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First colony of *Stomoxys calcitrans* (Diptera: Muscidae) successfully established under laboratory conditions in Argentina

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Primera colonia de *Stomoxys calcitrans* (Diptera: Muscidae) establecida bajo condiciones de laboratorio en Argentina

RESUMEN. Las moscas de los establos, *Stomoxys calcitrans* (L.) (Diptera:Muscidae) son insectos hematófagos que representan un problema, no solo por su hematofagia y transmisión de patógenos, sino además, porque su impacto económico en las producciones pecuarias es relevante. En Argentina aún no existe una cría de la plaga. El objetivo de este trabajo es establecer y describir la primera cría de *Stomoxys calcitrans* en el país y registrar la duración de cada estadio bajo condiciones controladas de laboratorio. Los adultos fueron exitosamente criados en una cámara de cría (28 ± 1 ºC y 47 ± 1 %RH) bajo un fotoperiodo de 14 h: 10 h (Luz: Oscuridad), mientras que los estadios inmaduros se criaron a 25 ± 2 ºC y luz natural. El ciclo desde los huevos hasta la emergencia de adultos duró 16,75 ± 2,9 días. El tiempo de desarrollo requerido para alcanzar el nuevo estado fue de: 2,0 ± 0,8, 6,75 ± 1,3 y 7,75 ± 1,7 días para huevos, larvas y pupas; respectivamente. Los adultos vivieron 16,5 ± 1,91 días. El período de preoviposición fue de 5,0 ± 0,8 días. La supervivencia de larvas y pupas fue de 93,28% y 70,25%, respectivamente. Estos resultados pueden ser usados como referencia por otras colonias que se establecieren en un futuro en el país.

PALABRAS CLAVE. Mosca de los establos. Ciclo de vida. Estadios inmaduros. Colonia de Laboratorio.

ABSTRACT. The stable flies, *Stomoxys calcitrans* (L.) (Diptera: Muscidae), are blood-sucking insects that represent a serious problem not only because of their hematophagy and transmission of pathogens, but also because of their considerable economic impact in animal production. No colony of these insects exists in Argentina. The objectives of this work were to establish the first *Stomoxys calcitrans* colony in this country, and to standardize and report the duration of each stage under controlled laboratory conditions. The adult flies were successfully reared in a growth chamber (28 ± 1 ºC and 47 ± 1 %RH, under a 14 h light: 10 h dark photoperiod); whereas the immature stages were kept at 25 ± 2 ºC and natural light. The cycle from eggs to adult emergence lasted 16.75 ± 2.9 days. The development time required to reach a new stage was: 2.0 ± 0.8, 6.75 ± 1.3 and 7.75 ± 1.7 days for eggs, larvae and pupae, respectively. Adult flies lived 16.5 ± 1.91 days. The preoviposition period lasted 5 ± 0.8 days. The larval and pupal survivals were 93.28% and 70.25%, respectively. These results could be used as a reference for other colonies that may be established in the future.

KEY WORDS. Stable fly. Life cycle. Immature stages. Laboratory colony.

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INTRODUCTION

Stomoxys calcitrans (Linneaus) (Diptera: Muscidae), also known as stable flies, are blood-sucking insects associated with livestock and wildlife throughout the world. They represent a serious problem not only because of their painful bites, hematophagy, and transmission of pathogens (Zumpt, 1973; D´Amico *et al*., 1996), but also because of their considerable economic impact (Campbell *et al*., 2001). A number of chemicals and application methods have been used to control stable and house flies while feeding upon host animals, most with limited success. Currently, there are no effective control measures available for stable fly larvae or adults in pastured livestock situations (Foil & Hogsette, 1994; Broce *et al*., 2005; Hogsette *et al*., 2008). Application of insecticides to pastured livestock is not practical because residual activity is short lived and stable flies spend the majority of their adult lives off the host (Farkas & Hogsette, 2000).

