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Artificial Rearing Methods and Observations of *Spodoptera litura* on Soybean Plants

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Abstract Under controlled artificial conditions, the growth cycle and behavioral characteristics of *Spodoptera litura* (striped stem borer) were observed with soybean plants as the sole feed. Experimental conditions: temperature around 25°C, relative humidity 23%, with a 12-hour light and 12-hour dark alternating cycle. It was observed that the duration from the pupal stage to the next generation pupation was about 45 days, with adult emergence taking about 15 days, mating and oviposition lasting about 2 days, egg hatching taking about 4 days, and the larval stage lasting 22 days, with the prepupal period lasting about 2 days. During the larval rearing process, providing sufficient space and food can reduce cannibalistic behaviors. Observing the reproductive organs of the pupae accurately distinguishes their sex. In environments with lower humidity, reproductive efficiency decreases, with the number of new individuals being only ten times that of the previous generation. Observing *Spodoptera litura* in an artificial environment provides basic data for biological research, contributing to green pest control strategies and resource utilization.
Keywords *Spodoptera litura*; Soybean; Natural feed; Rearing; Grow

1 Introduction

The striped stem borer (*Spodoptera litura*) belongs to the order Lepidoptera and the family Noctuidae, also known as the lotus-patterned noctuid or the striped noctuid (Islam et al., 2020). Unlike the incompletely metamorphosed dragonflies, *Spodoptera litura* undergoes complete metamorphosis, like butterflies and mosquitoes, with four distinct developmental stages: egg, larva, pupa, and adult (Zhong et al., 2024). It is a global agricultural pest, widely distributed in China, from Gansu in the west to coastal provinces and Taiwan in the east, from Liaoning in the north to Hainan Island in the south, where the pest has been recorded (Bragard et al., 2019). Data show that *Spodoptera litura* intermittently erupts in the northern Huai River region, while in the southern Huai River region, especially south of the Yangtze River, it occurs year-round, with increasing damage as one moves further south (Zhang et al., 2023). Hainan Island, due to its unique tropical climate, experiences this pest year-round (Baoqia, 2015).

From the perspective of generation, the *Spodoptera litura* occurs 4~5 generations per year in the Northeast and North China regions; The number of generations in East and Central China has increased to 5~7 (Zhang et al., 2023). Zhang et al. (2014) found that *Spodoptera litura* occurs 5~6 generations per year in Zhejiang Province, and its active period lasts from late April to early November; In Licheng District, Putian City, Fujian Province, the annual occurrence of *Spodoptera litura* can reach 6~9 generations, with overlapping generations, and the peak period is concentrated from May to October (Chen, 2010). According to a survey conducted by Qin et al. (2006), the host plants of the *Spodoptera litura* include 389 species (including varieties) from 109 families, including ferns (such as ferns, wood thieves, apples, etc.), gymnosperms (such as Chinese fir, cypress, ginkgo, etc.), monocotyledonous plants (such as chives, corn, banana, etc.), dicotyledonous plants (the most harmful), leguminous plants (such as soybeans and cowpeas), cruciferous plants (such as cabbage and Chinese cabbage), and other important economic crops (such as cotton and sweet potato).

The *Spodoptera litura* mainly causes harm by feeding on plants as larvae, and has the following characteristics: (1) Gluttony: high-density populations can gnaw on the entire crop leaf in a short period of time, leaving only leaf veins; (2) Dribbling behavior: often feeds into the heart and leaves of plants, causing tissue decay; (3) Transfer hazard: possessing the ability to actively migrate and diffuse; (4) Polyphagy: can simultaneously harm different organs such as leaves, flowers, and fruits. In recent years, monitoring data has shown that the economic losses caused by *Spodoptera litura* to agricultural production are becoming increasingly severe (Tuan et al., 2017; Thakur et al., 2024). For example, causing yield losses in crops such as cotton, corn, tobacco, and various vegetables, with damage levels ranging from 50% to 100% in some cases (Un Nisa et al., 2023). Zhang et al. (2023) believe that there are several factors contributing to the outbreak of *Spodoptera litura*: (1) the expansion of facility agriculture, such as the application of plastic greenhouses, providing abundant food sources and superior wintering environments for *Spodoptera litura*; (2) The arid climate is conducive to the growth of this population; (3) With the application of pesticides and other insecticides, older larvae have developed significant resistance to conventional pesticides; (4) The reform of the farming system has diversified vegetable varieties, expanded planting areas, and increased the multiple cropping index (the multiple cropping index of arable land has increased from 1.45 in 2018 to 1.62 in 2022).

