Aus dem Institut für Parasitologie und Tropenveterinärmedizin
des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

Occurrence and distribution of insecticide resistance in stable flies (*Stomoxys calcitrans*) on dairy farms in the Federal State of Brandenburg, Germany

Inaugural-Dissertation
zur Erlangung des Grades eines
Doktors der Veterinärmedizin
an der
Freien Universität Berlin

vorgelegt von
Sophia Reissert
Tierarzt aus Berlin

Berlin 2018
Journal-Nr.: 4078

mbvberlin | mensch und buch verlag

Aus dem Institut für Parasitologie und Tropenveterinärmedizin

des Fachbereichs Veterinärmedizin

der Freien Universität Berlin

Occurrence and distribution of insecticide resistance in stable flies (*Stomoxys calcitrans*) on dairy farms in the Federal State of Brandenburg, Germany

Inaugural-Dissertation

zur Erlangung des Grades eines Doktors der Veterinärmedizin

an der

Freien Universität Berlin

vorgelegt von

Sophia Reissert

Tierärztin aus Berlin

Berlin 2018

Journal-Nr.: 4078

Gedruckt mit Genehmigung des Fachbereichs Veterinärmedizin

der Freien Universität Berlin

Dekan:	Univ.-Prof. Dr. Jürgen Zentek
Erster Gutachter:	Prof. Dr. Peter-Henning Clausen
Zweiter Gutachter:	Univ.-Prof. Dr. Uwe Rösler
Dritter Gutachter:	Prof. Dr. Lothar H. Wieler

Deskriptoren (nach CAB-Thesaurus):
Stomoxys calcitrans, animal husbandry, livestock, insect control, insecticides, insecticides resistance, epidemiology, Brandenburg

Tag der Promotion: 08.11.2018

Bibliografische Information der *Deutschen Nationalbibliothek*
Die Deutsche Nationalbibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über <https://dnb.de> abrufbar.

ISBN: 978-3-86387-940-2
Zugl.: Berlin, Freie Univ., Diss., 2018
Dissertation, Freie Universität Berlin
D188

Dedicated to my parents

Table of contents

List of figures

List of graphics

List of tables

Abbreviations

AChE-R	acetylcholinesterase-resistance
BVL	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit - Federal Office of Consumer Protection and Food Safety
Ca^{2+}	Calcium
CDC	Centers for Disease Control and Prevention
cm	Centimetre
cm^2	square centimetre
DDT	dichloro-dipehenyl-trichlorethane
DNA	Desoxyribonucleic Acid
Dr	Doctor
ECHA	European Chemicals Agency
et al.	et alii, et aliae or et alia – and others
EU	European Union
F1 generation	First offspring generation
FET	Fisher's Exact Test
FU	Freie Universität
g	gram
g/L	gram per liter
GST	gluthathione-S-transferase
h	hour
i.e.	id est – that means
IGR	Insect Growth Regulator
Inc.	incorporation
IRAC	Insecticide Resistance Action Committee
kdr	knockdown-resistance
kg	kilogram
l	liter
L1-3	larvae 1-3
LC	lethal concentration
LD	lethal dose
lin-reg.	linear-regression
log-reg	logarithmic-regression
m	metre
mg	milligram
mg/kg	milligram per kilogram
mg/ml	milligram per milliliter
min	minute/s
ml	milliliter
mm	millimeter
µg	microgram
µl	microliter
MSD	Merck Sharp and Dohme
Na^{2+}	sodium
ng	nanogram
ng/µl	nanogram per microliter
p.	page
PBO	piperonyl butoxide
PCR	polymerase chain reaction
pen	reduced penetration
ppm	parts per million
sp.	Species
spp.	species pluralis

UBA	Umweltbundesamt – Federal Environment Agency
USA	United States of America
V2A-metall	austenitic stainless steel (Versuchsschmelze 2 Austenit-Metall)
vs.	versus
WHO	World Health Organisation
WHOPES	WHO Pesticide Evaluation Scheme
%	percent
°C	degree Celsius
λ	Lambda
χ^2	Chi square

1 Introduction

Stable flies (*Stomoxys calcitrans*) are serious pests of livestock, companion animals and humans worldwide. Nuisance by stable flies and the ensuing negative impact on livestock productivity play an important role in animal husbandry. Annoyance caused by their painful bites can reduce the productivity of livestock and lead to suffering in companion animals and humans. Additionally, stable flies are known vectors of blood-borne pathogens and are, therefore, potential transmitters of infectious diseases (Baldacchino et al., 2013).

Livestock production systems like dairy farms serve as an ideal livelihood and basis for the reproduction of flies. Stable flies disturb cattle in various ways. Since they induce pain they can reduce grazing time and provoke defensive behaviour like foot stomping, tail wagging, panniculus, and head throwing (Dougherty et al., 1993; Taylor et al., 2012b). This may lead to less time spent lying down and bunching (Berry et al., 1983). While bunching intends to decrease the attack rate of stable flies in the individual animal, it increases heat stress and the risk of injury (Catangui et al., 1993). It is estimated that the losses of the US cattle industry caused by *Stomoxys calcitrans* amounts to about 2.2 billion dollars annually (Taylor et al., 2012b).

Unfortunately, little is known about the effectiveness of insecticides against stable flies and the resistance in *Stomoxys calcitrans* in Germany. Thus, the objectives of this survey were to appraise the use and effectiveness of different insecticides and to assess the present status of occurrence and distribution of insecticide resistance in stable flies on dairy farms in the federal state of Brandenburg. Finally, it aimed at raising farmers' awareness and may assist in applying best-bet strategies for on-farm pest control in order to minimize both the use of insecticides and the risk of resistance development. The objective of the study was based on earlier results which indicated resistance in *Musca domestica* against several adulticides in livestock production systems in Germany (Hildebrand, 2017; Jandowsky, 2009; Jandowsky et al., 2010). However, as Sømme (1958) discovered in Norway, 74.5 % of flies observed in barns were stable flies. Thus, the assessment of the occurrence and distribution of insecticide resistance in stable flies in Germany seemed to be long overdue.

Since the Nobel prize winning discovery of DDT more than 50 years ago, the number of insects and mites worldwide that have developed strains resistant to one or more chemicals has increased to at least 504 (Georghiou, 1990).