Biological control is an alternative for chemical control, being investigations intended for control of livestock ectoparasites (Hogsette & Jacobs, 1999), including *S. calcitrans*. Micoinsecticides based on entomopathogenic fungi from the order Entomophthorales (Zygomycota) and Hypocreales (Ascomycota) may be formulated and included in an Integrated Pest Management Program (IPMP) (Furlong & Pell, 2005). In this sense, Singh & Moore (1985) have pointed out that progress in entomological research and success of IPMP depend on our ability to rear insects and establish colonies in the laboratory.

The purposes for which insects are reared include education, personal collections, stationary exhibits, insect zoos, butterfly houses, pets, human and animal food, agricultural and medical research, pest management and pollination (Leppla, 2002).

Although the stable fly is considered less amenable to laboratory rearing than the house fly (Schoof, 1964), it has been reared in laboratory conditions in order to study its biology and develop new methods of pest control (Berkebile *et al*., 2009). However, to the best of our knowledge, no colony of *S. calcitrans* has been established in Argentina, and in general little is known about stable fly biocontrol using entomopathogenic fungi (Moraes *et al*., 2008).

The objectives of this work were to establish the first *S. calcitrans* colony in Argentina and to standardize and report the duration of each *S. calcitrans* immature stage under constant laboratory conditions. The establishment of such a colony will allow us to understand pest biology parameters and to perform entomopathogenic fungi-host bioassays under controlled laboratory conditions.

MATERIALS AND METHODS

COLONY ESTABLISHMENT

For the establishment of the *S. calcitrans* colony, approximately 3,000 pupae were donated from the *S. calcitrans* Laboratory Colony of the Agricultural Research Service, United States Department of Agriculture (ARS-USDA), Gainesville, Florida, USA. It took us 6 generations to stabilize the colony and to obtain significant production for bioassays. Temperature (ºC) and Relative Humidity (%RH) were recorded twice a day.

REARING SYSTEM

Rearing and breeding guidelines for *S. calcitrans* were created in order to organize and establish the rearing system. A schedule was set up at the beginning of every month with all the activities to be performed. All trays and cages had dates indicating the estimated dates of pupa formation and adult emergence, respectively.

Adults: Estimated Adult Emergence Day (EADE) was calculated for the approximately 3,000 imported pupae and all generations after them under our laboratory condition. For this purpose the following equation was developed:

EAED = Estimated Day of Pupa formation (EDP) + 7days, where 7 days was a constancy obtained from Kunz *et al*. (1977) and Bailey *et al*. (1975). These authors had reared the stable fly in similar conditions as we did in this work. Also, we previously calculated EDP because this information helped us to separate on time the pre-pupa and mature larvae from the rearing larval medium. This information is given in detail in pupae paragraph. For this purpose, the following equation was developed:

EDP = Day of egg cultivation in medium + 7 days, where 7 days was a constancy obtained from Parr (1959).

The adults that emerged from the pupae provided by ARS were placed in adult cages. The adults rearing facility consisted of a room 4.82

m long x 2.32 m wide x 2.5 m high maintained at a temperature of 28 ± 1 ºC and 47 ± 1 %RH, under a 14 h : 10 h (Light : Dark) photoperiod. Metallic screened shelves held the adult cages which were 40 cm long x 40 cm wide x 40 cm high. The cages had an aluminum frame with a solid aluminum floor and a plug to drain after sanitation. The cages were covered with an aluminum screen mesh, except for half of the front which was covered with a cloth sleeve to allow access to the inside of the cage. Adult feeding methodology was based on studies carried out by Schoof (1964). Water and citrated bovine blood were offered as food in cotton balls inside cups. A fresh cup was placed in each cage daily, except on Fridays, when two cups were used to eliminate Saturday´s feeding. The blood was kindly provided by Campo Experimental de Animales de Producción of Centro de Investigación de Ciencias Veterinarias y Agronómicas (CICVyA), Instituto Nacional de Tecnología Agropecuaria (INTA). The blood obtained from adult bovines three times a month was collected from the jugular vein into a sterile transfusion bag containing 63 ml of citrated anticoagulant solution (Simple bag for blood code S-91. Laboratory P.L Rivero y cia. S. A. Industria Argentina) and kept at 4 ºC.

