The Sizes of *Sitophilus zeamais* in Different Life Stage

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Abstract: Knowing the developmental states of stored grain insects, especially for the immature stages hidden in cereal kernel, such as *Sitophilus zeamais*, could be in favor of monitoring early and controlling effectively. Atlas and scale parameters on different developmental stages of *S. zeamais* in wheat kernel were captured and described by three-dimensional microscope of super depth of field observation. The four instars of larva, pre-pupa, pupa, new adult, old adult in kernel could be seen by dissecting the infested kernels. The length, height, and width of larva body increased with insect development and centralized the sizes corresponding to the four instars of larva. The length of larva body centralized near 554, 746, 1 462, and 2 147 μm. The height of larva body centralized about 388, 575, 847, and 1 651 μm. The width of larva body centralized near 340, 598, 798, and 1 568 μm. Head capsule width was in same size for each exuviating period, centralized near 203, 317, 535, and 603 μm, and got big sharply with the instar changed. Developmental duration of four larva instars was about 21 days reared in wheat, less than 28 days reared in maize as published. The atlas and description should be helpful for monitoring the *S. zeamais* development hidden in cereal kernels and designing control strategy.

Keywords: Maize weevil; Immature stages; Morphology; Life stages; Sizes

1 Introduction

Maize weevil, *Sitophilus zeamais* Motschulsky, is one of the most destructive pests of stored cereals. It is particularly abundant in the tropic, sub-tropic and warm zones, and it could develop on several different cereals with preference for maize[1-2]. It is found in stored grain around world. Infestation of *S. zeamais* might facilitate the establishment of secondary insects, mites and stored product pathogens[3]. *S. zeamais* infests stored maize kernels, which promotes an increase in fungal infections by transporting spores and facilitating the penetration of hyphae through damage done in the grain, and then would lead to a reduction in the quality of kernels and germination[4-6].

Sitophilus zeamais and *S. oryzae* are sibling species. They can only be reliably identified by the dissection of the genitalia[7-8]. However, molecular techniques have been developed to identify insects[9-10]. Molecular and morphological characters to discriminate *S. oryzae* and *S. zeamais* and to confirm their reproductive isolation had been studied[7]. Recently, an integrative approach involving biology, morphology, morphometric and molecular characterization of various life stages for the accurate species discrimination of *S. oryza*e and *S. zeamais* by using strains from India had also been reported[1]. Moreover, there have been numerous reports on life history and biology of *S. zeamais* with the environment. Danho et al.[11] reported that the number of eggs laid increased when the amount of available grain increased. Furthermore, the oviposition of *S. zeamais* varies significantly among the cereal grains had been observed, and in that study the larval developmental period were not different significantly among maize, rice, sorghum and millet, about 22 days[12]. Life stage independent identifications are necessary to satisfy the phytosanitary and quarantine specific requirements[13]. Usually, the number of weevil adults was referred to decide this insect should be killed or not. In fact, the insect immature stage had been seating inside the grain kernels. Unfortunately, there were a few morphological characters of immature stage describing on *S. zeamais*[1]. The high-resolution images and size descriptions of various immature stages of *S. zeamais* should be useful to people who work on grain storage and other stored product protection.

2 Methods and Materials

Sitophilus zeamais strain was collected from grain depot located in Zhengzhou, Henan, China, and was cultured with wheat for serval generations. 300 adults in 14-d-old (n=300) were kept on 250 g wheat (12.5%±0.5% moisture content). The wheat with eggs was held at 28±0.5 °C and 75%±5% r.h. The wheat kernels were stained with dark spot dyed by acid fuchsin solution to identify kernels with egg plugs. These kernels (n=5/duration) were dissected after different durations to obtain the different life stages. All insects found were measured for body length, width, depth and head capsule width.

The specimens photographed using a digital microscope (Keyence®VHX-5000. Camera parameters 200 of amplification factor, 12.7 ms of shutter speed and 20 ms of film speed). The output pixels in 1 600×1 200 was achieved by three-dimensional z-stacking technology. Depth composite image was composed by setting splicing range and moving distance in Z-dimensional. The length, height, width of body, and head width were gotten by statistic of mean plus error range. All data were subjected to

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one-way ANOVA using SPSS 20.0. Significant differences between the mean sizes of different life stages were determined using Duncan's multiple range test ($P < 0.05$).