In 1961, it was reported that WHO programs had been so successful that one third of the world was entirely free of Malaria and the other third was close to its eradication (Brown, 1961). Between the years 2000 and 2015, malaria incidence was reduced by 37 % globally and by 42 % in Africa due to insecticidal nets and indoor residual spraying with insecticides (WHO,

2016). In 2015, the WHO, however, still calculated 214 million cases of malaria and 430,000 deaths. Several species of the Malaria vector *Anopheles spp.* have developed resistances against various insecticides (Corbel et al., 2007; Elissa et al., 1993; Ranson et al., 2001).

Insecticides that can no longer eradicate crop and livestock pests put an unnecessary strain on human health and the environment. Therefore, in 2018 the European Commission in cooperation with the European Food Safety Authority (EFSA) planned a blanket ban for the external use of three neonicotinoids as plant pesticides. This is expected to protect bees and to prevent the ensuing drastic decrease of natural pollination. The ban could already be activated in 2017 if EU member states assent (Kirchner and Liebrich, 2017).

In Denmark, an amendment of the Chemical Act during 1994 allowed to ban the import, sales and use of plant protection products (Environmental Protection Agency, 2017). The use of pesticides containing seven different active ingredients was prohibited, among them deltamethrin for outdoor use, which in Germany is still authorized in compliance with EU directive 98/8/EG (98/8/EC, 1998).

This study was intended to give an overview of the occurrence of insecticide resistance in livestock production systems in Germany using dairy farms in the Federal State of Brandenburg as a showcase. The results of this work are supposed to serve as a basis for purposeful, sustainable integrated pest management programs aiming at minimizing the use of insecticides in the future and thus, the development of insecticide resistance.

2 Literature review

2.1 *Stomoxys calcitrans*

The blood-feeding cosmopolitan stable fly *Stomoxys calcitrans* (Linnaeus, 1758) is a common problem in animal husbandry worldwide and can have a considerable negative impact on animal wellbeing, health and productivity (Berry et al., 1983; Taylor et al., 2012c).

2.1.1 Taxonomic classification

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Diptera
Suborder: Brachycera
Family: Muscidae
Genus: *Stomoxys*
Species: *Stomoxys calcitrans*

The genus *Stomoxys* includes at least 18 species (Zumpt, 1973).

Figure 1 Stable fly *(Stomoxys calcitrans)* with non-retractile proboscis.

2.1.2 Morphology

Both, female and male stable flies, are blood-sucking biting flies. Similar in size and colour the stable fly can be easily distinguished from the house fly by its short maxillary palps and its non-retractile proboscis adapted for penetrating the skin and imbibing blood (Masmeatathip et al., 2006). Horn flies, *Haematobia irritans* (Linnaeus 1758), which are also similar to *Stomoxys* in shape and colour have maxillary palps of the same length as their proboscis (Hale, 2011). The piercing sharp mouthpart of the stable flies held in a horizontal position is black and composed of three long sclerite parts as in other Muscidae: the labrum or upper lip, the hypopharynx and the horned labium or lower lip (Salem et al., 2012b). The labium comprises both the labrum and hypopharynx and terminates in a two-lobed labellum, which houses chemoreceptors, mechanoreceptors and prestomal teeth. The teeth are sharp, sclerotized and enlarged compared to those of other flies (Mullen and Durden, 2009). Stable fly imagoes of 4 to 7 mm in length (Zumpt, 1973) with an average length of 6.3 mm ± 0.6 mm (Salem et al., 2012a) can, furthermore, be differentiated from the house fly adults by their broader abdomen and the position of their wings. Their wings are longer than the abdomen, do not overlap and thus, form a triangle with wider angles. The position of the wings' veins are characteristic to the species. In stable flies the fourth vein shows an oblique curve at the distal end of the wing (Brain, 1912). On the second and third segment of the abdomen there is a checkerboard of dark spots (Masmeatathip et al., 2006) whose form and position allow to distinguish the different stable fly species (Zumpt, 1973). *Stomoxys calcitrans* presents three spots on each of the two rear abdominal segments and a relatively wide frons. The male genitalia consisting of the horned sclerite and the aedeagus, a pair of pre- and postgonits, can also be employed for differentiating *Stomoxys* spp. (Masmeatathip et al., 2006; Zumpt, 1973).

Stomoxys calcitrans possesses the typical eyes of an insect, two big compound ones at each side of the head and three ocelli at the vertex dorsal to the front (Peterson, 1916; Ranade, 1970). The ommatidia which the compound eyes are composed of form a net of hexagonal meshes (Houlbert, 1920). They contain a cluster of photoreceptor cells surrounded by support and pigment cells. At least two features distinguish male and female stable flies. The frontal index defined as the frontal space of vertex divided by the eye length, is smaller in males (medium of 0.3 ± 0.3 mm) than in females (medium of 0.55 ± 0.04 mm) (Masmeatathip et al., 2006). The presence of an anal sclerite in the male stable flies can also be employed to sex *Stomoxys calcitrans*.

Like all brachycera muscid flies *Stomoxys calcitrans* presents two antennae frontally and centrally on the head between the two compound eyes that are densely covered with microtrichia. The antennae consist of three segments: first a proximal scape, then a pedicel

where Johnston's organ is located and which features bristles for tactile functions. Thirdly, it is made of a distal flagellum which is characterized by a non-innervated arista (Salem et al., 2012b). The arista is a single hair with fine setae along its shaft. The third segment is profoundly topped with sensilla, superficial sense organs (Lewis, 1971; Sukontason et al., 2004). In stomoxine flies seven sensilla have been described: two trichoid, three basiconic, one clavate and one styloconic (Giannakakis and Fletcher, 1985; Lewis, 1971).

The stable fly's grey thorax features four longitudinal dark stripes. As is typical for the zoological class of insects their thoraces are composed of three sections, the prothorax, mesothorax and metathorax. The more developed membranaceous pair of wings is attached to the mesothorax whereas the second pair of so-called halters, which are rudimentary wings for more balance, originates from the metathorax.

