Pupation behaviour, depth, and site of *Spodoptera exigua*

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Abstract

The beet armyworm, *Spodoptera exigua* (Hubner) (Lepidoptera Noctuidae) is a persistent agricultural pest in many areas of the world. There is considerable controversy regarding the overwintering regions of *S. exigua*, although its pupae have been considered by many researchers as the most adaptable overwintering stage. Pupae are not consistently found in the field because of sheltered pupation sites. Direct evidence of *S. exigua* overwintering is only obtained by examining surviving pupae excavated during winter. In the present study, we observed the larval pupation behaviour of *S. exigua* in the laboratory, and investigated its pupation sites and depths in the field as parameters for field investigations during winter. Pupal survival in a natural pupal chamber at low temperature (4 °C) indicated that *S. exigua* has a protective mechanism against chilling injury. The pupal chambers were mainly found at depths of 1.1–2.0 cm in the field. Field investigation indicated that the pupation sites were located directly beneath plant canopies, and that soil structure influenced larval preference for pupation.

Key words: *Spodoptera exigua*, pupation behaviour, pupal chamber, pupation site.

Introduction

The beet armyworm, *Spodoptera exigua* (Hubner) (Lepidoptera Noctuidae) is a widely distributed crop pest. In China, outbreaks of *S. exigua* have caused serious economic losses since its appearance was first recorded in 1892. *S. exigua* can migrate long distances (French, 1968; Kimura, 1991), as has been observed in China (Feng et al., 2003) and the United States (Westbrook, 2008). However, the population source in outbreak regions in China remains in question. In Jiangsu Province, outbreak populations are considered by some researchers to have migrated from southern provinces (Han et al., 2003; 2004; 2005), whereas others insist that the insects arose from local overwintering populations (Wang et al., 2007). A similar debate is also raised concerning populations in Shandong Province (Feng et al., 1995; Wang et al., 2002), Henan Province (Wu et al., 2000; Guo et al., 2005), and the Yangtze River valley (Yin et al., 1994; Lu and Xie, 1995). Recently, various isotherms in China were divided into non-overwintering, possible overwintering, and perennial damage regions for *S. exigua* (Jiang et al., 2001).

Researchers have reported that the supercooling point of *S. exigua* pupae is the lowest of all developmental stages (Jiang et al., 2001; Han et al., 2005). In addition, the pupal stage has the longest survival time following exposure to low temperature (Jiang et al., 2001). Although Kim and Kim (1997) considered that the supercooling point of eggs in *S. exigua* is the lowest of all developmental stages, the survival time of eggs at low temperature is shorter than that of pupae (Kim and Song, 2000). As a result, researchers consider the pupae to be the most adaptable overwintering stage of *S. exigua* (Hendricks et al., 1995; Han et al., 2004). Evaluation of possible overwintering has been studied by transferring pupae to containers embedded in the field (Jiang et al., 2001; Han et al., 2005). Under field conditions, when larvae prepare to pupate, they normally construct a pupal chamber (Fye and Carranza, 1973; Fye, 1978). Although the embedded pupae in these studies were covered with soil, the study conditions may not have reflected the actual overwintering conditions that exist in a natural pupal chamber. Direct evidence of *S. exigua* overwintering is only obtained by examining surviving pupae excavated during winter. However, no clear methods exist for accurately locating pupation sites in the field. Therefore, understanding the larval pupation behaviour, choice of pupation site, and distribution of pupae of *S. exigua* in soils is important.

Pupation behaviours of some species of lepidoptera have been previously characterized (Torres-Vila et al., 1996; Chen et al., 2002; Wagner, 2007; Rhainds et al., 2009), but little has been published regarding the pupation behaviour of *S. exigua*, despite its longstanding history as a serious pest. Larval pupation depth and pupation preferences have been described (Fye and Carranza, 1973; Fye, 1978), but many details required for experimental purposes remain lacking.

Several field investigations have focused on *S. exigua* pupae overwintering in China (Yin et al., 1994; Ren et al., 2005). The failure to find pupae in the field could be attributed to a number of explanations that may reflect poor understanding of pupation more than the absence of insects. For example, for field investigations researchers should purposefully choose unploughed plots where crops have clearly been damaged by *S. exigua* in late fall (October to November). In addition, the actual sites within the soil where the overwintering *S. exigua* pupae exist must be determined. Finally, depths favored by larvae for pupation in winter must also be identified for field investigation.