3 Results and Analysis

All length, height, width of insect body and width of head capsule of the immature stages on different developmental days are shown in Fig. 1 and Table 1, in which the sizes were measured from three to five insect samples in infested wheat kernels. The ANOVA results indicated that there were significant differences for the size of different life stages of *S. zeamais*. The typical photography from several measured insect bodies of different immature stages is present in Fig. 2–Fig. 6 respectively.

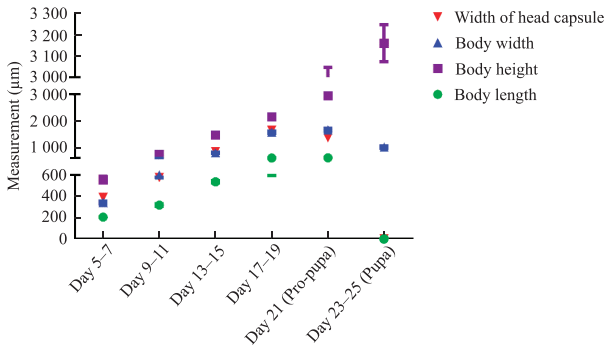


Fig. 1 Length, height, width of insect body, and width of head capsule of *S. zeamais* immature stages measured in different development days after oviposition

On lateral view larva external morphology of *S. zeamais* with no legs could be seen from day 5 to day 19 after oviposition. Larva, pre-pupa and pupa were cream coloured, excepted for the head and mouthparts of larva. After 25 days, the pupa became darker and yellow in which legs and antenna separated more from body (Fig. 3). The adults emerged from pupae about 27 days after oviposition. The adult transitioned from light yellow, dark yellow to brown over the next 4 days. The length, height and width of the body increased with developmental time (Table 1). On head-on view yellow and brown capsule, black ocelli and mouthparts of *S. zeamais* including maxilla and labipalpus, can be seen.