In every segment of the thorax there is a pair of legs, each composed of coxa, trochanter, femur, tibia and tarsus. The legs are of a dark brown colour with a pale tinge at the tips of femur and basal parts of tibia (Masmeatathip et al., 2006). The femurs of the first pair of legs are provided with hair on the dorsal as well as ventral parts. The femurs of the second and third pair of legs only possess hair on the ventral side whereas the hair of the third pair is rather short with the exception of the terminal one (Salem et al., 2012b). At the end of each tarsus there is a pair of claws and a hairy fleshy ball which allows the stable flies to find stability on slippery surfaces. A sensorial hair is of additional help.

The abdomen is divided into two parts: the pre-abdomen which consists of five segments clearly visible on the dorsal surface and the post-abdomen composed of seven segments transformed into a reproductive apparatus (Mullen and Durden, 2009; Salem et al., 2012b). The male possess an aedagus, an intromittent organ, which at rest is enclosed in a genital pouch. In the female, the terminal segments form a tubular ovipositor that is extended to lay eggs. Here, chemoreceptors can also be found (Mullen and Durden, 2009).

The digestive system of the stable flies is composed of an oesophagus, a crop, a proventriculus, midgut, hindgut and rectum (Lotmar, 1949). Stable flies store the ingested blood meal in the anterior midgut. Two hours following ingestion it is gradually passed to the posterior midgut where digestion occurs (Venkatesh and Morrison, 1980). Since *Stomoxys calcitrans* also sometimes feed on nectar, Lee and Davies (1979) documented that when a 10 % sucrose solution was ingested some of it went to the midgut but the main part remained in the crop. Stable flies generally deny feeding on blood when they are less than a day old but readily imbibe sucrose solutions when only three hours old (Lee and Davies, 1979). This might be due to the fact that the mouthparts have to harden adequately before being able to pierce skin (Hale, 2011).

Stable fly eggs are white, with a length of 0.9 to 1 mm and a width of 0.3 mm. They are elongated, conical at one end, convex at the ventral side and concave at the dorsal side. They

show two lengthwise rips that correspond to the zones where the egg dehisces when the larva hatches (Salem et al., 2012b). The stomoxine life cycle consists of three instars: L1, L2 and L3. The L1 is translucent and measures 1.5 to 2.5 mm whereas the L2 of 2.6 to 4.4 mm is crème-white and differs from the first instar by having anterior spiracles with 4 to 7 lobes. Posterior spiracles in second instars are weakly pigmented and have two slits in each (Hale, 2011). Third instars possess a light yellow to white colour, a length of 4.8 to 12 mm (Skidmore, 1985) and 12 cylindrical segments (Zumpt, 1973). Apart from their length, they can be separated from the L2 by the three sinuous slits in their spiracles. The first two segments cannot be easily distinguished (Zumpt, 1973) and contain a black sclerotic cephalopharyngeal skeleton and teeth, both visible through the integument (Salem et al., 2012b). The mandibles, modified to mouth hooks, the maxillary palpi and labial lobes allow the larvae to crawl and dig. Since the larvae lack eyes, they are provided with a pair of antennae. Antennae display an anterior dome which serves for olfaction whereas the maxillary palpi are innervated with mechano- and chemosensilla and scolopodia (Friesen et al., 2015). While each of the thoracic segments comprises Keilin's organs for hygroreception, the abdominal segments feature the anal pad, anus and papillae. Deprived of legs, they are provided with ventral creeping velts for locomotion (Friesen et al., 2015). Stable fly larvae can easily be distinguished from other muscid larvae by the form and assembly of their digitation and their posterior stigmata, especially present in L2 and L3, which are heavily sclerotized, subtriangular in shape and twice as far apart than the plate's width (Moon, 2002). Posterior spiracles are transformed from round spiracular discs with two straight slits in the first instar to a triangle with two and three sinuous slits in the second and third instar, respectively (Friesen et al., 2015). In pupation, the stomoxine third instar shortens rapidly to 5 to 10 mm of length and 2 mm in width and changes its colour from bright yellow to a chestnut colour (Brain, 1913). The integument becomes hard and dry underlining the form resemblance of a barrel. Only eleven segments are visible, the anterior one showing the thoracic, the rear one the posterior stigmata. The pupa turns almost black about a day before the imago is ready to hatch (Hale, 2011).

2.1.3 Life cycle

Stable flies are obligate blood-sucking livestock pests (Skidmore, 1985; Taylor et al., 2010). Since the larval body fat declines after two days post-emergence, adult female stable flies need an external protein source for oogenesis (Hale, 2011). Hence, for the females to produce their first cluster of eggs at least five blood meals are required (Chia et al., 1982). The first three blood meals stimulate the growth of the imago's fat body and the following two meals propagate a rapid ovarian growth accompanied by a decline in body fat (Chia et al., 1982).

Stable flies deprived of a blood meal three days after emergence show a lower life expectancy and females produce fewer and smaller eggs (Sutcliffe et al., 1993). Salem et al. (2012b) found that stable flies fed with honey and water only lived 10.7 ± 3.9 days whereas *Stomoxys calcitrans* males and females fed with honey, water and blood lived 23 and 24 days, respectively. While spermatogenesis in which spermatids evolve from fat fusiform cells to elongated ones was described to be independent from blood-feeding (Morrison et al., 1982), males are reported to require blood, too, for their accessory glands to produce the seminal fluid necessary for sperm transfer (Anderson, 1978). Males fed on sucrose solution were observed to produce low (Anderson, 1978) or no sperm transfer (Meola et al., 1977; Morrison et al., 1982). Meola (1977) demonstrated that only blood-fed flies were able to create and respond to the hydrocarbons which the female sex pheromones are composed of (Muhammed et al., 1975; Uebel et al., 1975). While male stable flies were documented to inseminate six females on average, females were observed to mate only once (Harris et al., 1966). Mating starts two days after hatching from the pupa, reaches its peak at five days after emergence and lasts for 5 minutes and 40 seconds on average (Anderson, 1978). Before that, males explore the females' heads with their mouth parts (Buschman and Patterson, 1981). Female stable flies begin to lay eggs after a minimum time period of five days (Ashrafi, 1964; Hogsette and Farkas, 2000). Sutherland (1979) states that the pre-oviposition time lasts 4.3 days at 30 °C and 11.7 days at 20 °C. Although oviposition encompasses a period of twenty days, egg production reaches its maximum peak when females are eight days old (Ashrafi, 1964). Lysyk (1998) discovered that stable fly females yielded less than 30 eggs at 15 and 35 °C and up to 700 eggs at 25 °C. Bailey et al. (1975) observed that females produce an average of 292 eggs during their life span. Ten to eleven batches of an average of 35.5 eggs were oviposited at a time, one single mating appearing to be sufficient (Schoof, 1964). Ashrafi (1964) detected a more active mating behaviour resulting in an increased egg production when illumination was prolonged to fourteen hours a day. Oviposition sites are elected by olfactory stimuli (Jeanbourquin and Guerin, 2007).

