Onderstepoort J. vet. Res., 46, 223–227 (1979)

SOME EFFECTS OF TEMPERATURE ON THE ADULTS, EGGS AND PUPAE OF *STOMOXYS CALCITRANS* LINNAEUS (DIPTERA: MUSCIDAE)

B. SUTHERLAND, Veterinary Research Institute, Onderstepoort 0110

ABSTRACT

SUTHERLAND, B., 1979. Some effects of temperature on the adults, eggs and pupae of *Stomoxys calcitrans* Linnaeus (Diptera: Muscidae). *Onderstepoort Journal of Veterinary Research*, 46, 223–227 (1979).

Adults could only live and reproduce to their full capacity at temperatures between 20 °C and 30 °C. At 15 °C the females laid no eggs, the adult life span was relatively short and the reproductive capacity of females kept at 35 °C was low. The thermal histories of the flies had no apparent effect on their later reactions to temperature in any of the parameters tested.

The viability rates of *S. calcitrans* eggs exposed to temperatures between 10 °C and 40 °C exceeded 84%, but 45 °C was lethal. The optimum temperatures for incubation of the eggs was 30 °C.

Pupae of *S. calcitrans* seemed to tolerate temperatures between 20 °C and 30 °C, but their mortalities increased markedly outside this temperature range. Tests showed that pupal mortalities increased linearly with increasing periods of exposure to a temperature of 15 °C.

Résumé

QUELQUES EFFETS DE LA TEMPÉRATURE SUR LES ADULTES, LES OEUFS ET LES PUPES DE STOMOXYS CALCITRANS *LINNÉ (DIPTERA: MUSCIDAE)*

Les adultes n'ont pu vivre et se reproduire à leur capacité maximale qu'à des températures comprises entre 20 °C *et* 30 °C. *A* 15 °C, *les femelles ne pondaient pas d'oeufs, la longévité de l'adulte était relativement bréve et la capacité reproductive de femelles gardées à* 35 °C *était faible. Les antécédents thermiques des mouches n'ont pas eu d'effet apparent sur leurs réactions ultérieures à la température dans aucun des paramètres essayés.*

Les taux de viabilité des oeufs de S. calcitrans *exposés à des températures comprises entre* 10 °C *et* 40 °C *ont dépassé* 84%, *mais une température de* 45 °C *était léthale. La température optimale pour l'incubation des oeufs était de* 30 °C.

Les pupes de S. calcitrans *ont apparemment toléré des températures comprises entre* 20 °C *et* 30 °C, *mais leurs mortalités ont augmenté considérablement en-dehors de cette gamme de températures. Des expériences ont montré que les mortalités pupales augmentaient de façon linéaire avec l'augmentation des temps d'exposition à une température de* 15 °C.

INTRODUCTION

Berry & Kunz (1977) found that the maximum life span (MT_{100}) of female *Stomoxys calcitrans* decreased from 85 days to 63 days at environmental temperatures of 15,2 °C and 23,8 °C respectively. Hafez & Gamal-Eddin (1959) found that its egg production dropped from 200 eggs per female at 30 °C to 95 eggs per female at 22 °C. Temperature also affects the incubation period of the eggs of the stable-fly, since Hummadi & Maki (1970) found that this period is shorter at 30 °C than at 24 °C. Kunz, Berry & Foerster (1977) proved that the viability of the eggs of this species decreased with elevations in incubation temperatures.

Todd (1964) claimed that in New Zealand *S. calcitrans* overwintered in the pupal stage, a claim which supports the statement by Chung, Ryu, Kwon & Im (1975) that the pupae of this species may moult at ambient temperatures as low as 11 °C. Bailey, Whitfield & La Brecque (1975), however, recorded a 92% mortality rate in *S. calcitrans* pupae exposed for 6 days to 6 °C.

This investigation was aimed at measuring some of the effects of 8 different constant temperatures on the adult, egg and pupal stages of *S. calcitrans*. Attempts were made throughout to determine whether the thermal histories of the flies had any effect on their later reactions towards temperature.

GENERAL PROCEDURE

All the *S. calcitrans* adults, eggs and pupae used in this investigation were obtained from a large stock colony raised in an incubator room at 27 °C at the Veterinary Research Institute, Onderstepoort. Three sub-colonies were also kept at 15 °C, 20 °C and 30 °C respectively to provide flies for experiments designed to determine whether their thermal histories had any effect on their later reactions to temperature. The adult flies in all the colonies and experiments were fed on citrated cattle blood (Sutherland, 1978a).