This study used soybean plants as the sole feed source to simulate natural conditions indoors and observed the full lifecycle of *Spodoptera litura*, aiming to investigate its growth cycle, behavioral habits, and the impact of rearing conditions on its reproductive capacity. The findings will provide experimental data and theoretical support for future green pest control, precise identification, and resource utilization strategies.

2 Materials and Methods

2.1 Collection and screening of insect sources

The sources of *Spodoptera litura* used in this experiment were collected from soybean fields, and a total of 14 mature larvae were captured. They were placed in glass culture bottles and fed with fresh soybean leaves, and 13 individuals successfully pupated, with a pupation rate of 92.9%, and one larva died in the pre-pupae stage. After the insect sources were brought into the room, they were first raised individually for observation for one generation, and then they were confirmed to develop normally and be free of pathogens before being incorporated into large groups for breeding. This screening measure can avoid problems such as premature aging and lack of vitality of the breeding population due to latent infection.

2.2 Rearing environment and instruments

The indoor rearing of *Spodoptera litura* needs to simulate the suitable environmental conditions for its growth in the wild as much as possible. Temperature is an important factor affecting the development rate. The experimental room uses air conditioning to maintain a constant temperature of about 26℃, and the measured temperature is about 25.5℃. Due to the influence of air conditioning refrigeration, the air humidity is about 23%. It is transferred to a container with a layer of about 20% moist fine sand inside to increase the humidity of the local microenvironment. In terms of light cycle, fluorescent lamps are used to supplement lighting to simulate the natural circadian rhythm, and the light cycle is controlled to 12 hours of light: 12 hours of darkness.

Instruments and supplies: temperature and humidity controlled air conditioner (Midea brand), digital temperature and humidity meter (precision ±0.1 °C/±2% RH), white plastic emergence containers, black plastic soybean cultivation containers, nylon rearing net boxes, brushes, standard measuring tools (ruler, etc.), petri dishes, cotton, iron shovel, hoe, glass bottles, digital camera, and charger. Reagents: honey (food grade), tap water.

2.3 Feeding method

Feed: In this experiment, soybean leaves were selected as the main feed. Soybeans have the advantages of rich nutrition, short growth cycle, and natural ingredients. *Spodoptera litura* showed a significant feeding preference for leguminous plants. Its host selectivity was: Leguminosae> Cruciferae> Araceae> Cucurbitaceae> Malvaceae> Amaranthaceae (Zou et al., 2016).

Soybean seeds are purchased from ordinary commercial products. To ensure year-round supply, while soybeans are potted indoors, small plots of land are opened up outdoors for rotational sowing to continuously provide fresh leaves required by larvae of all ages. When feeding natural feed, care should be taken to keep the leaves fresh. The base of the harvested branches and leaves can be inserted into a bottle with water or fresh leaves can be replaced frequently. Young larvae usually gather on the back of the leaves to feed on the surface mesophyll. It is necessary to ensure that the leaves do not dry up to increase their feeding rate. Scientific formulation, high-frequency feeding, and frequent replacement and cleaning are the key to ensuring the high survival rate and normal development of *Spodoptera litura* larvae.

2.4 Experimental methods

2.4.1 Soybean seed pre-treatment and planting

Seed pre-treatment: Soybean seeds stored in refrigeration were activated in a 25°C light incubation box for 2 hours, then transferred to a cool place to cool down (Didorenko et al., 2023). Sandy loam was filled to 2/3 of the height in plastic planting containers, and approximately 15 soybean seeds were evenly sown in each container, with a covering soil thickness approximately equal to the diameter of the seeds, followed by thorough watering.

Considering the large food intake of *Spodoptera litura* larvae, additional soybean plants were planted outdoors for backup: a plot of land was selected, surface weeds were removed, soil was deeply turned, and shallow furrows (5 cm wide, 2 cm deep) were dug at 10 cm intervals. After watering, seeds were sown, and soil was leveled. The seeds sprouted in approximately 4 days.

2.4.2 Larvae rearing and emergence

The entire experiment was conducted in an insect rearing room. The temperature was maintained around 25.5°C with a relative humidity of 23%, using natural light plus daylight lamps with a 12-hour light and 12-hour dark cycle.

Thirteen pupae were placed in white emergence containers and covered with nylon mesh, with soybean plants placed nearby. Daily observations were made on the color changes of the pupae (from light green to black to dark brown), and the plants were watered every three days to keep them fresh, maintaining a soybean plant fresh weight moisture content of ≥80%.

After the adults emerged, 10% honey water (1 mL honey + 9 mL tap water) was prepared and placed in open petri dishes with soaked cotton for nutrition. The honey solution was replaced daily to enhance adult reproductive vitality. The body length of the adults was measured using image analysis software (ImageJ v1.53), with n=20 samples.

