Improved Visualization of Fat Body Cell Conditions and Abundance in the Southern Green Stink Bug (Hemiptera: Pentatomidae)[1]

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Abstract Fat bodies are a crucial source of energy for insect survival and reproduction. Differing states, or conditions, of fat body cells and amounts have been previously reported for southern green stink bug, *Nezara viridula* L., but clear supporting images are lacking. Further, in ongoing studies elucidating the ecology of southern green stink bug in the southern United States, additional fat body cell conditions in the abdomen were observed that had not been previously reported, and findings are presented here. Four fat body cell conditions were characterized based on appearance as: (1) *cloudy, white, and oily*; (2) *spongy, white*; (3) *spongy, yellow*; and, (4) *honeycomb*. Amounts of fat body cells in abdomens were categorized as: *abundant*, *intermediate*, and *lean*. This report improves the sole extant schematic of fat body cell conditions by presenting images relating to newly identified fat body cells conditions, and provides the first visuals depicting the amounts of fat body cells that can be encountered in adult southern green stink bugs. These findings are critical components in elucidating the biology and ecology of the southern green stink bug.

Key Words fat body cell condition, *Nezara viridula*, physiology, fat body reserves

Stink bugs (Hemiptera: Pentatomidae) have reached elevated pest status in the Cotton Belt of the southern United States following boll weevil eradication efforts, implementation of transgenic cotton varieties, and use of species-specific insecticides (Williams 2003). The southern green stink bug, *Nezara viridula* L. (Hemiptera: Pentatomidae), is a cosmopolitan pest of cotton, soybeans, other row crops, and fruits and nuts (Todd 1989, and references therein). Because of the elevated pest status, efforts are underway to determine the seasonal and overwintering survival of this pest in the southern United States.

Population potential of insects is influenced by the insect fat body, which is composed of masses of cells whose contents contribute to critical life processes such as reproduction, migration, and overwintering survival (Chapman 1998). These cells may be loosely arranged or closely compacted within the abdomen. Contents of these fat body cells vary depending on an insect's developmental stage and nutritional status. In newly-eclosed adults, these cells commonly contain extensive lipid droplets, glycogen accumulations, and protein granules (Chapman 1998) which are used during the insect's lifespan. Typically, the developmental status of the reproductive systems and

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condition and amount of fat body cells in an insect are indicators commonly used to determine whether adult insect populations are actively reproducing or preparing for dormancy (Spurgeon et al. 2003). Thus, in an effort to elucidate the biology and ecology of southern green stink bug adults in a temperate region, Esquivel (2009) documented differing developmental stages for male and female reproductive systems. However, accurate and clear descriptions of the types and amounts of fat body cells associated with southern green stink bug are lacking. Whereas several studies frequently discuss the "amount" of fat body cells in southern green stink bug (Kiritani 1963, Pitts 1977, Banerjee and Chatterjee 1985, Seymour and Bowman 1994, Jones and Westcot 2002), only Kiritani (1963) provides a rudimentary visual schematic of 2 fat body cell "types", hereafter referred to as physiological condition. None of these previous studies provided visuals allowing more objective quantification of the amount of fat body cells or identification of physiological condition of fat body cells in southern green stink bug. Based on the latter and preliminary observations in ongoing studies elucidating the ecology of southern green stink bug in the southern U.S., it was determined that clear descriptions of fat body cells (i.e., physiological condition and amounts) associated with southern green stink bug were needed. Improved fat body descriptions, in conjunction with reproductive development records (Esquivel 2009), are critical to properly determine the biotic potential and survival of insects during the row crop growing seasons and the overwintering period. The objective of this report is intended to improve upon the extant schematic of 2 fat body cell physiological conditions by presenting images relating to newly-identified physiological conditions of fat body cells, and provide cornerstone visuals depicting the amounts of fat body cells that can be encountered in adult southern green stink bugs.