The experiments were carried out in a series of 8 incubators set at 5 °C intervals from 10 °C–45 °C, with a relative humidity of 60–80% and a photoperiod with an 8-hour light and 16-hour dark cycle. The temperature and humidity in each incubator were monitored with a thermo-hygrograph.

In assessing the effects of temperature on the adults, parameters such as the MT_{50} and MT_{100} mortality levels [the mean time lapse (days) required to achieve a natural mortality of either 50% or 100%, whichever the case might be], pre-oviposition mortality and the length of the pre-oviposition period, the number of eggs produced per female, the viability rates of these eggs and reproductive potentials were used (Sutherland, 1978a). In the case of the egg and pupal stages, the effects of temperature were measured in terms of viability and mortality respectively. Additional experiments were carried out with pupae. The viability of eggs laid by the adults that emerged from pupae exposed to different temperature treatments was also determined, and the mortalities in pupae from the same age-group after varying periods of exposure to 15 °C were recorded.

Each experiment was repeated 3 times and, unless otherwise stated, an ordinary analysis of variance was done on the experimental data to determine any "very significant" (P=0,01) or "significant" (P=0,05) differences that had resulted from the various treatments.

Received 2 October 1979—Editor

PART 1: ADULTS

Materials and Methods

Mixed batches of flies, less than 18 h old, were transferred to 0,027 m³ gauze cages simply by allowing them to fly from paper cups containing pupae. Not more than 150–200 adults were kept per cage.

To determine whether the thermal histories of the abovementioned colony of flies had any effect on their longevity or reproductive rates, the F_1 generation of flies from a sub-cc ony maintained at 15 °C and the F_4 generation of flies from a sub-colony at 30 °C were exposed to 15 °C and 30 °C respectively.

Results

The results of the experiments are summarized in Table 1. The thermal histories of the flies had no effect on any of the parameters listed, as no significant differences occurred when flies from the stock colony and those from the sub-colonies maintained at 15 °C or 30 °C were subsequently exposed to 15 °C or 30 °C respectively.

MT_{50} and MT_{100} mortality levels

At 45 °C none of the flies survived for longer than 4 h and the effect of this temperature on the mortality rates of these flies will not be taken into consideration nor discussed further in this section.

The male MT_{50} values were very significantly (P=0,01) lowest at 10 °C (4,9 days), 35 °C (3,4 days) and 40 °C (2,6 days), and no significant (P=0,05) differences occurred between these treatments. Very significantly low MT_{50} values were recorded for males exposed to 15 °C (9,2 days) and 30 °C (9,0 days). At the MT_{50} level, male lived very significantly the longest at 20 °C (23,8 days), but those at 25 °C lived only 13,7 days.

At the MT_{50} mortality level, females lived for the shortest time at 40 °C (3,4 days) and 35 °C (4,6 days). The MT_{50} value for females kept at 20 °C was very significantly the longest (26,9 days), while at 25 °C this value was 13,5 days; this only differed significantly from the 10,1 day period recorded at 15 °C. At 20 °C the females lived significantly longer than the males. At all the other temperatures both sexes survived equally long.

At the MT_{100} level both males and females lived the shortest time at 40 °C (males 4,3 days; females 5,3 days) and the longest at 20 °C (males 47,0 days; females 39,0 days).

Pre-oviposition mortality and period

All the females kept at 10 °C, 15 °C, 35 °C and 40 °C died without laying eggs. Pre-oviposition mortality was very significantly lowest (P=0,01) amongst females kept at 20 °C (9,1%) and 25 °C (16,0%).

Very significantly, the shortest pre-oviposition period was recorded for females kept at 30 °C (4,3 days), and the longest at 20 °C (11,7 days).

Number of eggs produced per female

There was no significant difference between the mean numbers of eggs laid by females kept at 20 °C, 25 °C and 30 °C. Females kept at 35 °C, however, laid significantly (P=0,05) fewer eggs.

Egg viabilities

Significantly (P=0,05) more eggs produced by females maintained at 30 °C were viable than those produced and incubated at other temperatures.

Reproductive potential

The reproductive potential of flies kept at 10 °C, 15 °C, 40 °C and 45 °C was nil, and that of flies at 35 °C was very significantly lower (P=0,01) than those of flies at 20 °C, 25 °C and 30 °C. The reproductive potentials of the latter 3 groups did not differ significantly.