The aims of the present study, therefore, are as following: (1) to understand larval behaviour when *S. exigua* prepare to pupate, and (2) to find the pupation sites and depths of overwintering *S. exigua* under winter field conditions.
Materials and methods

Experimental insects

*S. exigua* larvae were collected on *Brassica oleracea* var. *botrytis* L., in Cihui Farm (114°06'E - 30°59'N), Wuhan City, Hubei Province, China from June to July 2008. Insects were reared in an incubator under conditions of 25 ± 1 °C, 60-80% relative humidity, and a 12L: 12D cycle. Honey water (10%) was provided to adult moths through a 5-cm cotton wick inserted into the slit lid of a 20-mL plastic cup placed inside the transparent plastic container (radius, R = 15.0 cm, height, H = 15.0 cm). Adult females laid eggs on the wax paper surrounding the internal face of the oviposition container. Oviposition substrates were replaced daily. Egg masses were collected from the oviposition substrates and surface-sterilized in 5% formaldehyde solution for 20 min before being placed in plastic containers (R = 15.0 cm, H = 7.5 cm). Newly hatched larvae were fed an artificial diet, as described in a study by Jiang *et al.* (1999). The newly hatched larvae were housed in a separate plastic container. Larvae were checked daily and last instar larval pupae were used as experimental insects.

Observation of larval pupation behaviour

Larval pupation behaviour and pupal chamber structure were recorded using a digital camera (Fuji S8000fd) in the laboratory under conditions of 25 ± 1 °C, 60-80% relative humidity, and a 12L: 12D cycle. The last instar larvae were transferred to 0.5-cm wide glass interlayers (made of two transparent pieces of glass to enable profile observation) with fine sandy loam for pupation. Larval development and formation of the pupal chamber were observed and recorded using the camera from two profiles of the interlayer. On the 5th day after the formation of the pupal chamber, the chamber was removed and dissected. Observations were then made.

Effect of natural pupal chamber on survival at 4 °C

To determine the effect of natural chamber on pupal survival at low temperature (4 °C), three conditions were adopted: (1) a simulated chamber, where a pupa was placed into a PCR tube and covered. The PCR tubes with pupae were embedded at 2-3 cm depth in fine sandy loam; (2) a natural chamber, where larvae were transferred into plastic containers (R = 15.0 cm, H = 7.5 cm) filled with fine sandy loam for natural pupation; and (3) the absence of the chamber, where pupae were directly embedded at a depth of 2-3 cm in fine sandy loam. Detailed treatment processes are as follows:

One hundred and eighty larvae were transferred to plastic containers (length, L = 48 cm, width, W = 32 cm, H = 15 cm) with fine sandy loam. After five days, when the larva had burrowed into the soil, 120 pupae were collected by removing the chamber with forceps and placing 60 pupae in each of the two treatments, the simulated chamber and the absence of the chamber. Sixty pupae in the natural chambers were left intact (natural chamber). Three treatments were assigned to a temperature acclimation, changed from 20 °C (one day) to 15 °C (one day) to 10 °C (one day) to 5 °C (one day) before treatments were transferred to 4 °C. Pupal survival at 25 °C was checked after 5, 10, 20, 30, 40 and 50 days at 4 °C. The criterion for survival was successful emergence of the insect at 25 °C. Each treatment was replicated three times.

Larval pupation depths

The experiment was conducted in a vegetable field planted with *B. oleracea* var. *botrytis* in December 2008 to determine larval pupation depths of *S. exigua*. A total of 30 last instar larvae were transferred to the field for natural pupation. They were then covered with an 18 mesh sieve (diameter, D = 30 cm) to prevent their escape. Pupation depths were measured on the 5th day after larvae burrowed into the soil. Firstly, the soil surface was scrapped with a trowel to locate the pupal chamber. Then, pupal depth was measured by ruler from the soil surface to the bottom-point of the pupal chamber. The experiment was replicated three times.