Materials and Methods

Source of insects. To identify representative samples of fat body cell types in wild southern green stink bug adults, a total of 1514 wild insects was dissected (see below for dissection method) from 2004 - 2009. Insects were obtained using blacklight traps (n = 662 adults), hand-collections from pearl millet (*Pennisetum glaucum* (L.) R. Br.) (n = 242) and castor beans (*Ricinus communis* L.) (n = 21), and by sweep net in alfalfa (*Medicago sativa* L.), cotton (*Gossypium hirsutum* L.), and soybeans (*Glycine max* (L.) Merr.) (n = 296 insects total from the 3 crops) and local wild vegetation (n = 293). For the sweep net collections, methods are described in studies documenting wild host plant associations for *Lygus lineolaris* (Palisot de Beauvois) and *Pseudatomoscelis seriatus* (Reuter) (Esquivel and Mowery 2007, Esquivel and Esquivel 2009, respectively). For the blacklight collections, traps were serviced 2 - 3 times per week and were operational throughout the sampling years.

As evidenced by the numbers above, insects from the field proved to be difficult to locate and collect, especially during local fall and winter seasons. Thus, to account for insects that would presumably be exposed to local overwintering conditions, cohorts of nymphs and adults were reared in the laboratory under simulated local fall and winter temperatures based on 100-yr averages for the region (NOAA 2009). This rearing regimen held eggs and first and second instars at constant 29.7°C, 14:10 [L:D] photoperiod, whereas third through fifth instars and subsequent adults (n = 426) were held at 10:14 [L:D] photoperiod under simulated overwintering temperatures (NOAA 2009) (shortened day-length has previously been reported to induce 'diapause' during overwintering periods [Ali and Ewiess 1977]). Average overwintering temperatures

were calculated using the initial date of 1 October. For comparison with the shortened day-length regimen, separate cohorts of nymphs and subsequent adults (n = 515) were reared at constant 29.7°C and 14:10 [L:D] photoperiod. For both rearing profiles, first instars were provided green beans (*Phaseolus vulgaris* L.) only, subsequent instars were provided green beans and soybean seeds whereas individual adults were only provided a section of green bean (approx. 2.5 mm in length). As insects perished, insects were dissected to determine reproductive status and to characterize fat body cell condition and amounts.

Understandably, given the variable sources of wild insects and manipulation of abiotic factors for insects held in the laboratory, one would expect that the effects of differing insect sources and abiotic and biotic factors on the physiology of the southern green stink bug would be presented here. However, this report is intended to establish reference images for subsequent reports that, in conjunction with assessments of reproductive status (Esquivel 2009), will indeed more thoroughly address the influence of abiotic and biotic factors on the biology and ecology of the southern green stink bug in temperate regions.

Dissection method. A paraffin-filled glass Petri plate bottom (100 × 20 mm diam) was imprinted with 4 'dissection wells'; one well per quadrant of the plate bottom. The wells were formed by pressing the moistened bottom of a glass vial (25 drams) into the cooling paraffin, being careful not to burst the surface of the recently-heated paraffin. Individual southern green stink bug adults were pinned dorsally through the thorax into a dissection well filled with distilled water, and the dissection plate placed on the illuminated stage of an Olympus SZ60 stereomicroscope (Olympus Ltd., Kalamazoo, MI). After pinning the insect, 2 pairs of forceps were used to sequentially remove the pairs of wings, the scutellum, and the dorsal abdominal cuticle.

To facilitate repeating the dissection method, the technique presented here is based on the assumption that the researcher is a right-handed individual. Essentially, 1 pair of forceps was used for grasping the body part of interest whereas the other pair was used to prevent the insect from being pulled apart by applying downward pressure at identified locations. To remove the wings on the right side, the right pair of forceps was used to separate the wings, subsequently grasping the hemelytron at the base whereas applying pressure dorsally on the scutellum with the left pair of forceps. The hemelytron was then pulled away from the right side of the insect body; this procedure was repeated for the hindwing on the right side. Inversely, for removal of the wings on the left side, the left pair of forceps was used to separate the wings, subsequently grasping the hemelytron at the base whereas applying pressure dorsally on the scutellum with the right pair of forceps. The hemelytron was then pulled away from the left side of the insect body; this procedure was repeated for the hindwing on the left side. To remove the scutellum, the left pair of forceps was used to apply pressure dorsally to the rear of the abdomen whereas using the right pair to grasp the tip of the scutellum, located over the middle of the abdomen, and pull the scutellum away from the abdomen. The scutellum was pulled up and toward the head region, and, without completely removing the scutellum, allowed to swivel at the point where the scutellum joins the body. Whereas still holding the scutellum with forceps in the right hand, the left pair of forceps applying pressure at the rear of the abdomen was brought up to apply pressure at the juncture of the thorax and abdomen. Whereas holding the left pair of forceps across and perpendicular to the length of the insect body and depressing at this juncture, the scutellum was simultaneously pulled away and removed from the body. To remove the dorsal abdominal cuticle, one prong from the right pair of