The life cycle is composed of several stages: the egg, three larval stages, the pupa and the imago (Zumpt, 1973). After 12 to 80 hours the eggs hatch (Hale, 2011; Larsen and Thomsen, 1940; Skidmore, 1985). Kunz (1977) observed the egg hatch time to range from 36 hours at 23.9 °C to 22 hours at 35 °C. Stable fly larvae hatch after 19 hours and five days at a temperature of 28 °C according to Simmons (1944). The development from one developmental stage to another is greatly influenced by temperature and humidity (Lysyk, 1998). The total maturation can vary from 12 to 150 days (Berry et al., 1976). This was confirmed by Gilles (2005) who observed that the life cycle required 13 and 71 days at temperatures of 30 and 15 °C, respectively, to be completed. At temperatures above 30 °C again longer developmental times were described (Lysyk, 1998) with a temperature optimum of 30 °C (Kunz et al., 1977;

Sutherland, 1979; Thomsen and Thomsen, 1937). Even a higher barometric pressure is observed to lead to an increase in biting activity (Voegtline et al., 1965) and therefore, to an increase in egg production. Temperatures above 35 °C and below 12 °C are expected to be less productive or even fatal (Kunz et al., 1977; Larsen and Thomsen, 1940), a temperature above 45 °C is lethal to eggs. Smith (1985) found that the egg mortality was 20.6 %. Nine to 14.2 % were attributed to egg sterility, 1.1 % to predation and 8.3 % to unknown causes such as desiccation (Berkebile, 1995). The water content must be above 36 % in order to survive (Abasa, 1983). At 25 °C the life cycle was described to be of a length between 17 (Gilles et al., 2005) and 19 (Salem et al., 2012b) days. However, immature stage stable flies were found to develop under several different physical conditions (Rasmussen and Campbell, 1981). Development temperature ranges from 21.10 to 25.25 °C, pH from 7.13 to 8.23 and humidity from 22.90 to 65.02 %.

Often larval development is associated with unhygienic husbandry conditions (Berkebile et al., 1994; Broce et al., 2005; Meyer and Petersen, 1982). Decaying organic material mixed with animal waste products as well as hay balls are very advantageous breeding sites (Berkebile et al., 1994; Foil and Hogsette, 1994; Hall et al., 1983; Hogsette et al., 1987; Lysyk and Krafsur, 1993; Salem et al., 2012b; Skoda et al., 1991; Zumpt, 1973). It was demonstrated that certain bacteria play an essential role in larval development (Albuquerque and Zurek, 2014; Romero et al., 2006; Talley et al., 2009). After hatching, the larvae pupate for a duration of eleven to 30 days (Bishopp, 1913; Brain, 1912; Newstead, 1906). Melvin (1931) found that at controlled temperatures of 25 °C and 30 °C, pupation occurred after 15.7 and 13.6 days, respectively. Mitzmain (1913) discovered that maturity was reached after seven to eight days at 30 and 31 °C and Hummadi and Maki (1970) observed the larvae to develop in 10.9 days at 30 °C. Berry et al. (1977) noted the maximum time span for larval development to be 156 days at 10.9 °C with a survival rate of 70 %. The subsequent pupal development is completed after 3 to 26 days (Bishopp, 1913; Brain, 1912; Jones and Smith, 1966; Lodha, 1961). At 27 °C Lodha observed the pupal stage to be completed after three to five days. According to Kunz (1977), at 23.9 °C 95 % of the adult stable flies emerge from the pupae. Melvin (1931) found the emergence rate to be of 99.8 % at 25 °C and 100 % room humidity (Berkebile, 1995) whereas it dropped to 10 % at 25 °C and 73 % room humidity. At temperatures between 15 and 20 °C mortality increases (Sutherland, 1979). At a temperature of 30 °C emergence rates of 30.5 (Kunz et al., 1977) to 35.5 % (Sutherland, 1979) were described. Sutherland (1979) noted 45 °C to prevent emergence at any relative humidity. Adults of stable fly were observed to live between 46 and 53 days (Bishopp et al., 1915; Glaser, 1924). At temperatures above 45 °C adult *Stomoxys* were only able to live for four hours (Sutherland, 1979). Low humidity increases the mortality rates at temperatures between 18.3 and 35 °C (Wang, 1970).

Stable flies cease to be active at 12 °C in the tropics (Hafez and Gamal-Eddin, 1959; Kunz, 1976) and at 10 °C in the subtropics (Buschman and Patterson, 1981). Nevertheless, stable fly imagoes often take refuge in artificially heated buildings or farms (Larsen and Thomsen, 1940; Sømme, 1961) when the average temperature remains between 15 and 20 °C and does not go below 0 °C. If that is not possible, stable flies overwinter as pupae (Hewitt, 1909) or quiescent larvae (Hale, 2011). Quiescent larvae are characterized by a reduced metabolism and survive in substrates such as open silage storage systems (Greene et al., 1989; Meyer and Petersen, 1983; Scholl et al., 1981; Todd, 1964; Williams et al., 1980), piles of fermenting vegetation (Simmons and Dove, 1941) and manure (Berkebile et al., 1994), hay balls (Broce et al., 2005), peanut litter (Simmons, 1944), straw stacks (Bishopp, 1913; Bishopp, 1920) and pig manure (Mellor, 1919) which provide adequate moisture and warmth.