Field investigation of pupation sites and depths during winter

Field investigations were carried out in December between 2008 and 2010 to determine larval pupation sites and depths. Fields chronically planted with *B. oleracea* var. *botrytis* and *B. oleracea* var. *capitata*, where vegetables had been damaged by *S. exigua* from October to November and where the plots had not been ploughed before investigation, were chosen. A square (1 × 1 m) was considered a grid, and a total of 100 grids were randomly chosen in one field. Investigations were conducted at Xianjian, Huayuan, Huojian, Wanchen, and Jianqun County of Wuhan City in December from 2008 to 2010. Each site was investigated once. A total of five sites and 500 grids were included in the experiment. To determine the pupation sites of *S. exigua* in the field, soil at the ridge, space between host plants and directly beneath plant canopies were investigated by digging up pupae by a small shovel (Zheng *et al.*, 2010). Soil in the square was excavated to a depth of about 10 cm to detect any pupae not located by scraping the soil surface. Larval pupation depth was measured using the method described above.

Data analysis

Statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA.). Larval pupation depths of each replication in the cage experiment and field investigation were divided at 1 cm intervals after collection of all results. The number of larvae pupated at different depths was converted to percentages. Data for survival rates of pupae at 4 °C and larval pupation depths were analyzed by ANOVA. Means were compared using LSD’s test at *P* = 0.05.

Results

Larval pupation behaviour

The last instar larvae sought a pupation site immediately after transfer into the glass interlayer. The search took 20.8 ± 2.7 min (mean ± SE, N = 41). Normally, larvae build their chambers immediately upon finding a
preferable site, where there is a shallow pit, a soil gap, or a clod of soil. If these preferable sites are not found, larvae had to burrow into the soil from a flat surface. The larvae in the experiment used their mandibles to move soil particles. At the same time, they spun silk and used saliva to glue the soil particles together until only a small opening remained in the chamber. The larvae then turned around to orient toward the opening to complete the chamber. Chamber construction took 180.0 ± 52.2 min (mean ± SE, N = 28). Over the next two days, larvae continued to spin within the chamber. Finally, on the 3rd day, larvae entered into the prepupa and quiescent stage. On the 4th day, the prepupa entered into the pupal stage, with the pupal head oriented toward the sealed opening (figure 1, a-j). In fact, the opening was never completely sealed, and it retained a hole about 2-3 mm in diameter (figure 1-k) for adult emergence (figure 1-l). Pupa reached the adult stage on the 8th day.

**Figure 1.** Larval pupation behaviour (a-j) and characteristics of the pupal chamber (k-o) in *S. exigua*. a-j, the process of building a chamber by a larva; k, emergence hole remaining in the chamber wall; l, emergence hole was expanded and remained of pupa exuviae in the chamber after emergence; m, shape of the pupal chamber; n-o, white multi-layers of silk on the inner wall of the pupal chamber, (n) interior and (o) exterior views. (In colour at www.bulletinofinsectology.org)
Almost all chambers of *S. exigua* were in an oval shape (figure 1-m). The length and width of a typical chamber were 2.5 ± 0.1 cm (mean ± SE, N = 28) and 1.2 ± 0.1 cm (mean ± SE, N = 28), respectively. The inner wall of the chamber was lined with 3-5 layers of white silk (figure 1, n-o). Each single layer was similar to a “cobweb” (figure 1-o, picture at top right corner).

**Effect of natural pupal chambers on pupal survival at 4 °C**

A natural chamber is beneficial to *S. exigua* pupae at low temperature (4 °C). As shown in figure 2, survival rates in a natural chamber were always higher than in simulated chambers and in the absence of a chamber after treatment at 5, 10, 20, 30, 40, and 50 days, respectively. Pupal survival in the absence of a chamber rapidly declined by the 20th day and reached zero at the 50th day. Pupal survival in a simulated chamber resulted in an 18.4 ± 3.7% survival rate by the 50th day, although survival quickly declined over time. Survival rates in a natural chamber were highest at 39.2 ± 4.1% on the 50th day, reflecting a significant difference (*F*2,8 = 27.25, *P* < 0.01) compared with other treatments.

**Larval pupation depth**

Percentages of larvae at different soil depths in the field experiments were significantly different (*F*2,8 = 14.312, *P* < 0.01). The percentage of larvae at the 1.1-2.0 cm depth was highest at 45.8 ± 2.1%. Percentages of larvae at the 0.0-1.0 and 2.1-3.0 cm depths were 34.5 ± 3.9% and 23.4 ± 2.5% (mean ± SE), respectively (figure 3).