forceps was inserted at the juncture of the cuticle with the thorax. Insertion of forceps was only to break the cuticle at the juncture with the thorax, thus, only a small length of the prong was inserted. Whereas using the left pair of forceps to apply pressure on the anterior-lateral right side of the abdomen, the inserted prong of the right pair of forceps was moved horizontally and perpendicular to the length of the insect body along the underside of the cuticle toward the opposite left side of the abdomen, and away from the left pair of forceps. This prevented the abdomen from being pulled along with the inserted prong. Similarly, after breaking the cuticle at the juncture of the abdomen and thorax, one prong from the right pair of forceps was inserted at the dorso-lateral juncture of the abdomen and thorax and, whereas using the left pair of forceps to apply pressure along the top of the cuticle, the right pair of forceps was moved underneath and along the length of the right side of the abdomen to the rear of the abdomen. After the cuticle had been cut along the side of the abdomen, the left pair of forceps was used to hold the cuticle at the cut to begin pulling the cuticle up and away from the abdomen. Whereas gently pulling the cuticle, however, care was taken to use the right pair of forceps to disengage, or disconnect, any tracheae with attached fat body cells that may have been clinging to the underside of the abdominal cuticle. When the cuticle was pulled up with only the opposing left side attached, and the cuticle oriented vertically to the abdomen, the right pair of forceps was used to apply pressure internally along the attached left side to enable pulling away of the cuticle in its entirety using the left pair of forceps.

Fat body characterization and imaging. Assessments of the physiological condition of fat body cells and amount of fat body cells in the abdomens were made immediately after removal of the dorsal abdominal cuticle. Physiological conditions were characterized based on the external appearance of the fat body cells (i.e., as influenced by the relative abundance of contents within the cells), because studies involving dissections require rapid assessments. Biochemical analyses were not conducted, but may be pursued pending outcome of ongoing studies examining relationships of fat bodies and insect survival under varying abiotic conditions. Findings regarding various physiological conditions of the fat body cells and amounts of fat body cells comprising the insect fat body in the abdomen are presented here. The images of fat body cell conditions and amount of fat body cells in the abdomen were captured using Lumen*era* INFINITY software and camera (Model: INFINITY 1 - 3C; Lumen*era* Corporation, Ottawa, Ontario K2E 8A7, Canada) mounted on the Olympus SZ60 stereomicroscope. The camera was interfaced with a Windows-driven PC, which was used to operate software and record selected images.

Results

Four physiological conditions of fat body cells (Fig. 1) were observed in adult southern green stink bugs. These conditions were characterized as: (1) *cloudy, white and oily* - white and opaque with contents throughout the fat body cell, exhibiting an oily-like appearance (Fig. 1A); (2) *spongy, white* - white and translucent with minimal, if any, contents within the fat body cell (Fig. 1B); (3) *spongy, yellow* - yellow and translucent with minimal, if any, contents within the fat body cell (Fig. 1C); and, (4) *honeycomb* - white or translucent with, presumably, a pattern of contents coagulating immediately underneath the fat body cell surface, thereby producing a distinct reticulated pattern or honeycomb-like appearance (Fig. 1D).