2.1.4 Occurrence and distribution

Stable flies are cosmopolitan livestock pests that can be found close to husbandry systems throughout the whole world. Its northern borderline extends from southern Alaska across central Canada (Huckett, 1965), Norway, Finland and through northern China (Brues, 1913). The southern boundary runs through Chile, South Africa and New Zealand (Brues, 1913; Skidmore, 1985). Stable flies were long believed to preferably feed on cattle blood (Anderson and Tempelis, 1970). Anderson and Tempelis (1970) observed that when catching flies on poultry farms none would have fed on birds. When feeding on herbivores the stomoxine egg-production and life span were described to be greater than when feeding on omnivores and carnivores. Subsequently, Sutherland (1978) noted that no eggs were laid when *Stomoxys* fed on chicken blood. However, although this might only be a reflection of their opportunistic feeding behaviour (Hale, 2011), stable flies were later discovered feeding on American white pelicans infected with West Nile Virus (Johnson et al., 2010). Likewise, Friesen (2012) observed that stable flies artificially fed on chicken blood showed a 20 % higher oviposition rate than when fed on cattle or horse blood. Nevertheless, stable flies showed a 50 % longer fecundity when provided with cattle blood only.

Unlike house flies, stable flies prefer a breeding substrate that is composed of manure as well as some decomposing plant material in contrast to pure manure (Meyer and Petersen, 1983; Schmidtmann et al., 1989). Boire et al. (1988) demonstrated that cattle and horse manure generated a higher pupal production and pupae of higher weight. Although normally stable flies gather around favourable feeding and breeding sites, long distance flights also occur. Bailey et al. (1973) noted that stable flies were capable of flying at least 3.2 km in search of a blood meal. Hogsette et al. (1987) concluded that stomoxines looking for a blood meal travel

5 km or more. Taylor et al. (2010) observed 50 % of the stable flies beyond 1.6 km of their natal site, whereas only 5 % had dispersed beyond 5 km. Molecular analysis led to the assumption that they could fly even longer distances (Jones et al., 1991; Szalanski et al., 1996). Hogsette and Ruff (1985) were able to catch stable flies in Florida 225 km from their starting point due to transport wind after the passage of a cold front.

2.2 Harmful effects and economic impact of *Stomoxys calcitrans*

2.2.1 *Stomoxys calcitrans* as a nuisance insect

Stable flies are part of the most damaging arthropod pests of cattle worldwide (Taylor et al., 2012b). They preferably feed on the legs or abdomen of an animal (Cortinas and Jones, 2006). Since they lack an anaesthetic in their saliva, their stings cause a lot of pain (Cortinas and Jones, 2006). Schofield and Torr (2002) observed that stable flies never fed to repletion and that only 24 % reacted to host defensive behaviour. The authors compared this behaviour to that of tsetse flies which were more responsive to the host behaviour. Since stable flies have a shorter life span and are oviparous in contrast to the larviparous tsetse flies, they take greater risks to obtain a blood meal (Schofield and Torr, 2002). Although cattle, other livestock animals and horses seem to be preferred, dogs and humans are also attacked in their absence (Bishopp, 1913). Along seashore and beaches they are often observed to cause pain and irritation to humans in recreational areas (Hansens, 1951). Especially in cattle, their painful stings as well as the concomitant blood loss of 11 to 15 µl per average feeding (Schowalter and Klowden, 1979) cause stress and defensive movements like head and ear movements, tail swishes and the panniculus reflex (Dougherty et al., 1995) as well as gatherings for mutual protection. By retrenching into water or stomping their feet, cattle and horses try to chase stable flies away. This often causes swollen joints (Bishopp, 1913). However, persistent attacks due to high stable fly density can lead to habituation and, thus, to a decrease in defensive behaviour in cattle and other animals (Mullens et al., 2006). Stable flies often provoke cutaneous lesions due to hypersensitive reactions (Moorhouse, 1972). Related to abundance of the stable flies, this can lead to reduced weight gain in cattle and swine or a decline in milk production in dairy cows (Campbell et al., 1987; Campbell et al., 2001; Campbell et al., 1977; Catangui et al., 1993, 1997). Studies showed that dairy cattle treated with insecticides produced more milk (Baker, 1918; Bishopp, 1913; Freeborn et al., 1925) and had longer lactation periods (Bruce and Decker, 1958; Morgan and Bailie, 1980). Nevertheless, as Berkebile (1995) pointed out, several of those earlier studies lacked an adequate comparison between treated and untreated dairy cattle (Cheng and Kesler, 1961; Miller et al., 1973; Todd, 1964). Furthermore, since

insecticides, especially petroleum based ones, that were used as veterinary medicinal products, can increase the body temperature as well as the respiration rate (Freeborn and Regan, 1932; Melvin, 1932), some of them are expected to cause more harm than the stable flies themselves. Campbell et al. (1977) found that 50 stable flies per calf resulted in reduced weight gains by 0.9 kg/day due to the concurrent reduction of food intake. Berry et al. (1983) concluded that the decrease in weight gain is of 0.652 % for each stable fly per front leg. However, changes in weight gain differ between different cattle breeds (Catangui et al., 1993). Dougherty (1995), on the other hand, noted that stable flies caused cattle to increase their dry matter intake and bite mass. Wieman (1992) discovered that the heat stress caused by the cattle bunching when annoyed by flies is more responsible for the reduction in weight gain than the stable flies themselves. Schwinghammer (1986) noted an increase in physiological stress such as raised cortisol concentrations, heart and respiration rates as well as rectal temperature when cattle was exposed to 25 to 50 stable flies. Estienne (1991), however, observed that ten, 20 or 30 stable flies per animal did not cause any physiological stress nor any alteration in food consumption. Economic thresholds of two stable flies per leg in feedlot cattle (Campbell et al., 1987) and three per leg in pastured calves (Campbell et al., 2001) have been defined. Bruce and Decker (1958) observed a 0.7 % decrease in milk production due to stable fly nuisance. Barré estimated in La Réunion the daily milk loss per cow to be of 0.5 to 1 l in highly infested farms (Barré, 1981). In the United States the economic impact on the cattle industry was estimated to be of 400 million dollars per year (Kunz et al., 1991) and of more than 300 million dollars in the milk industry (Taylor et al., 2012b). In total, Taylor (2012b) calculated the annual economic loss in the US cattle industry due to stable fly nuisance to be of over 2,000 million dollars. Furthermore, Geden and Hogsette (2001) assumed that over 40 million dollars were spent in 1997 on ectoparasite control in horses.