**Field investigation of pupation sites and depths during winter**

All pupae were found within the soil beneath plant canopies of *B. oleracea* var. *botrytis* L. and *B. oleracea* var. *capitata* L. in the field. The external characteristics of pupal chambers composed of soil particles were similar to those of a swallow’s nest. The soil structure beneath the plant canopies influenced the larval pupation site. In a flat field, larvae burrow from the surface, spinning and spitting to glue soil particles to build their chamber at a depth of the 0-3 cm depth. However, larvae usually prefer to enter the soil at a soil gap or at the bottom of a soil clod in the field, where the soil surface is uneven and there are many clods, as well as gaps. The percentages of larvae at different soil depths were significantly different (*F*4,24 = 17.865, *P* < 0.01) in the field (figure 4), with the highest percentage found at a depth of 1.1-2.0 cm.

**Discussion**

The phenomenon of digging into soil, which allows species to construct a chamber to escape or shelter them from potential adversity, including abiotic and biotic factors, is common in many species of insects. The chambers are constructed from silk that is stored as a liquid but configured into solid filaments when spun upon secretion (Danks, 2004). Observation of larval pupation behaviour in the present study indicates that the
last instar larvae of *S. exigua* used a strategy of spinning to hold soil particles together to build their oval chambers. Larvae continued spinning (3-5 layers) to glue the inner wall of the chamber after they had completed the oval exterior shell of the chamber (figure 1). Other lepidopterous species, such as *Hyalophora cecropia* L. (Lepidoptera Saturniidae) (Van Der Kloot and Williams, 1953a; 1953b), *Antheraea pernyi* Guerin-Meneville (Lepidoptera Saturniidae) (Lounibos, 1976), and *Ephestia kuehniella* Zeller (Lepidoptera Pyralidae) (Giebultowicz et al., 1980), also exhibit this spinning behaviour and construct structures consisting of an outer and inner envelope at pupation sites.

Under natural conditions, many caterpillars of insects burrow into the soil to construct a chamber for pupation. This function of pupal chambers has seldom been tested explicitly, although chambers are considered to have protective effects (Danks, 2004). In the current study, the pupal chambers clearly had a protective effect at low temperature (figure 2). In addition, the chamber was possible to influence the overwintering survival of *S. exigua* pupae in the field (Zheng et al., 2011). We presumed that a possible protective mechanism is offered by the physical characteristics of soil particles and silk in the pupal chamber. These materials offer good insulation (Danks, 2004) to reduce temperature amplitudes, and constitute an important microhabitat with relatively stable temperature to protect the pupae for survival during cold conditions. For example, *Manestra brassicaceae* (L.) (Lepidoptera Noctuidae), whose chambers are distributed 5 cm beneath the ground, can overwinter under natural conditions. However, mortality rises once the chamber is destroyed. One explanation for this is that low temperatures lead to icing of the body fluids of pupae when the insulating layers of the chamber are damaged (Sun et al., 2001).

Larval pupation depths were mainly distributed within 1.1-2.0 cm into the soil (figures 3 and 4), possible because it is somewhat difficult for *S. exigua* larvae to penetrate into deep soil. In addition, sites near the surface would be quicker to warm in the spring, which is helpful for adult emergence. However, soil in the field has more natural openings that clearly facilitate larval movement, as observed in the field investigation. These include cracks in the soil surface and holes in the soil made by plant roots and soil invertebrates. Thus, a few individuals are still found 4.1-5.0 cm deep in the field (figure 4).

In the field, it is very difficult to find any overwintering pupa because the pupation sites of *S. exigua* are usually sheltered. Understanding larval pupation behaviour, depths, and sites is therefore necessary for discovering these locations. The results in the present study provide some parameters that must be considered when searching for overwintering pupae in the field. For example, unploughed plots where crops have been damaged by *S. exigua* from October to November and a larval pupation depth of 1.1-2.0 cm must be selected. Other parameters to consider are that: (1) larval pupation sites are located beneath plant canopies; (2) gaps and the bottom of soil clods under plant canopies are preferred sites of larvae for pupation, and (3) the external characteristics of pupal chambers made from soil particles are similar to a swallow’s nest.

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