2.3.3 Adult reproduction and egg hatching

Two days (48 hours) after emergence, the adults began mating and laying eggs. The eggs were mostly laid on the nylon mesh, pot walls, and the surfaces of soybean leaves in clusters, and they hatched in situ without human intervention, allowing natural hatching (Islam et al., 2020). Egg diameter was measured using a micrometer (n=50).

2.4.4 Larvae rearing management

The newly hatched larvae (1.02±0.15 mm in body length) are black in color and were transferred to the undersides of soybean leaves using a soft brush or paintbrush. The larvae were continuously observed to ensure they did not fall off or move, and they were promptly returned to the leaf surface if necessary. Rearing density was kept ≤5 larvae per plant (during the 3-4 leaf stage), and the food was replaced daily to ensure sufficient food supply. Large larvae (≥4th instar) require more food, and thus potted or field-grown soybeans were prepared in advance. The larvae were then reared in individual containers (1 larva per container) to reduce density and prevent cannibalism. The body length growth curve was observed and recorded daily.

2.4.5 Pupal treatment

After defecating, the mature larvae enter the prepupal stage and should be separated for individual rearing to prevent cannibalism or disturbance during pupation. The larvae were observed for molting, deformation, and color changes. Each pupa was placed in sterilized sand for pupation, and the pupal length was measured 24 hours after pupation (Jun and Hong, 2005). To prevent dryness from causing abnormal pupation, the pupae were considered for transfer to moist sand or petri dishes to maintain humidity.

3 Results and Analysis

3.1 Duration from Pupa to Pupa

The experiment recorded the complete generation development cycle of *Spodoptera litura* under artificial control environment (temperature of about 25.5 ℃, humidity of 23%, natural light cycle). The average developmental period from the first generation pupae to the second generation pupae is about 45 days (45.2 ± 2.1 days), specifically manifested as a pupal period of about 15 days (15.3 ± 0.8 days), an adult oviposition period of about 2 days (2.1 ± 0.3 days), an egg period of about 4 days (4.2 ± 0.5 days), and a larval period of about 24 days (23.6 ± 1.7 days). Compared with the growth cycle (38-39 days) reported in literature for natural feed such as green vegetables and Chinese cabbage, the development period under soybean feeding conditions is significantly prolonged (Figure 1) (Ramaiah and Maheswari, 2018; Hashmi et al., 2023).

Figure 1 The four developmental stages of *Spodoptera litura*: egg, larva, pupa and adult

The overall observation results indicate that the larval stage occupies more than half of the entire life cycle and is the stage with the most significant changes in energy consumption and growth rate. The entire process is slightly longer than other natural feed sources such as cabbage and green vegetables (the incubation period of *Spodoptera litura* on cabbage is about 37~40 days) (Mao et al., 2008). Under experimental conditions, the lifespan of adult insects is relatively short (about 4 days), which may be related to the low air humidity in the experimental environment (the optimal humidity is around 75%). Low humidity poses challenges to the smooth emergence of pupae and the survival of adult insects (Wei et al., 2008).

A total of 132 insect pupae were collected in the experiment, which increased by about 10 times compared to the first generation of 13 insect pupae, demonstrating a certain level of reproductive ability. However, compared to other studies that achieved a reproductive efficiency of more than 20 times under optimized humidity conditions (60-80%), the reproductive effect of this experiment is still relatively low, which further confirms that humidity is an important factor affecting its developmental success rate (Sun et al., 2015).

Spodoptera litura larvae are highly sensitive to both food quality and quantity. In this experiment, when the rearing density was too high or soybean plants were insufficient, cannibalistic behavior among larvae was commonly observed. Abnormal behaviors during the larval stage, such as fighting and pseudo-death falls, could also affect their developmental efficiency. Tu et al. (2010) recommended implementing individual separation strategies under high-density rearing to reduce stress and mortality.

3.2 Gender differences in pupae

In this experiment, the most intuitive "genital observation method" was used to distinguish the male and female characteristics of 13 experimental armyworm pupae in order to evaluate their sex characteristics. The results showed that the genital area of male pupae usually presents obvious protrusions with a dark brown color, while that of females is relatively smooth and lighter in color (Figure 2). This method is simple and intuitive, and can be used as an effective sex recognition tool before adult emergence (Chowdary et al., 2024).

Figure 2 Male and female *Spodoptera litura* pupae

In comparison, other characteristics such as weight, volume, and body color did not exhibit stable gender differentiation in this experiment, and further larger sample sizes and statistical validation are required. Related studies have also pointed out that the morphology of reproductive organs is one of the most accurate methods for distinguishing between male and female *Spodoptera litura*, particularly in the late larval or pupal stages (Chen et al., 2010). This technique has been widely applied in artificial breeding and mating experiments for other insects such as the cabbage white butterfly and cotton bollworm, improving experimental efficiency and data reliability (Sun et al., 2010).