TABLE 1 The effects of various constant temperature treatments on the mean mortality and reproduction of adult *Stomoxys calcitrans*

Temperature (°C)	Mortality (MT)						Reproduction				Mean reproductive potential
	MT_{50} (days)			MT_{100} (days)			Pre-oviposition		Eggs		
	Male	Female	Adult	Male	Female	Adult	Mortality (%) (P)	Period (days)	Number (E)	Viability (%) (V)	
10	4,9	5,9	5,4	13,7	13,0	13,3	100,0	—	—	—	0
15	9,2	10,1	9,7	35,7	32,3	34,0	100,0	—	—	—	0
15*	9,4	10,0	9,7	34,7	30,7	32,7	100,0	—	—	—	0
20	23,8	26,9	25,5	47,0	39,0	43,0	9,1	11,7	57,8	88,4	4 644
25	13,7	13,5	13,6	21,0	21,0	21,0	16,0	7,0	58,8	89,5	4 420
30	9,0	8,8	8,9	21,0	23,0	21,8	34,2	4,3	52,0	95,3	3 260
30†	8,7	8,5	8,6	22,3	21,3	21,8	33,5	4,3	55,0	95,4	3 489
35	3,4	4,6	4,1	10,3	10,3	10,3	78,2	6,7	5,5	88,8	106
40	2,6	3,4	3,0	4,3	5,3	4,8	100,0	—	—	—	0
45	All flies died within 4 hours						100,0	—	—	—	0

* F_1 generation from the sub-colony maintained at 15 °C

† F_4 generation from the sub-colony maintained at 30 °C

$$\text{Mean reproductive potential} = \frac{(100-P) \times E \times V}{100}$$

PART 2: EGGS

MATERIALS AND METHODS

Eggs were obtained from the stock colony by exposing 60 mm Petri dishes filled with oviposition medium for 30 min in cages containing gravid females (Sutherland, 1978a). Immediately after collection, all the eggs were pooled. The eggs were lifted from the medium with a soft camel's-hair brush and transferred in batches of ±100 on to moist filter paper strips in Petri dishes, covered and incubated for 8 days. Preliminary trials had shown that any viable eggs would hatch within this period. At least 3 of these dishes were exposed to each of the 8 test temperatures. During incubation all the filter paper strips were kept moist to prevent desiccation of the eggs. After 8 days each dish was rinsed separately with 70% ethanol into a clean 60 mm Petri dish with a counting grid affixed to its base. With the aid of a very fine dissecting needle the number of eggs that had hatched was determined under a stereoscopic microscope.

To determine the effects of the thermal histories of the flies, eggs from the 2 sub-colonies maintained at 20 °C and 30 °C respectively were also collected and then incubated at the same temperature as the sub-colony from which they had originated.

RESULTS

A constant temperature of 45 °C was lethal to the eggs (Table 2), but large numbers hatched at all the other experimental temperatures. The thermal histories of the adults apparently had no effect on the viability of their eggs since no statistically significant differences occurred between the mortality rates of eggs from the sub-colonies maintained at 20 °C and 30 °C respectively and those of eggs from the stock colony when exposed to the latter 2 temperatures (Table 2).

TABLE 2 The mean viability rates of eggs of *Stomoxys calcitrans* at various constant temperatures

Temperature (°C)	Mean egg viability (%)
10	84,9
15	85,4
20	88,7
20*	88,5
25	89,4
30	95,4
30†	93,9
35	88,9
40	86,0
45	0

* Eggs collected from the sub-colony maintained at 20 °C
† Eggs collected from the sub-colony maintained at 30 °C

PART 3: PUPAE

MATERIALS AND METHODS

Exposure of pupae to 8 different test temperatures

Pupae were collected from the stock and 2 sub-colonies from a number of selected larval breeding jars in which the development of the immature stages was regularly monitored. All dark-brown pupae were collected twice daily with a pair of fine forceps. On each occasion the total yield of pupae from the pre-selected larval breeding jars in each of the 3 fly colonies was used to make up a single test group.

Three groups, each containing 100–150 pupae, were exposed in 60 mm Petri dishes to each of the test temperatures. In the incubators, Petri dishes were kept in 1 000 mℓ glass beakers containing large wads of cotton wool which were kept moist throughout the exposure periods. The beakers were covered with squares of fine muslin secured by elastic bands.