2.2.2 *Stomoxys calcitrans* as a vector of pathogens

The blood-sucking nature of the stable fly makes it a potential mechanical as well as biological vector of skin and blood borne pathogens especially of livestock, but occasionally also humans. As a consequence of its nuisance host animals gather close to each other facilitating the probability for the flies to move from one host animal to another in case of interrupted feeding. Thus, when feeding is interrupted, *Stomoxys calcitrans* is likely to reassume its blood meal on another host and thereby, inject some of its saliva that might be mixed with infected blood (Baldacchino et al., 2013). It was also noted that stable flies keep some blood in their crop which offers a friendly environment for potential pathogens that could be regurgitated and then transmitted during the next blood meal (Coronado et al., 2004). Straif et al. (1990) found

that *Stomoxys calcitrans* was able to transmit erythrocytes and pathogens mechanically and by regurgitation *in vitro*. As spontaneous regurgitation was rarely observed and at that time the *in vivo* transmission was not described for all of the following pathogens, it was concluded that for successful transmission the mobility and degree of infectivity of the pathogens were important factors. Since stable flies can have an immunosuppressive impact as a consequence of stress, energy losses and food intake on their hosts, they can enhance the pathogenesis of infected animals and reduce the resistance of potential hosts (Desquesnes and Dia, 2004).

Attempts to transmit Potomac horse fever, a disease caused by *Ehrlichia risticii*, (Burg et al., 1994; Burg et al., 1990) and lumpy skin disease, a viral disease of cattle, (Chihota et al., 2003) by the stable fly have failed. Nevertheless, a successful transmission of bovine leucosis virus has been shown (Buxton et al., 1985). Bovine diarrhea virus which can lead to abortions and foetal abnormities in cattle was proven to be retained by stable flies for up to three days (Tarry et al., 1991). Furthermore, *Stomoxys calcitrans* has been demonstrated of being capable to transmit Retroviridae which can cause Equine infectious anemia disease (Foil et al., 1983; Green et al., 1996; Hawkins et al., 1973; Scott, 1922), Asfarviridae which can cause African swine fever virus (Mellor et al., 1987), Bunyaviridae which can cause Rift Valley fever virus (Hoch et al., 1985), Bovine Herpes virus (Gibbs et al., 1973), and possibly Flaviviridae which can cause West Nile virus fever (Doyle et al., 2011), Reoviridae which can cause Blue Tongue virus (Krinsky, 1976) and Rhabdoviridae which can cause Vesicular stomatitis virus (Ferris et al., 1955; Smith et al., 2009). Bacteria like *Bacillus anthracis* (Hugh-Jones and Blackburn, 2009; Schuberg and Böing, 1914; Schuberg and Kuhn, 1912; Turell and Knudson, 1987), *Anaplasma marginale* (Guglielmone, 1995; Kocan et al., 2003; Scoles et al., 2005; Urdaz-Rodriguez et al., 2009), *Pasteurella multocida, Erysipelothrix rhusiopathiae, Francisella tularensis* (Krinsky, 1976), *Dermatophilus congolensis* (Macadam, 1977; Richard and Pier, 1966) as well as *Enterobacter sakazakii* (Mramba et al., 2007) have also been demonstrated to be transmittable by stable flies. De Castro et al. (2007) reported the presence of 33 different species of bacteria such as *Escherichia coli, Staphylococcus aureus* and *Staphylococcus intermedius* on the cuticle, mouthparts and abdominal alimentary tract of stable flies. *Coxiella burnetii* could also be collected from *Stomoxys calcitrans* (Nelder et al., 2008). Apart from tsetse flies, stable flies have also been shown to be able to mechanically transmit the unicellular flagellated protozoan trypanosomes. Stable flies are capable of passing on *Trypanosoma brucei, T. vivax, T. congolense* and *T. evansi* (Desquesnes and Dia, 2003a, b, 2004; Mihok et al., 1995). Bouet and Roubaud (Bouet and Roubaud, 1912) demonstrated that *T. evansi* might not only be mechanically transmitted with immediate effect but also after contact with a delay of 24, 48 and 72 hours. While delayed transmission had never been observed in tabanids, these experiments showed the stable flies' potential capacity of it. The

apicomplexan protozoon *Besnoitia besnoiti* causing bovine Besnoitiosis has also been shown to be mechanically transmittable by stable flies (Bigalke, 1968; Liénard et al., 2011; Liénard et al., 2013). Berberian (1938) reported a successful transmission of *Leishmania tropica* by *Stomoxys calcitrans*. Stable flies were found to be vectors and intermediate hosts of the nematode *Habronema microstoma* (Yarmut et al., 2008) when its nematode DNA was discovered in different anatomical parts of both field and laboratory flies (Traversa et al., 2008). The embryonated eggs or larvae of the nematode are ingested by stable fly larvae where they develop to the L3 stage. When the stable fly imago hatches the pupae the infective *Habronema* larvae are excreted from them during the feeding process and deposited around nostrils, lips, or wounds of the host (Baldacchino et al., 2013).

2.3 Control measures against *Stomoxys calcitrans*

Numerous stable fly control methods have been established over the years due to the economical as well as sanitary harm they can cause. Consequently, those control measures encompass mechanical, physical, biological and chemical methods.

2.3.1 Stable hygiene

Recent studies revealed that mechanical control measures and the reduction of breeding sites facilitate minimizing stable fly breeding and, thus, stable fly populations (Keiding, 1986; Thomas et al., 1996). In addition to the mechanical removal of potential breeding material, the accumulation of liquid manure can be avoided by accelerating the slurry flow rate (Ribbeck et al., 1987). A good stack management of the dung hill can enhance destroying larvae and breeding sites due to biothermical processes (Ribbeck et al., 1987). It was reported that several farmers succeeded reducing the stable fly population by burning residues of hay and straw (Hogsette et al., 1987) or by covering it with tarps (Fay, 1939) or black polyethylene foil in order to induce a heat development leading to the death of larvae (Hogsette et al., 1987). Spreading the manure as a thin layer on agricultural fields (Burg and Axtell, 1984) or storing it at a dry place with the manure showing no more than 50 % of moisture is also documented to prevent fly development (Watson et al., 1998). Composting increases the inner waste temperature and decreases the moisture content resulting in not being apt enough anymore for flies (Machtinger, 2011). Therefore, aged swine compost was reported not to aid house fly breeding (Larrain and Salas, 2008). Watson et al. (1998) integrated poultry manure at different depths in soil and observed that no depth was significantly better in reducing fly development than the treatment of the surface. Putting hay on mobile wagons and frequently relocating round hay

bales, however, might cut down stable fly breeding (Broce et al., 2005). Schmidtmann (1991) discovered that sawdust and gravel bedding were most effective in preventing flies from breeding.