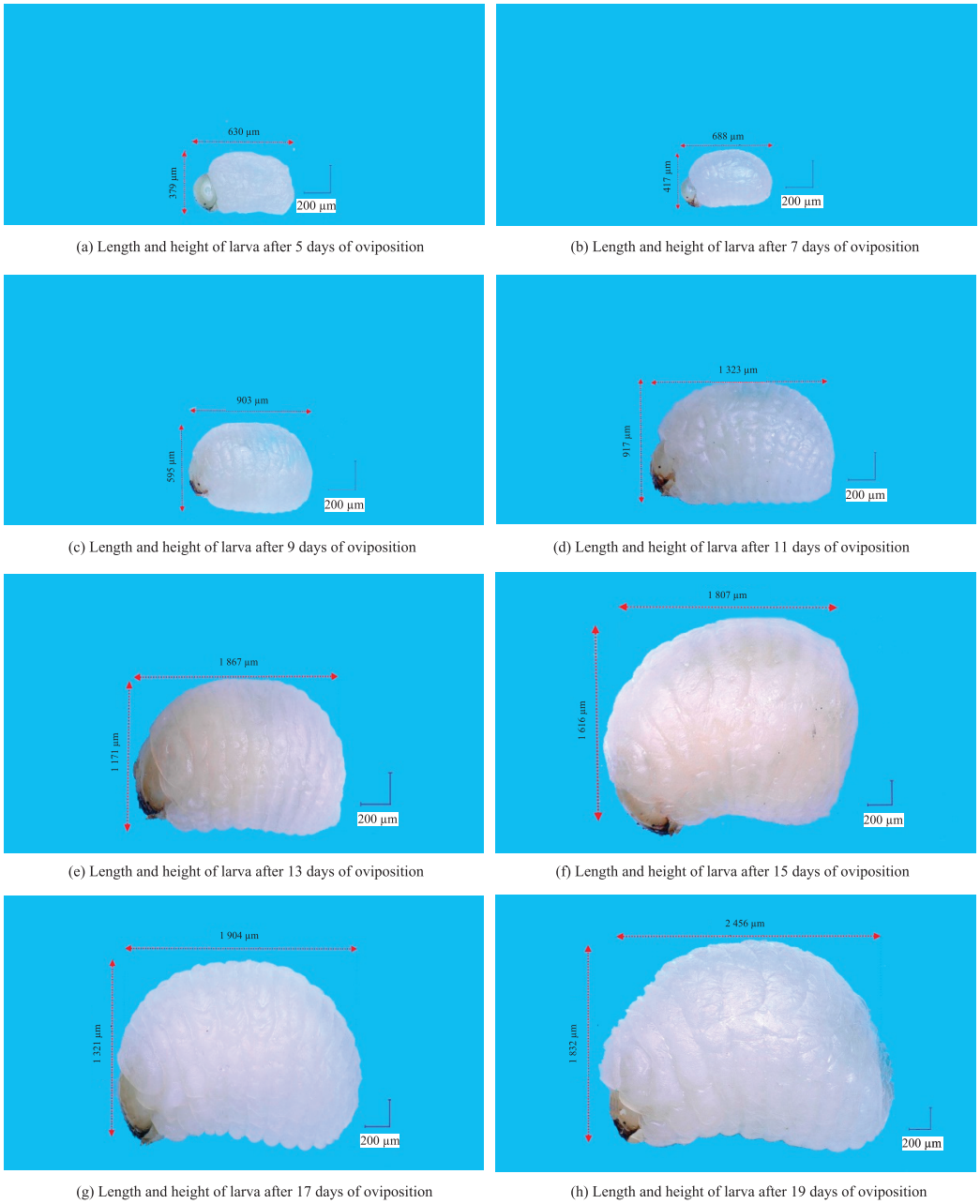
4 Discussion

There were four larva instars of *S. zeamais* that can be judged by the scale parameters, including the length, height and width of larva body in four groups of centralized sizes. The pre-pupa, young adult and old adult can be seen also clearly. Atlas of immature stages of the insect dissected from wheat kernels should be useful to know the insect development and to decide the management strategy. It is also reference to insect population monitoring and control. Devi et al.[1] reported the developmental periods of 1st, 2nd, 3rd, and 4th instar of *S. zeamais* reared in maize were 5.8, 7.0, 8.4, and 7.5 days. Obviously, the four instar developmental duration of *S. zeamais* reared in wheat in this paper was shorter sharply than that reared in maize. Similar observations were also made by Sharifi et al.[14] who reported that the four periods of larval growth for *S. zeamais* reared in wheat, were 3.6, 4.7, 4.8, and 5.0 days, respectively. Furthermore, a positive correlation was observed between the body length and width of the larval instars and body size of the male and female[1]. Here, *S. zeamais* hidden in wheat had a shorter developmental period than that reared in maize. The scale parameter and development period of the insect immature stages had been gotten from wheat, although they should be variable in other cereals and quality condition, especially for development duration.

Table 1 Mean length, height, width of insect body and width of head capsule of *S. zeamais* immature stages (n=5) measured at different times after oviposition, held at 28±0.5 °C, r.h.= 75%±5% on wheat

Measurement	1st larval instar (day 5–7)	2nd larval instar (day 9–11)	3rd larval instar (day 13–15)	4th larval instar (day 17–19)	Pre-pupa (day 21)	Pupa (day 23–25)
Body length	555±124^{A}	746±200^{B}	1 462±116^{C}	2 147±419^{D}	2 938±421^{E}	3 161±338^{F}
Body height	389±51^{A}	575±101^{B}	847±83^{C}	1 651±452^{E}	1 369±111^{F}	/
Body width	341±43^{A}	598±86^{B}	798±88^{C}	1 568±154^{D}	1 665±190^{F}	1 014±137^{G}
Width of head capsule	204±9^{A}	317±46^{B}	535±32^{C}	603±27^{D}	613±21^{D}	/

Note: Means ± SD followed by the same letter within a row are not significantly different from each other at 5% probability level.



(a) Length and height of larva after 5 days of oviposition

(b) Length and height of larva after 7 days of oviposition

(c) Length and height of larva after 9 days of oviposition

(d) Length and height of larva after 11 days of oviposition

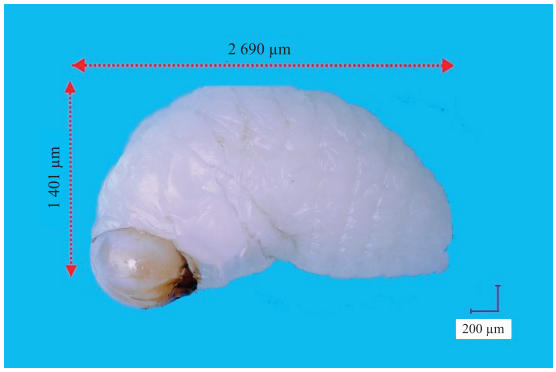
(e) Length and height of larva after 13 days of oviposition

