink bollworm males and female in the field by spraying rubidium in cotton fields in California. Their results showed that some marked moths dispersed up to 400m outside of the cotton field. In one experiment during 1976, the sex ratio of moths emerging from field-picked bolls was 1:1, but males outnumbered females roughly 3:1 in light traps within the cotton field and roughly 10:1 at distance of 400-1,600 m outside of the cotton field. The higher ratio of males to females outside of the field suggests that males were more likely to leave the field than were females. However, Noble (1936) reported that it was biologically possible for fertilized pink bollworm moths to continue deposition of fertile eggs after as much as seven days of isolation from males, using wind currents and rising to a considerable height. Pink bollworm dispersal was greater in the first and last generations than in the middle of the growing season (Van Steenwyk, 1978, Henneberry and Naranjo, 1998). The laboratory study by Wu et al. (2006) also highlights the pink bollworm with potentially more flight propensity. These references hint the strength of pink bollworm as a strong candidate for dispersal around the area and highlight the possibility of this pest as alien specie in the Yangtze River Region of China from Pakistan.

Liu et al. (2009) tested this long-held hypothesis and genotyped a total of 527 individuals from 14 sites at 13 micro satellite loci. They analyzed these data with traditional statistics as well as with Bayesian methods. The loci were, for the most part, highly polymorphic. The allelic richness of Chinese populations at six loci was greater than those of the Pakistani and American populations. Significant deficits of heterozygote were recorded for all 14 populations, and null alleles were the most probable factor contributing to these deficits. Pair wise Fst estimates showed that there was significant differentiation among the pooled Chinese, Pakistani, and American populations, and there was structure within most of the Chinese populations. The Bayesian analysis revealed that the combined Chinese, American, and Pakistani populations formed separate clusters, and the nine Chinese populations were divided into two clusters. Allelic frequency distributions showed that private and shared alleles within Chinese *P. gossypiella* were derived only partly from the Pakistani and American populations. The microsatellite-based genetic analyses suggested that the Chinese *P. gossypiella* populations originated from multiple sources. Invasions are rapid evolutionary events in which populations are usually subjected to a founder effect during the colonization event (Sakai et al., 2001). If populations in new environments are founded by a few individuals, allelic diversity will be reduced by genetic drift and inbreeding compared to the source populations (Nei et al., 1975).

However, introductions from multiple genetically distinct sources should show enhanced allelic diversity. Multiple introductions from distinct native-range sources are common in biological invasions, which
could be caused by either single mixed introductions or by sequential introductions. There have been many examples of exotic populations that have high genetic diversity as a result of multiple introductions and hybridization (Allendorf and Lundquist, 2003; Kolbe et al., 2004; Suehs et al., 2004). The problem of establishment after introduction and subsequent dispersal still arises. Our present results showed that the larval, pupal periods, fecundity and male, female longevity were significantly different between the two different geographical populations under investigations except the hatching period for the eggs of these strains was found at par between them. Which support the idea of Liu et al. (2009), their studies regarding the allelic frequencies of the shared alleles among the pooled Chinese, Pakistani, and American population indicated that the Chinese populations were partly from the Pakistani and American populations. The Pakistani populations contributed more sources to the Chinese populations than the American populations according to the number of the shared alleles among these pooled populations. These results led into hypothesis that alien strains of pink bollworm having variation in biology will have no chance for random mating with the local strain. If it is true then the migrated specie of pink bollworm may not led to the successful establishment in the new environment. Our results regarding the mating compatibility between these two different strains clued successful chance for the random mating between the populations under investigations. However, another assumption that the moths of overlapping generations emerged at different time in the field would not be able for successful possible mating between the different strains. The results that different age affected the mating percentage negatively between the strains. But, the subsequent fecundity resulted from these crosses was dramatically at par to crosses made between the moths of the same age. Moreover, the mating percentage, mating time, rituals, feeding time of virgin moths was also found slightly different between these two strains. These characters explain the theoretical success of the migration based establishment theory and may be considered in devising the management strategy of pink bollworm.

CONCLUSION AND RECOMMENDATIONS

These findings can be helpful in redesigning the management strategy based on the random mating of the overlapping and migrating generations of the different invasive strains of pink bollworm in the cotton growing areas of the world.

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