Adults emerging from each group of test pupae were transferred daily to separate 0,027 m^3 gauze cages. All the flies were kept under the same conditions as those in the stock colony and the viability of their eggs was determined at 27 °C, using the experimental procedure of Sutherland (1978a). When the test pupae yielded too few adults, large batches of pupae were exposed to the relevant test temperatures and the resulting adults were used for the egg viability studies only. After eclosion of the adults the remaining pupae in each group were kept at the particular temperature for 14 days longer. They were then opened with a dissecting needle under a stereoscopic microscope to determine the mortality rates.

Pupae from the 2 sub-colonies maintained at 20 °C and 30 °C were also exposed to 20 °C and 30 °C respectively, using the same experimental procedure.

Exposure of pupae for varying periods to 15 °C

Twenty-one groups of 100–150 pupae from the stock colony were used and collected, as described above. Eighteen of these groups were placed at 15 °C and 3 at 27 °C, with a relative humidity of 60–80% and a photoperiod with an 8-hour light and 16-hour dark cycle.

The 3 groups at 27 °C were kept at this temperature until all the adults had emerged. Of those at 15 °C 3 randomly selected groups were transferred, after exposure to this temperature for 2, 4, 6, 9, 12 and 14 days respectively, to 27 °C. After the last adult in each group had emerged, the remaining pupae were kept at 27 °C for 14 days longer to determine the percentage mortality.

The mean mortality rates of those pupae exposed initially for varying periods to 15 °C were corrected for the mean control mortality rate recorded at 27 °C using the formula of Abbott (1925). The corrected mortality rates were then plotted on graph paper.

RESULTS

Pupae exposed to 8 different constant temperatures (Table 3)

None of the pupae survived a constant temperature of 45 °C, and high mortalities were recorded at 40 °C, 35 °C and 15 °C. The lowest mortality (7,6%), which occurred amongst pupae at 25 °C, differed very significantly (P=0,01) from that at 20 °C (14,0%) on one hand, and at 30 °C (35,5%) on the other. No significant differences were recorded between the responses of pupae from the stock colony and those from the sub-colonies when they received the same temperature treatments (i.e. 20 °C and 30 °C).

The temperatures at which the pupae developed apparently had no significant effect on the viability of eggs laid by the adults that emerged subsequently.

Pupae exposed for varying periods to 15 °C (Fig. 1)

The pupal mortalities corrected by means of Abbott's formula, for the mean 13,9% mortality at 27 °C, increased linearly with increasing periods of exposure to 15 °C.

TABLE 3 The effects of various constant temperature treatments on the pupal stage of *Stomoxys calcitrans*

Temperature (°C)	Mean pupa mortality (%)	Mean egg hatchability (%)
10	82,2	93,3
15	75,8	93,1
20	14,0	93,2
20*	14,1	93,3
25	7,6	93,8
30	35,5	94,0
30†	34,9	93,8
35	80,7	93,6
40	97,3	94,7
45	100,0	—

*F_4 generation of pupae from the sub-colony maintained at 20 °C
†F_4 generation of pupae from the sub-colony maintained at 30 °C

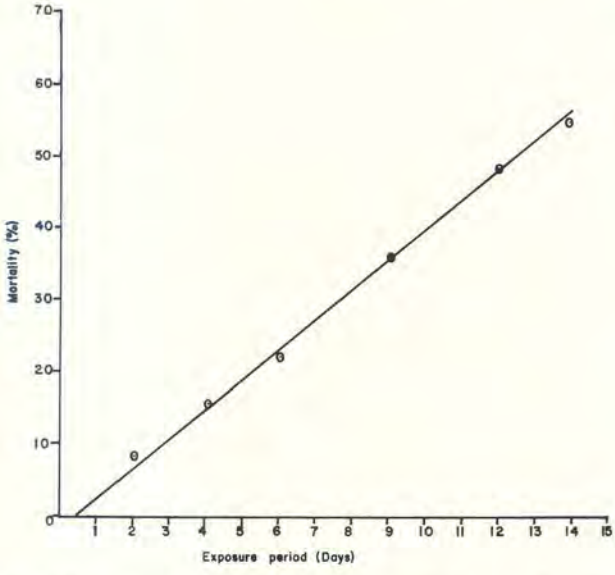

FIG. 1 The mean mortalities of *Stomoxys calcitrans* pupae exposed to 15 °C for varying periods (fitted by eye)