Eggs: The cups of blood that provided food also provided an oviposition medium, since the females laid their eggs on the surface of the blood-soaked cotton. The eggs were separated from cotton by rinsing and collected into a beaker where they could settle. The harvested eggs were set into a test tube to measure their quantity in milliliters. Then, approximately 1ml of eggs (approximately 10,000 eggs) was rinsed onto a tray of rearing medium.

Larvae: The larva rearing facility consisted of a room 5 m wide x 4 m long x 2 m high at 28 °C ± 1 ºC. Metallic screened shelves held the larval rearing trays. The trays were 55 cm long x 45 cm wide x 8.5 cm high, and they were covered with a voile cloth secured tightly with a rubber band and held at 25 ± 2 ºC. We adapted the larval medium according to its availability in the local market, and we used ingredients within reach of Buenos Aires, Argentina. The larval medium formula was based on Hogsette (1992) and consisted of 75% wheat bran, 15% texturized soya, 9.75% brown sugar, and 0.25 % dry yeast, and it was mixed with water (1 kg of medium with 750 ml of water), so that a few drops of fluid could be squeezed from it. The trays were held for 14 days until pupation was nearly complete. This synchronization of the larval cycle eliminated the weekend work.

Pupae: Stable fly larvae remain in the larval medium to pupariate, but go through a wandering stage to select the best microhabitat for development of pupae. Mature stable fly larvae search for a dark habitat with approximately 68% RH. This allowed us to separate larvae from the medium by benefiting from this behavior. About 24-48 hours before EDP, we removed the cloth cover and exposure to light and the larvae moved downward. Three-quarters of the ´upper´ larvae rearing medium were removed using a trowel to concentrate the pre-pupa and mature larvae. Larvae again moved downward and more media could be carefully removed. This resulted in the formation of “larvae nests” and later of the puparia.

Smaller trays were made so that half of the tray contained the rearing medium and the other half moist cotton. Once larvae reached the pupal stage, they were separated from the rearing medium, by using the shelf method as described by Berkebile *et al.* (2009).

Pupae were placed into plastic trays and, for controlling the process in our schedule, the above-mentioned equation of Estimated Adult Emergence Day (EAED) was calculated.

Pupae were placed into the new clean adult cages until the adults emerged.

Once the colony was stabilized with 6 generations, we could maintain cages with several EAED from different cohorts and designed a schedule.

LIFE CYCLE

For bioassays carried out in the laboratory on all immature stages of the stable fly, material was taken from our laboratory colony and maintained in the same conditions as the original colony.

One-hundred 1-day-old pupae were placed in rearing adult cages. The emerged adults were fed daily with bovine citrate blood and provided with water *ad libitum*. Both blood and water were offered in cotton balls inside cups. Females laid their eggs in the blood cups from which they fed. The duration of each stage was monitored from the time at which 50% of the pupae reached an adult stage (Quesada-Moraga & Santiago-Alvarez, 2001). The time at which

the first adult appeared in the cage was also recorded. Longevity and preoviposition period were monitored during the life of the adults. Also, the survival percentage was calculated. After 15 days from pupae placed in the cage, they were collected and the number of open (emerged) and close (not emerged) pupae were counted. To calculate this parameter we used the following formulae: number of pupae reaching adult stage (open pupae) x 100 / total of pupa (open and close pupae)

The eggs were separated from cotton by rinsing, and then collected into a beaker where they could settle. After that, approximately 5,000 eggs (0.5 ml) were rinsed together onto a tray of rearing medium. The egg-hatching was monitored daily, and the egg-hatching time was recorded from the time when the very first larva appeared in the rearing medium trays. The age of larvae was expressed as days after egg hatch (dAEH), adapted from Basso *et al.* (2011).