(f) Length and height of larva after 15 days of oviposition

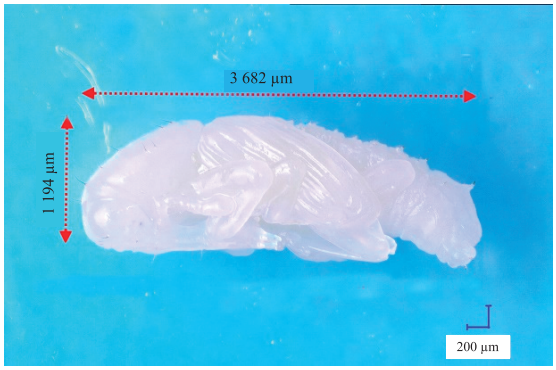
(g) Length and height of larva after 17 days of oviposition

(h) Length and height of larva after 19 days of oviposition

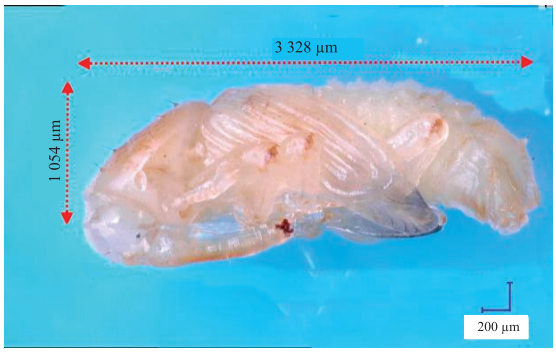
Fig. 2 The body length and height of larvae of *S. zeamais* reared in wheat kernel with different development time



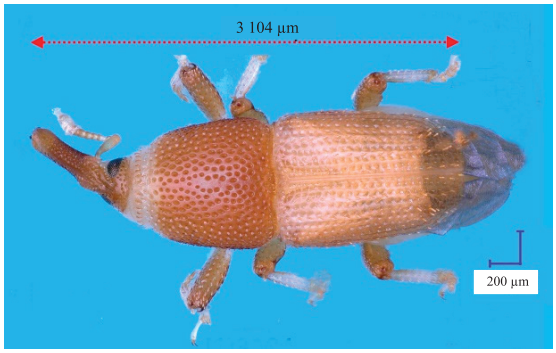
(i) Length and height of pre-pupa after 21 days of oviposition



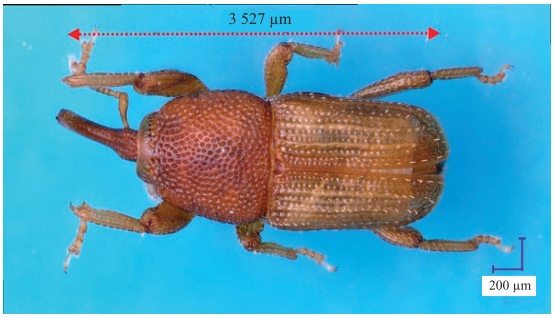
(j) Length and height of pupa after 23 days of oviposition



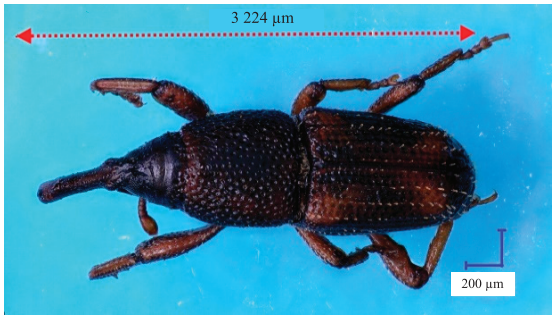
(k) Length and height of pupa after 25 days of oviposition