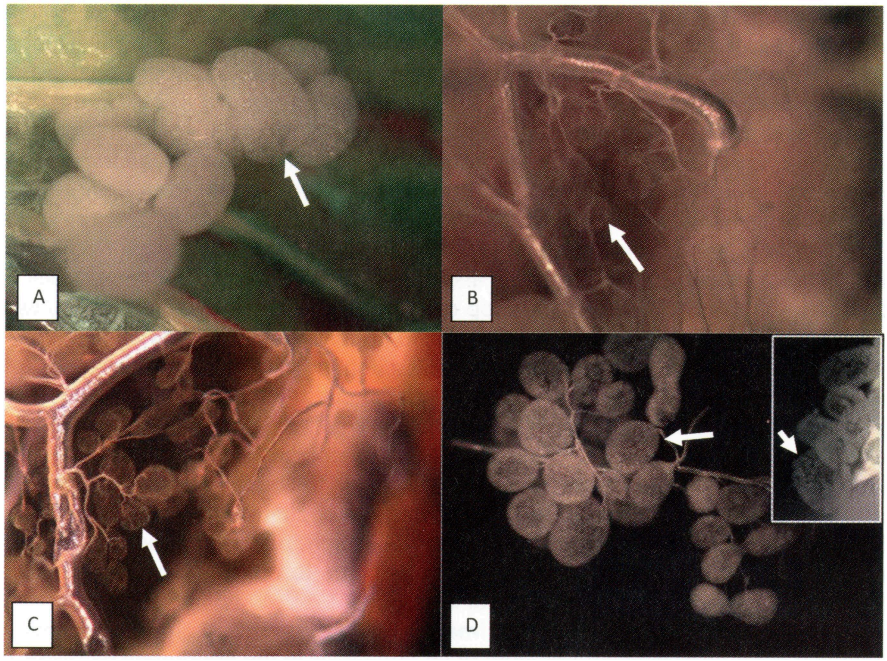

Fig. 1. Physiological conditions of fat body cells (highlighted by arrows) observed in adult southern green stink bug. (A) *Cloudy, white, and oily* – white and opaque with reserves seemingly throughout individual fat body cells, producing an oil-like appearance (at 110.90×). (B) *Spongy, white* – white and translucent with minimal, if any, reserves within individual fat body cells (at 110.90×). (C) *Spongy, yellow* – yellow and translucent with minimal, if any, reserves within individual fat body cells (at 88.00×). (D) (including inset) *honeycomb* – white or translucent with, presumably, a pattern of reserves coagulating immediately beneath the fat body cell surface, thereby exhibiting a distinct reticulated pattern or honeycomb appearance (at 110.90×).

Amounts of fat body cells in individual specimens examined were variable, but a general pattern of distribution was evident (Fig. 2). This pattern led to the classification of fat body cell quantities as: *abundant* – dorsal fat body cells completely covering all organs, with organs not readily visible (Fig. 2A); *intermediate* – dorsal fat body cells irregularly distributed and covering organs, with organs partially visible through dispersed fat body cells (Fig. 2B); and, *lean* – dorsal fat body cells relegated to periphery of abdomen, with organs readily visible (Fig. 2C).

Each of the fat body cell conditions and levels of abundance (Figs. 1 and 2, respectively) were observed in southern green stink bugs irrespective of source. Because ages and history of wild insects were unknown, and to demonstrate the changes that fat body cells undergo, Fig. 1 presents images based on known-age insects reared in the laboratory under simulated historical temperate fall and winter temperatures

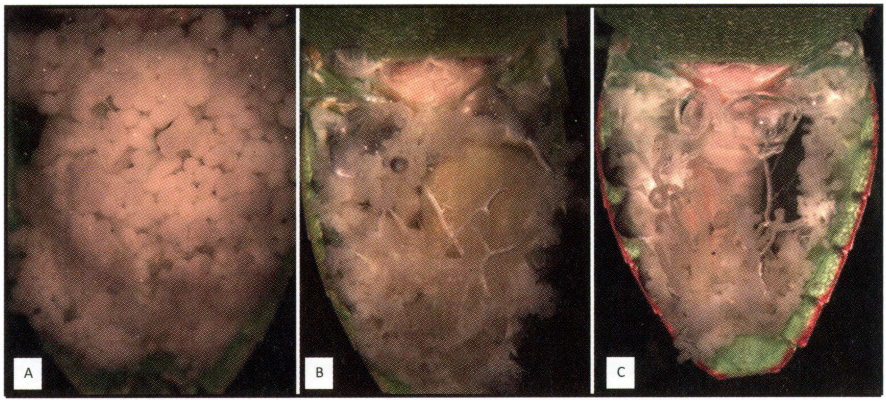

Fig. 2. Representative dorsal view of ratings for the amounts of fat body cells in the abdomens of adult southern green stink bugs. (A) *Abundant* – dorsal fat body cells completely covering all organs, with internal organs not readily visible (at 26.40×). (B) *Intermediate* – dorsal fat body cells irregularly distributed and covering organs, with organs partially visible through dispersed fat body cells (at 21.12×). (C) *Lean* – dorsal fat body cells relegated to periphery of abdomen, with organs readily visible (at 21.12×).