General Discussion

Adults

S. calcitrans is an inhabitant of the temperate zones of the world (Zumpt, 1973) and is therefore exposed to a wide temperature range. Extreme temperature fluctuation may be tempered by the natural habitat to allow the survival of this species under adverse conditions. According to Berry, Foerster & Campbell (1978) *S. calcitrans* seeks warm protected locations to survive the cold winter of the northern United States. Indeed, according to Hafez & Gamal-Eddin (1959), 'it seems that the occurrence of *S. calcitrans* around stables and byres or houses is only for protective purposes'.

The life span of adult *S. calcitrans* is dependent on the environmental temperatures to which it is subjected. Since the total life span (MT_{100}) of living organisms is subjected to considerable individual variation, the MT_{50} mortality level is a truer indication of life span. At this level, adult *S. calcitrans* lived markedly longer at 20 °C than at any other temperature. According to the MT-values of the adults, a normal life span occurs at temperatures between 15 °C and 30 °C. The finding that temperatures above 40 °C are lethal supports the observation by Jack (1939) that *S. calcitrans* adults survived for only 3 hours at 42 °C.

Bursell (1974) states that extremes in temperature more readily affect reproduction than any other physiological process in insects. In the experiments described here *S. calcitrans* laid eggs only at temperatures between 20 °C and 35 °C. The females may be unable to lay at 15 °C or lower because they are inactive and take in too little food at these low temperatures. The low reproductive capacities of females at 35 °C and the total absence of any egg production above this temperature may be caused by increased digestion rates and consequent exhaustion of the nutrients necessary for reproduction (Bursell, 1974).

If reproductive potential is used as a measure of the suitability of various temperatures for adult *S. calcitrans*, it is evident that this fly can only live and reproduce at its full capacity at temperatures between 20 °C and 30 °C.

Eggs

As a sessile stage in the life cycle of a eurythermal species, which lays its eggs in the superficial layers of its breeding medium, the eggs of *S. calcitrans* are likely to be resistant to a wide range of environmental temperatures. The results of this study verify this supposition as it was found that eggs survived constant temperatures between 10 °C and 40 °C. This resistance to temperature extremes is probably an inherent property of the eggs of the stable-fly, as the thermal histories of the parent flies had no significant effect on the eggs they produced.

It is not possible to compare the egg viabilities in this study with those given by Gamal-Eddin (1963) and Kunz *et al.* (1977), as these authors do not state what food their experimental flies received and adult nutrition plays a role in egg viability (Du Toit, 1975; Sutherland, 1978a). Gamal-Eddin (1963), however, found the viability rates of the eggs of the stable-fly to be 100% at a relative humidity of 50% when exposed to 16 °C, but only 56% when exposed to 30 °C and the same relative humidity. Kunz *et al.* (1977) determined the viability rates of these eggs to be 90% and 84,2% at 23,9 °C and 35 °C respectively.

Pupae

A comparison of the pupal mortalities recorded in this study with those given by various other authors is complicated by a number of factors. As *S. calcitrans* has a coarctate pupa, it is possible that the pharate adult may spend some time in the protective puparium before eclosion. The age of the pupa and the pharate adult may therefore play an important role in any biological study on this developmental stage. Since the handling of young, creamy-white pupae results in high mortalities of this developmental stage (Du Toit, 1974), the collection method used in this study was adopted.

Larval nutrition also influences pupal mortality in *S. calcitrans* (Du Toit, 1974; Sutherland, 1978b) and this may account for the difference in mortalities in this study and in that of Kunz *et al.* (1977). These authors did not state the larval breeding medium used in raising their experimental pupae, but they found the pupal mortalities of this species to be 80,6%; 86,6% and 66% at ambient temperatures of 23,9 °C; 29,4 °C and 35 °C respectively.

The results of this study show that *S. calcitrans* pupae are better adapted to temperatures between 20 °C and 30 °C than to those above or below these limits, so that the habitat selected by the fully-fed larvae will influence the subsequent survival of pupae.