3.3 larval feeding preference

Observation shows that the newly hatched larvae are black in color and prefer to live in groups. They concentrate on feeding on leaf flesh near the egg mass and mainly stay on the back of soybean leaves, forming irregular transparent patches. As the insect age increases, feeding behavior tends to disperse. Middle and older larvae can fully feed on whole leaves and even invade pods and tender stems. From this, it can be seen that the *Spodoptera litura* has extremely strong feeding adaptability and plant destruction ability (Abbas et al., 2025).

Spodoptera litura exhibits significant dietary advantages in leguminous, cruciferous, and solanaceous crops, with a particular preference for protein rich leaf tissues (Di et al., 2021). In high-density populations, its damage to the same plant can quickly spread to pods or even roots, causing serious impacts on yield.

Yang et al. (2009) also suggested that "behavioral interference" could be introduced during the field stage by planting low-preference plants, which could slow down the damage rate, in combination with inducing plants or biopesticides for control.

3.4 Larval shyness and aggressive behavior

The behavioral characteristics of *Spodoptera litura* larvae vary at different stages of their age (Li et al., 2021). After being startled, the first instar larvae often quickly scatter and run away or spin silk and fall, showing a "startle response". Older larvae shrink into a ball and pretend to be dead, falling to the ground while spitting out bodily fluids. This is a defensive behavior of pretending to be dead.

However, under high-density rearing or food shortage conditions, frequent cannibalistic behaviors among larvae were observed, with even pupae being attacked. This behavior suggests that when space is limited and resources are scarce, the larvae become more aggressive and territorial, showing a strong territorial instinct (Wen et al., 2025). Such cannibalistic behavior leads to decreased survival and pupation rates.

Wen et al. (2022) found that *Spodoptera litura* larvae became more aggressive under high-density rearing due to food scarcity and cramped space. To improve artificial rearing efficiency, Tu et al. (2010) recommended that after the third instar, larvae should be managed individually in separate containers and provided with enough food, which can reduce mortality and alleviate larval stress.

4 Discussion

This study observed the developmental cycle, behavioral characteristics, and reproductive ability of *Spodoptera litura* under artificially controlled environmental conditions, selecting soybean plants as the sole source of feed, and evaluated its survival challenges and adaptation in low to medium humidity environments. The process from pupae to the next generation of pupae takes a total of 45 days, which is longer than the incubation period of Hardik and Dolly (2020) in high humidity environments (65%~75% RH) (approximately 35~38 days). In a low humidity environment (23%), the success rate of *Spodoptera litura* during pupation and eclosion stages will decrease, making it more prone to difficulties in molting during the pupal stage, increased rates of adult deformities, and even death. This result is consistent with existing research, indicating that humidity is one of the most critical environmental factors affecting the life cycle of *Spodoptera litura* (Divakara and Manjulakumari, 2015).

During the larval rearing process, significant feeding competition and cannibalism were observed among the larger larvae, especially under high-density rearing conditions. This cannibalistic behavior reduced individual survival rates and resulted in fewer pupae and adults in subsequent generations. Thillainayagam et al. (2022) pointed out that *Spodoptera litura* larvae exhibit "density-dependent aggressiveness," and it is recommended to separate individuals or provide ample space and food under high-density rearing to reduce intraspecific competition.

Although soybean was chosen as the natural feed in this experiment, which is practical to some extent, the reproductive efficiency was still lower than that achieved with artificial formulated diets, which have been widely used in recent years. For example, artificial feed based on chickpeas and wheat germ has been shown to increase the average egg-laying capacity of female moths to over 2,500 eggs, whereas the data in this experiment only reached about 132 pupae (10 times the number from the previous generation of 13 pupae) (Gupta et al., 2005). Therefore, in the future, attempts can be made to add protein sources and vitamins to the natural feed, combined with humidity control, to optimize the rearing results.

This study also observed the extensive destructive behavior of *Spodoptera litura* on soybean leaves, pods, and tender stems, confirming its status as a high-risk field pest. Using viral agents, such as nucleopolyhedrovirus (NPV), during the early instar stages has been proven effective, and mating disruption using sex pheromones can also reduce egg counts and population sizes at the source (Yang et al., 2009). On the other hand, the larvae of *Spodoptera litura* have a high protein content, and after detoxification or heat treatment, they can be used as animal feed or fertilizer, potentially realizing a "pest-to-benefit" resource conversion pathway, as demonstrated by successful experiences in the utilization of insect resources like yellow mealworms and legume pod borers.

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Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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