Groups of 50 newly hatched one dAEH larvae were placed in smaller larval trays (30 cm long x 22 cm wide x 7 cm high), with half of each tray containing rearing medium and the other half moist cotton. Pupae were separated using the shelf method as described by Berkebile *et al.* (2009). The duration of all the larval stadia was monitored from the time at which 50% of the larvae reached the pupal stage until 50% of the larvae reached the following stage. As demostrated for other dipterans, the criterion used to establish the onset of pupal stage was the deposition of the new cuticle (Bocaccio & Quesada-Allué, 1989). The survival percentage (number of larvae reaching pupal stage x 100 / total of larvae) and the time at which the first pupa appeared in the cage were recorded. Every procedure was repeated 4 times for each stage under study. All biological parameters were expressed as mean and standard errors.

RESULTS

LIFE CYCLE

The period required to complete the life cycle (from eggs to adult emergence) was 16.75 ± 2.9 days. Table I shows the development time of the different stages, the approximate day when these changes occurred, day "zero" of the cycle being the day when the eggs were set onto the larval medium for hatching. Also, it shows the survival percentage of larvae and pupae when reaching next stage.

Adult flies lived 16.5 ± 1.91 days. The preoviposition period lasted 5 ± 0.8 days. The development time of the first individual to reach a new stage was: 6.75 ± 1.5, 2.0 ± 0.8, 6.0 ± 1.6 days for the first adult, larva and pupa; respectively.

DISCUSSION

There are several works reporting different techniques for rearing the stable fly, *S.calcitrans* (L.) (Glaser, 1924; Melvin, 1932; Eagleson, 1943; Campau *et al.*, 1953; Champlin *et al.*, 1954; McGregor & Dreiss, 1955; Goodhue & Cantrel, 1958; Parr, 1959; Gingrich, 1960; Schoof, 1964; Christmas, 1970; Bailey *et al.*, 1975; Bridges & Spates, 1983).

However, to the best of our knowledge, there is no record of stable fly being reared in Argentina. Perhaps one reason for this is that the stable fly is considered less amenable to laboratory rearing than the housefly (Schoof, 1964; Jones, 1966; Watson *et al.*, 1995; Skovgård & Steenberg, 2002).

According to Kunz & Schmidt (1985), the most certain method to establish a laboratory colony is to obtain pupae from an existing colony. In our study, to establish the colony, approximately 3,000 pupae were kindly supplied by The ARS- USDA Laboratory Colony (Gainesville, Florida, EE.UU). We had attempted to incorporate stable flies from the field many times before, but the various attempts were unsuccessful. This would be explained as blood-sucking flies from field which did not adapt well to the laboratory way of feeding, very different from the natural one.

Another reason for the difficulty in establishing a stable fly colony could be the temperature, an important factor directly influencing the development of the stable fly stages. A previous study showed that as the temperature in the colony increased, the time required for each life stage to transform to the next was shortened (Kunz *et al.,* 1977), and most authors agree that temperatures between 31 and 35 ºC would be detrimental to the development of the immature stages. For this reason, in our study, the rearing of the stable fly was carried out in a regulated chamber at 28 ± 1 ºC and 25 ± 2 ºC for adults and

Table I. L Development time and survival (mean ± standard error) required to reach each new stage in the *Stomoxys calcitrans* colony. NC: not calculated.

Period	Development time (Days)	Life cycle day since egg setting	Survival (%)
Egg –larvae	2.0 ± 0.8	2nd	NC
Larvae- pupae	6.75 ± 1.3	8th	93.28 ± 3.5 (n = 200)
Pupae-adult	7.75 ± 1.7	14-15th	70.25 ± 6.0 (n = 400)
Egg-adult emergence	16.75 ± 2.9	17th	NC

immature stages, respectively. We found that stable flies spent 16.75 ± 2.9 days to complete the cycle from egg to adult emergence, whereas in similar temperatures other authors found different results that ranged from 19.0 (Bailey *et al.,* 1975), 18.0 (Christmas, 1970), 13.2 (Kunz *et al.*, 1977) to 12.0 days (Schoof, 1964) at 25.0, 25.0, 29.4 and 26.5 °C, respectively. Among authors we found a wide range of HR, and in some cases the photoperiod is not described.