2.3.2 Physical control measures

Mechanical control measures resulting in a good stable hygiene achieve a decrease in fly abundance to some extent (Kočišová et al., 2002). However, if the pest pressure is too high, stable flies can also be coped with by physical measurements. Especially calf holding areas, where manure management is rather difficult, serve as an important breeding source for flies (Meyer and Petersen, 1983; Schmidtmann, 1991). Catching adult flies with fly traps (Broce, 1988; Hogsette and Ruff, 1990; Pickens, 1995; Pickens and Miller, 1987; Thimijan et al., 1970; 1972; Williams, 1973) in these areas may provide an additional relief to calves (Kaufman et al., 2005). Miller et al. (1993a; 1993b) documented sticky pyramid traps to reduce stable fly populations on dairy farms. Kaufman et al. (2001a) reported that spider webs, high-capacity sticky traps, are able to attract and capture large numbers of stable (Kaufman et al., 2005) and house flies in dairy calf greenhouses (Kaufman et al., 2001a). Broce (1988) and Hogsette and Ruff (1990) found alsynite traps, which reflect ambient light at a UV wave length attractive to stable flies (Agee and Patterson, 1983), and Williams traps were comparably efficient in decreasing stable fly pressures. Alsynite traps that were combined with permethrin have also been investigated on and proven to be effective (Hogsette and Ruff, 1996; Koehler and Patterson, 1982). Taylor and Berkebile (2006) found other materials to be as attractive to stable flies as alsynite ones. Accordingly, materials like adhesive-coated corrugated plastics (Cilek, 2003) and coloured coroplast sticky traps (Beresford and Sutcliffe, 2006) have been proven to work against stable flies. However, Foil and Younger (2006) documented UK Trigger targets to catch a higher number of stable flies than alsynite cylinder traps. Laveissière and Grebaut (1990) discovered Vavoua traps employed for sampling tsetse flies in Africa, baited with octenol, to be at least as successful as the Williams trap in catching stable flies. The Broce trap was significantly less effective than the Vavoua, Nzi and Williams traps for catching stable flies on La Réunion island (Gilles et al., 2007). Furthermore, fly screens (Kühlhorn, 1961) and suitable ventilation engineering (Kühlhorn, 1965) can hinder flies from entering the stables. Since *Stomoxys* are diurnal insects they are attracted to the ultraviolet radiation that electrocuting traps emit and thereby electrocuted when coming into contact with the electrifying wires (Weidhaas and Haile, 1978). Electrocuting traps are reported to be well suited and effective due to the large number of insects they can catch (Hienton, 1974; Pickens, 1991).

According to Foil and Younger (2006) and Hogsette et al. (2008), treated cloth targets are a more recent and effective method than the standard alsynite sticky traps.

2.3.3 Biological control measures

Due to the increasing amount of ineffective chemical control measures caused by the growing resistance development in flies, some farmers feel encouraged to include biological control measures into their integrated pest management. While biological control measures are proven not to be a sole method for fly control, combined with chemical and mechanical control measures they can enhance the overall effectiveness (Machtinger, 2011). Among the natural enemies of flies are fungi, mites, spiders, parasitic and predatory hymenoptera, birds (Kühlhorn, 1983), nematodes (Geden, 2012; Smith et al., 1987), bacteria (Lysyk et al., 2010) and viruses (Coler et al., 1993; Moussa, 1978). Simmons and Dove (1941) even reported fire ants (*Solenopsis geminata* (F.)), a predacious wasp (*Vespula squamosa* [Drury]) and a predacious bee (*Xylocopa micans* [Lepeletier]) to capture or feed on stable fly eggs. Pteromalid wasps like *Spalangia cameroni* [Perkins]*, Spalangia nigroaena* [Curtis]*, Spalangia endius* [Walker]*, Muscidifurax* spp., *Pachycrepoideus vindemiae* [Rondani], *Urolepis rufipes* [Ashmead], *Nasonia vitripennis* [Walker], an ichneumonid *Phygadeuon fumator* [Gravenhorst], a diapriid *Trichopria* sp. and a coleopteran staphylinid *Aleochara* sp. (Skovgård and Jespersen, 1999) which parasitize on fly pupae were shown to be effective against house and stable flies in several studies (Birkemoe et al., 2009; Legner and Brydon, 1966; Legner and Olton, 1971; Morgan et al., 1975; Petersen and Meyer, 1983; Rutz and Axtell, 1979). However, several authors (Geden and Moon, 2009; Greene et al., 1989; Jones and Weinzierl, 1997; Pawson and Petersen, 1988; Petersen and Meyer, 1983; Quarles, 2006; Skovgård and Jespersen, 1999) suggested that if pteromalid wasps are considered as a biological control measure, first one should determine which species is suitable for the specific habitat and the climate aimed at. For some species were not as viable and therefore effective as others under certain environmental circumstances like temperature and humidity. Furthermore, an existing parasitoid population can inhibit the installation of a new species (Legner et al., 1990). Other dipterous predators like *Muscina stabulans* and *Hydrotaea aenescens* have been reported to mostly attack *Musca domestica* or *Fannia* larvae (Legner et al., 1967) but not *Stomoxys calcitrans* larvae.