(l) Length of adult in wheat kernel after 27 days of oviposition

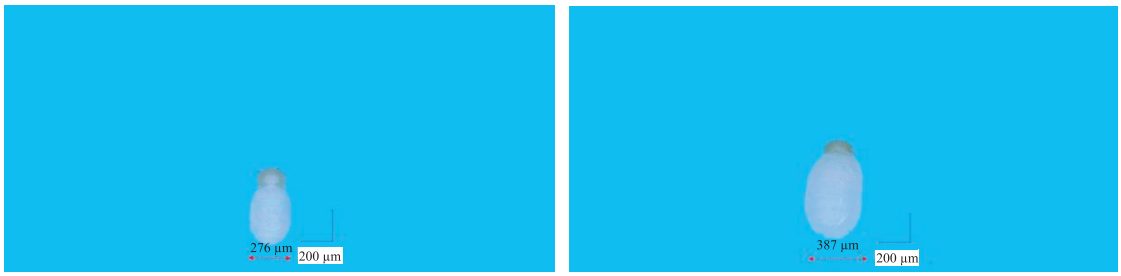


(m) Length of adult in wheat kernel after 29 days of oviposition



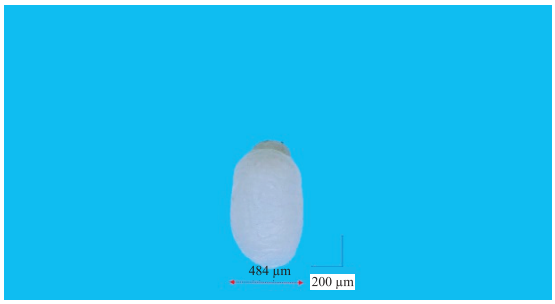
(n) Length of adult in wheat kernel after 31 days of oviposition

Fig. 3 The body length and height of *S. zeamais* hidden in wheat kernel in different development time

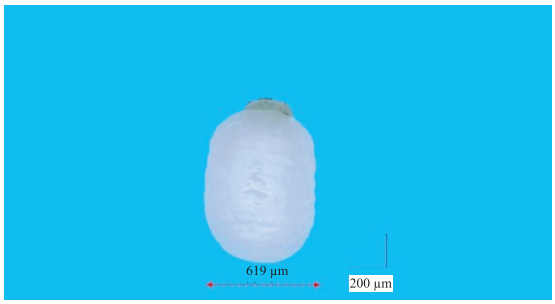


(a) Width of larva after 5 days of oviposition

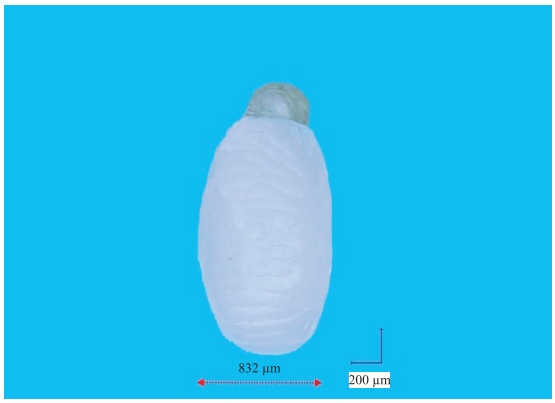
(b) Width of larva after 7 days of oviposition



(c) Width of larva after 9 days of oviposition



(d) Width of larva after 11 days of oviposition



(e) Width of larva after 13 days of oviposition

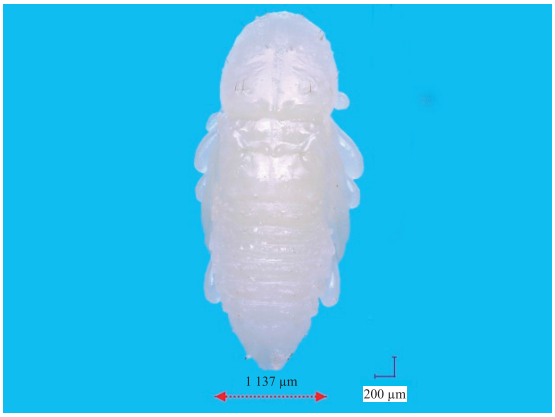
(f) Width of larva after 15 days of oviposition