Neither Kunz *et al.* (1977) nor Schoof (1964) described data relating to RH and photoperiod used. Christmas (1970) used a similar range of RH (45-55%) as we did but no data about photoperiod is described. Bailey *et al.* (1975) reared stable flies in a chamber with RH: 70% under a 12 h: 12 h (light: dark). In agreement with Ashrafi (1964), we tried a longer photoperiod with 14 hours of illumination every day to intensify mating activity in the culture, resulting in increased egg production.

According to Bailey *et al.* (1975), the time required in the various developmental stages of the stable fly was not sharply demarcated. In our study the eggs began to hatch after 2 ± 0.8 days. Bailey *et al.* (1975) also found that at 21.5 ± 1 °C eggs began to hatch after 1 day and all hatching occurred after 2 days. In agreement with the same author, the larval stage was the only one present in our population from the 2nd to the 6th day of the cycle. In our study, pupation began on the 8th day with 93.28 ± 3.5% survival, while Bailey *et al.* (1975) found that the pupation began on the 6th day. In our colony, adults began to emerge on the 14th day with 70.25 ± 24% survival and had completed emergence by the 21st -22nd day. By the day 21st also the oviposition began and the cycle started again. This result is consistent with that of Bailey *et al.* (1975). It is interesting to point out that in comparison with other studies, all stages of the stable flies developed more slowly and took more time to reach the next stage under our laboratory conditions. It should be noted that we had to adapt, or improve, the facilities and the elements of the rearing medium, considering their availability and cost in the local market. The most famous stable fly larval medium is Chemical Specialties Manufacturers' Association (CSMA, Ralston Purina, St. Louis, MO), but in Argentina this product is not available. Hogsette (1992) formulated diets similar to CSMA with constituents available year round in the Gainesville area, USA. We modified diets, by using ingredients within reach of Buenos Aires, Argentina. With respect to protein constituents, we replaced alfalfa meal (20-50%) by texturized soya (15%), not only because of its availability but also its economical market price. Fiber in stable fly diets generally exceeded 25% (Christmas, 1970; Bridges & Spates, 1983), so we prepared diet with 75% wheat bran. No corn meal was added, as opposed to Diet 2 (corn meal 30%), 3 (corn meal 20%) and 4 (corn meal 15%) described by Hogsette (1992) because the use of corn meal resulted in fungal growth and bacterial fermentation very quickly, so we decided not to add it as a diet constituent and replaced it with brown sugar 9.75%.

In order to establish an efficient technique for the mass production of any insect, a complete understanding of its laboratory biology would be necessary (Bailey *et al.,* 1975). Furthermore, continuous laboratory production of high quality insects depends on specialized knowledge, experience and talent, entailing both art and science, and business management skills (Singh & Ashby, 1985; Singh & Clare, 1992).

Advances in entomological research and the success of IPMP require the provision of high quality insects. Satisfying this demand depends on the ability of researchers to breed insects and establish colonies in the laboratory (Bartlett, 1984; Leppla *et al.*, 2009). Therefore, in this

work stable flies were reared for the first time in Argentina. Our results could be useful for other stable fly colonies that may be established in the future. Moreover, this information can provide additional knowledge about the biology of the pest and also, the production of the stable fly will help us to carry out bioassays with entomopathogenic fungi to develop new biocontrol methods for this pest.

Further studies are likely to provide new insights into the biology, rearing and biocontrol of the stable fly, especially in Argentina, where developing and applying eco-friendly products for this pest still remain a challenge.

With this study we could achieve the establishment of the first *S. calcitrans* colony in Argentina and standardize and report the duration of each *S. calcitrans* immature stage under constant laboratory conditions.

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