under short photoperiod. Figure 1A depicts cloudy, white and oily fat body cells observed in a 30-d-old adult, and these cells are identical to those observed in newly-eclosed adults reared under long photoperiods, as well as wild adults of unknown age. Influence of insect age needs to be explored because the spongy, white fat body cells (Fig. 1B) and spongy, yellow fat body cells (Fig. 1C) also were observed in 30-d-old and 41-d-old adults, respectively. Wild adults collected in October and November possessed honeycomb fat body cells identical to those seen in the adults held under local simulated overwintering conditions (Fig. 1D). Fat body cells observed in a 93-d-old adult are shown in Fig. 1D, with fat body cells observed in a 118-d-old adult shown within the inset. Additional analyses are required to determine the effect of age on presence of fat body cell condition and amount, but it is proposed that the fat body cells classified as spongy, white or spongy, yellow are likely intermediate stages between cloudy, white, oily fat and honeycomb fat, where the spongy-type fat body cells reflect utilization of the contents within the fat body cell for survival.

Discussion

Images presented herein provide a more complete and clear assessment of differing conditions of fat body cells and quantities that can be found in southern green stink bug adults (Figs. 1, 2). Collectively, these images are a vast improvement over the sole previous schematic of 2 fat body cells (Fig. 3). The images in Fig. 1 result from insects held in the laboratory, but given that all the fat body cell conditions and abundance of cells in the abdomens shown here were also seen in wild insects of unknown age and other known-age insects (J.F.E., unpubl. data), Figs. 1 and 2 are good

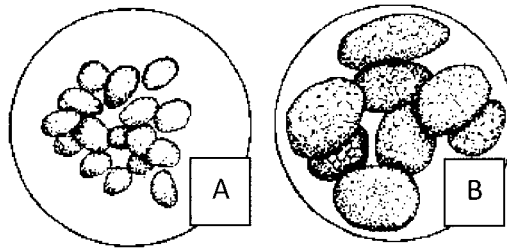

Fig. 3. Extant schematic depicting fat body cells in southern green stink bug. (A) "Fat body cells of normal female." (B) "Fat body cells just before or during hibernation." [Adapted and reprinted with permission from Kiritani (1963)]

representations of fat body cell conditions and abundance of fat body cells, respectively, encountered in southern green stink bug adults.

Presumably, 2 of the fat body cells shown here (Fig. 1) reflect those by Kiritani (1963) (Fig. 3); however, because of the two-dimensional orientation and lack of color originally presented, it is difficult to determine with certainty which 2 fat body cell types observed in the current study may be representative of those depicted by Kiritani (1963). Regardless, Kiritani (1963) presents, "Fat body cells of normal female" (Fig. 3A) and "Fat body cells just before or during hibernation" (Fig. 3B), with the latter being referred to as "the hypertrophy of fat body cells." Observations of the spongy, white fat body cells and the honeycomb fat body cells in this report seem consistent with the virtual absence of markings (Fig. 3A) and the more distinct pattern of markings (Fig. 3B), respectively, presented by Kiritani (1963). However, Fig. 3B could also be interpreted to be representative of the cloudy, white, oily fat body cells observed in the current study.

Another attempt at describing fat bodies, but without supporting images (Banerjee and Chatterjee 1985), complicates the current understanding of types and amounts of fat body cells associated with southern green stink bug. For example, the terminology used such as "relative size…of fat" implies that Banerjee and Chatterjee (1985) measured the fat bodies, but they did not measure the size of fat body cells individually or collectively, per se. Instead, Banerjee and Chatterjee (1985) provide 3 grade ratings of the *amounts* and *types* of fat as follows: "(1) fat thinly spread over the dorso-lateral corners of the abdomen and without free fat globules; (2) about half of the abdominal cavity around the inside of the body wall occupied by a continuous layer of fat and often with free fat globules; and (3) all or nearly all of the abdominal cavity occupied by fat and with numerous free fat globules." The *amounts* described by Banerjee and Chatterjee (1985) generally agree with observations in the current study – lean, intermediate, and abundant – which would be equivalent to the 1, 2, and 3 ratings, respectively. However, the descriptions regarding the *types* of fat are open to interpretation. First is the issue of the types of fat identified, and, secondly, what is the interpretation of the "fat" and "free fat globules"? Without supporting images for ratings 1, 2, and 3 (Banerjee and Chatterjee 1985), how does one interpret "fat…without free fat globules", "fat…with free fat globules, and "fat and with numerous free fat globules", respectively? The fat identified as "with numerous free fat globules" suggests the equivalent of cloudy, white, oily fat (Fig. 1A). Similarly, the fat identified as "without free fat globules"