(g) Width of larva after 17 days of oviposition

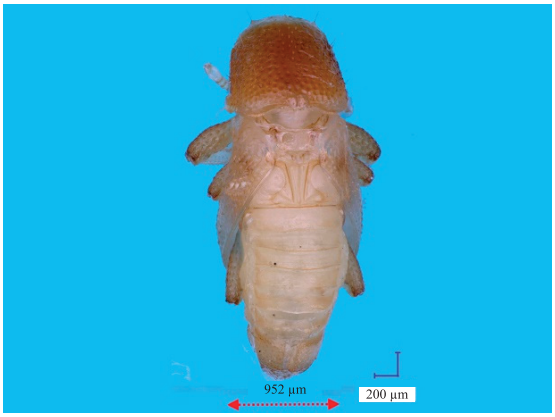
(h) Width of larva after 19 days of oviposition

Fig. 4 The body width of immature stage of *S. zeamais* hidden in wheat kernel in different development time

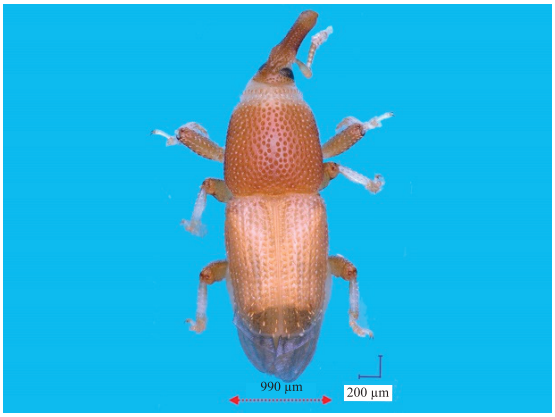
(i) Width of pre-pupa after 21 days of oviposition



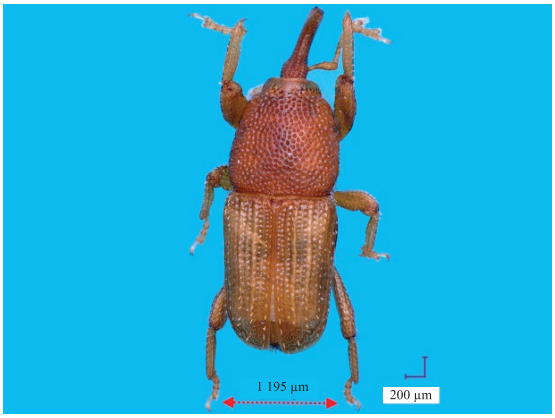
(j) Width of pupa after 23 days of oviposition



(k) Width of pupa after 25 days of oviposition



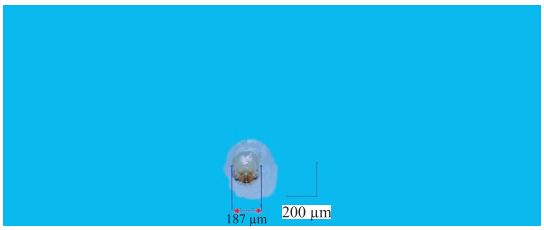
(l) Width of adult in wheat kernel after 27 days of oviposition



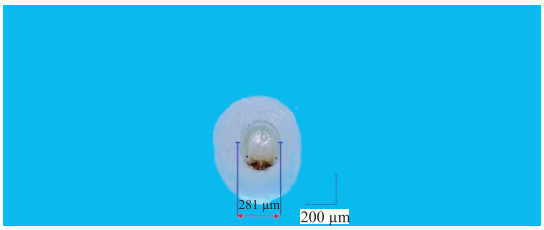
(m) Width of adult in wheat kernel after 29 days of oviposition

(n) Width of adult in wheat kernel after 31 days of oviposition

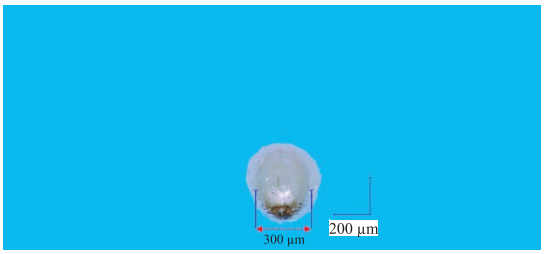
Fig. 5 The body width of *S. zeamais* hidden in wheat kernel in different development time



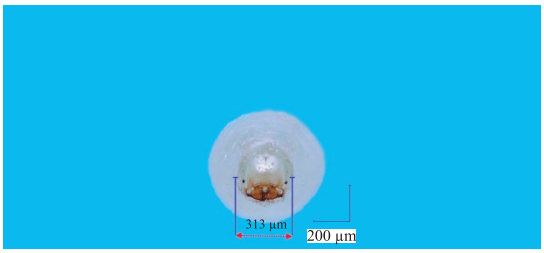
(a) Width of larva capsule after 5 days of