The thermal histories of the flies in this study had no apparent effect on the reaction of their pupae to temperature, nor did the action of temperature on the pupae affect the viability of the eggs produced by the resulting adults. When pupae from the same age-group, raised initially at 27 °C, were exposed for progressively longer periods to 15 °C, pupal mortality also increased linearly, which suggests that this temperature had a cumulative detrimental effect on the pupae. This means that in the fly's natural habitat, short-term changes from the optimum temperature may only kill a certain portion of the pupae present. This proportion will be directly related to the duration and depend on the intensity of the temperature change.

ACKNOWLEDGEMENTS

The author is indebted to:

1. Prof. H. E. H. Paterson, University of the Witwatersrand, Dr I. G. Horak, University of Pretoria, Dr C. J. Howell and Mr A. M. Spickett for criticism of the manuscript.

2. Prof. K. R. Solomon, University of Guelph, for assisting in the statistical analysis of the experimental data.

3. Miss Jane B. Walker for editing the manuscript.

REFERENCES

ABBOTT, W. S., 1925. A method for computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18, 265–267.

BAILEY, D. L., WHITFIELD, T. L. & LA BRECQUE, G. C., 1975. Laboratory biology and techniques for mass producing the stable fly, *Stomoxys calcitrans* (L.) (Diptera: Muscidae). *Journal of Medical Entomology*, 12, 189–193.

BERRY, I. L. & KUNZ, S. E., 1977. Mortality of adult stable flies. *Environmental Entomology*, 6, 569–574.

BERRY, I. L., FOERSTER, K. W. & CAMPBELL, J. B., 1978. Overwintering behaviour of stable flies in manure mounds. *Environmental Entomology*, 7, 67–72.

BURSELL, E., 1974. Environmental aspects: Temperature. pp. 1–41. *In:* Rockstein, M.(Ed.), The Physiology of Insecta, (2nd Ed.), 2, New York & London: Academic Press.

CHUNG, K. H., RYU, J., KWON, S. H. & IM, M. S., 1975. Study on stable fly eradication by sterile-male technique. (5). On the population density of the stable fly, *Stomoxys calcitrans* L. (In Japanese). *The Korean Journal of Entomology*, 5, 13–16.

DU TOIT, G. D. G., 1974. Aspects of the biology of *Stomoxys calcitrans* Linnaeus (Diptera: Muscidae). M.Sc. Dissertation. University of Stellenbosch, Stellenbosch.

DU TOIT, G. D. G., 1975. Reproductive capacity and longevity of stable flies maintained on different kinds of blood. *Journal of the South African Veterinary Association*, 46, 345–347.

GAMAL-EDDIN, F. M., 1963. Experimental studies on the developmental stages of two blood-sucking flies (*Stomoxys calcitrans*, Lin. and *S. sitiens* Rond.) in Egypt (Diptera: Muscidae). I. The egg stage. *Journal of the Arabian Veterinary and Medical Association*, 23, 309–318.

HAFEZ, M. & GAMAL-EDDIN, F. M., 1959. Ecological studies on *Stomoxys calcitrans* L. and *sitiens* Rond. in Egypt, with suggestions on their control. *Bulletin of the Entomological Society of Egypte*, 43, 245–283.

HUMMADI, M. K. & MAKI, B., 1970. Studies on the breeding of *Stomoxys calcitrans* stable fly. *Bulletin of Iraq Natural History Museum*, 4, 21–26.

JACK, R. W., 1939. Studies on the physiology and behaviour of *Glossina morsitans*, Westw. *Memoirs of the Department of Agriculture of Southern Rhodesia*, 1, 1–203.

KUNZ, S. E., BERRY, I. L. & FOERSTER, K. W., 1977. The development of the immature forms of *Stomoxys calcitrans*. *Annals of the Entomological Society of America*, 70, 169–172.

SUTHERLAND, B., 1978a. Nutritional values of different blood diets expressed as reproductive potentials in adult *Stomoxys calcitrans* L. (Diptera: Muscidae). *Onderstepoort Journal of Veterinary Research*, 45, 209'–212.

SUTHERLAND, B., 1978b. The suitability of various types of dung and vegetable matter as larval breeding media for *Stomoxys calcitrans* L. (Diptera: Muscidae). *Onderstepoort Journal of Veterinary Research*, 45, 241–243.

TODD, D. H., 1964. The biting fly *Stomoxys calcitrans* (L.) in dairy herds in New Zealand. *New Zealand Journal of Agricultural Research*, 7, 60–79.

ZUMPT, F., 1973. The stomoxyine biting flies of the world. Stuttgart: Gustav Fischer Verlag.