could be the observed spongy, white fat (Fig. 1B). This would be accurate if the "free fat globules" were the equivalent of the reserves within the fat body cells as described in the current study. If this is indeed the case, Banerjee and Chatterjee (1985) may have inadvertently and exclusively tied the amounts and types of fat body cells together. That is, only those insects graded as 3 would have the fat "with numerous free fat globules" and likewise with the other 2 grades. However, this is not always the case because, for instance, varying amounts of fat body cells have been observed in adults possessing cloudy, white and oily fat cell bodies (J.F.E., unpubl. data).

Additional data are being collected to determine the role of the identified fat body cell conditions and amounts of fat body cells in relation to southern green stink bug survival. Fat bodies are a source of energy for survival and reproduction (Snodgrass 1993), and these likely undergo changes in appearance as this resource is used, as suggested by Fig. 1. Because use of this resource is in a state of flux, fat body cells in a given insect may not all be a specific type. That is, some insects may be transitioning to honeycomb-type fat whereas still having remnants of the cloudy, white and oily fat (Fig. 1D).

Fecundity, health, and potential overwintering survival of insects are usually determined by the condition of fat body cells, amount of available fat body cells, and reproductive condition. The findings presented here complement the identification of gonadal development in southern green stink bug (Esquivel 2009). When gonadal development is assessed in conjunction with fat body cell types and amount of cells comprising the fat body, estimates may be deduced regarding survival and reproductive potential. However, these characters are not mutually exclusive. For instance, females with a full complement of eggs may be healthy but the amount of fat body cells is affected by the number of eggs present. A newly-eclosed female may have a rating of abundant fat body cells but the rating may change to intermediate or lean as egg clutches develop. Similarly, fully reproductive males that have not mated have been observed to possess fully extended ectodermal sacs (Esquivel 2009) occupying over half the abdomen, thereby affecting the amount of fat body cells. Similar occurrences have been observed in other regions of the world for southern green stink bug, with the amount of fat body cells oscillating depending on reproductive status (Kiritani 1963, Banerjee and Chatterjee 1985).

It is generally accepted that fat bodies are a critical energy resource for insect survival during times of scarce food resources or harsh environmental conditions. Numerous studies are available regarding reproductive development and diapause induction in the pentatomids; yet, these studies fail to clearly describe (Ali and Ewiess 1977, Banerjee and Chatterjee 1985, Musolin and Saulich 2001, Jones and Westcot 2002, Coombs 2004) or mention the physiological conditions or amounts of fat body cells encountered (Harris et al. 1984, Musolin and Numata 2003a, b, 2004, Musolin et al. 2007). In most cases, terms such as, "well-developed fat body", "fully developed fat body", or other similarly ambiguous descriptors, are generally used to describe the insect fat bodies – and *amounts* of fat body cells observed are not always provided. Alternatively, authors commonly cite Kiritani (1963) as criteria for respective studies. For the former, subsequent researchers are at a disadvantage in determining what constitutes a "well-developed fat body" or "fully developed fat body" because, without supporting images, the 2 terms are wholly open to interpretation. For the latter (i.e., Kiritani 1963), it has been shown that ample improvement was needed.

Images and concise descriptions presented here of physiological conditions of fat body cells and amounts of fat body cells within adults provide more accurate

assessments of fundamental resources likely affecting the southern green stink bug. Additionally, these results are likely applicable to related hemipteran species because cloudy, white, and oily fat body cells have been observed in *Euschistus servus* (Say) adults (J.F.E., pers. obs.). These findings are critical components for providing a foundation to elucidate the biology and ecology of the southern green stink bug, and related species, in the southern U.S.

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