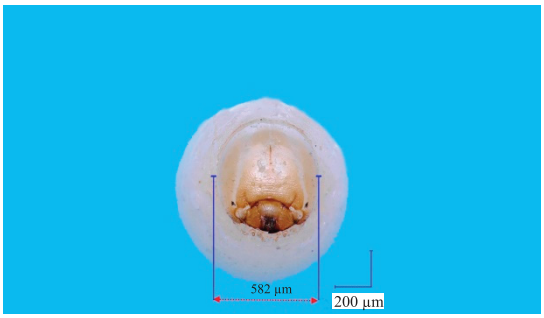
(b) Width of larva capsule after 7days of oviposition



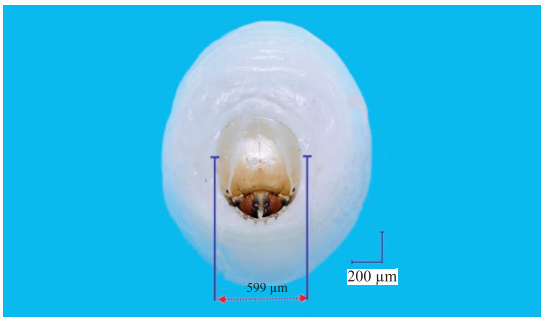
(c) Width of larva capsule after 9 days of



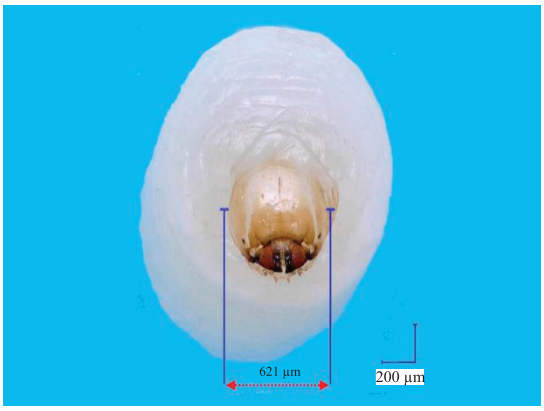
(d) Width of larva capsule after 11 days of oviposition



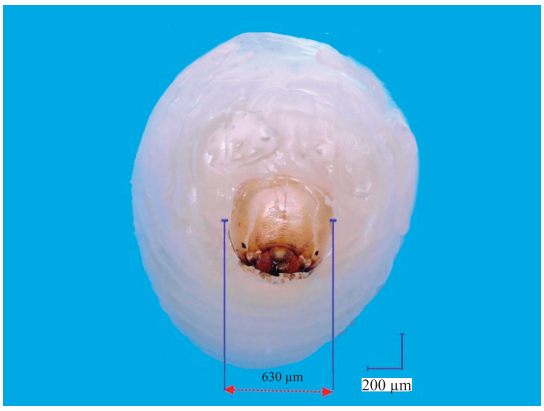
(e) Width of larva capsule after 13 days of oviposition



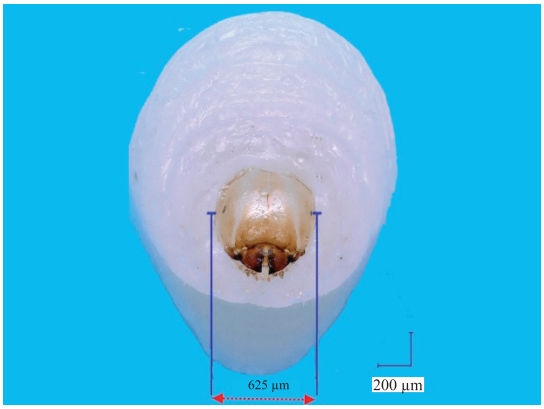
(f) Width of larva capsule after 15 days of oviposition



(g) Width of larva capsule after 17 days of oviposition



(h) Width of larva capsule after 19 days of oviposition



(i) Width of larva capsule after 21 days of oviposition

Fig. 6 Width of larva capsule after different days of oviposition

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Conflict of Interest

The authors declare that there is no conflict of interest.

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