The fungus *Entomophthora muscae* has already been employed as a biological control measure against insects (Hildebrand, 2017; Vogel, 1968). While house flies are very susceptible to *Entomophthora muscae,* which penetrates the fly body through the hemocoel and proliferates through the whole organism harming also the central nervous system, stable

flies are reported not to react to them (Kramer and Steinkraus, 1981). However, stable fly eggs are susceptible to the fungus *Metarhizium anisopliae* (Moraes et al., 2008). *Beauveria bassiana*, known to cause white muscardine disease, was shown to be pathogenic to *Stomoxys calcitrans*, too. However, stable flies are less susceptible than house flies (Watson et al., 1995). Additionally, mites are often found to be phoretically attached to flies which appears to prevent them from emigrating (Beresford and Sutcliffe, 2009). However, Kinn (1966) reported *Macrocheles muscadomesticae* being predacious on both *Musca domestica* eggs and first instar larvae to be unimportant in controlling large stable fly populations. As an alternative to synthetic insecticides, *Bacillus thuringiensis* in a spray or feed-through formulation have been developed and tested on poultry and dairy farms against house and stable flies. *Bacillus thuringiensis* is a Gram-positive bacterium that produces protein based crystals that can be ingested by the insects. Inside their intestines those endotoxins destroy the intestine cells (Gill et al., 1992). They can even have a negative effect on the larvae, the hatching or pupation rate (Labib and Rady, 2001; Tharwat et al., 1994). Gingrich (1965) found that stable fly larvae could be controlled by *Bacillus thuringiensis* var. *thuringiensis* (Berliner) as feed additive, although they appeared to be less susceptible than *Musca domestica* (L.) and *Haematobia irritans* (L.). Lysyk et al. (2010) reported 95 % of 85 *Bacillus thuringiensis* (Berliner) isolates to result in less than 50 % mortality, 93 % in less than 25 % mortality in stable flies. Nevertheless, five isolates were highly toxic to *Stomoxys calcitrans.*

Geden et al. (2011) showed that the double stranded DNA *Musca domestica* salivary glands virus of the virus family Hitrosaviridae also affected selected fitness parameters of *Stomoxys calcitrans*. Infected house flies develop greatly enlarged salivary glands caused by the massive viral reproduction and females become sterile due to a virus-induced impaired protein digestion and a following down-regulation of vitellogenesis (Lietze et al., 2007). Virus injected stable flies suffered from a higher mortality and a lower fecundity rate than control flies. They also deposited less faecal spots than healthy flies (Geden et al., 2011). However, *Stomoxys calcitrans* did not develop salivary gland hypertrophy, which probably led to a virus titre two orders of magnitude lower than the one in *Musca domestica*. Nonetheless, extracts of stable fly salivary glands and ovaries were infective to house flies. It is assumed (Lietze et al., 2013) that in order to be transmitted the virus is able to disrupt the fly's peritrophic membrane and, thus, to facilitate oral infection from a feeding bait. Another plausible option is that the virus might be equipped with sharp materials to enhance infection through a damaged cuticle after topical aerosol applications (Lietze et al., 2013).

Furthermore, although they produce additional waste products that can aggravate pest management control measures, swallows are found to contribute to the reduction of nuisance flies in cattle barns (Stuyck, 2014).

Imai (1987) succeeded to reduce the resistance levels to Fenitrothion and Diazinon to 1/6 and to other organophosphates (Fenthion, Calclofos, Dichlorvos) by half in *Musca domestica* field populations by releasing susceptible house flies. This scenario is likewise imaginable for stable flies. As Patterson et al. (1981) demonstrated, 99.9 % of an enclosed stable fly population can be eliminated by releasing sterile male flies. Over a period of 18 months, they released 100,000 sterile male insects five days a week on St. Croix, U.S., Virgin Islands. After that, the population was zeroed out with the exception of few fertile flies that were believed to have come from surrounding islands with the wind or had been imported with livestock or pets. So this could be thought as part of future integrated pest management as well.

2.3.4 Chemical control measures

Over the last centuries, chemical control constituted the mainstay for managing nuisance insects in livestock production systems. In 1972, it was pointed out that the use of big amounts of fly traps would be more expensive than the use of insecticides (Thimijan et al., 1972). However, the toxicity to non-target species and environmental pollution as well as the increasing resistance levels overtime have cast some doubt on insecticides as the primary control for nuisance insects (Cilek and Greene, 1994; Kočišová et al., 2002; Pickens and Miller, 1987).

There are chemical insecticides of a variety of forms and modes of action. The form of application can be gaseous that can be fumigated, liquid, or powdery, or in form of pellets, impregnated strips, toxic baits or fabrics. Natural and synthetic insecticides can be distinguished as well as repellent and lethal substances. Repellents are substances that are able to prevent insects from sitting on or piercing through body surfaces. With their repulsive inherent smell, repellents cover up attractants produced by body scented secretions and thereby chase away insects. In livestock production systems, insecticide impregnated ear tags are often employed as repellents. However, against stable flies they are of limited use. Stable flies prefer to feed on the lower extremities, which are only reached by small quantities of the insecticide. Additionally, since stable flies spend only a few minutes a day on the host for blood feeding, their time of exposure to the insecticide is relatively short.

Within the lethal form of insecticides there are larvicides and adulticides. Among the adulticides, there are contact, feed-through and respiratory insecticides. Since *Stomoxys calcitrans* in contrast to house flies almost exclusively feeds on blood, feed-through insecticides like neonicotinoids and spinosyns are not considered to be effective against stable flies and adding of attractants is deemed necessary (Carlson et al., 1971). Apart from larvicides, those ectoparasitic drugs are usually based on neurotoxic mechanisms provoking

paralysis or death. If the time of contact with the insecticide is too short or if the substance intake is too low, however, the organism can recover from its effect (Richter and Steuber, 2010).

2.3.4.1 Legal framework

In the EU, insecticides, larvicides and acaricides can be authorized either as biocides or as veterinary medicinal products. Whether a product is regulated by the Regulation (EU) No. 528/2012 as a biocidal product or whether it falls under the Directive 2001/82/EC as a veterinary medicinal product is to be decided on a case-by-case basis. Those decisions are based on the guidance document of the European Commission on borderline products and on the jurisdiction of the European Court of Justice. The main criteria for such a borderline setting are the compliance of a product with the relevant definition and the intended purpose of a product. In case of incertitude for a specific product, however, the competent authorities of either the Medicinal Products Directive or the Biocidal Products Directive should be consulted. According to Art. 1 of Directive 2001/82/EC a veterinary medicinal product is defined as “any substance or combination of substances presented as having properties for treating or preventing disease in animals or any substance or combination of substances which may be used in or administered to animals with a view either to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action, or to making a medical diagnosis” (2001/82/EC). In Germany, the national authorisation procedure is based on the German drug law (Arzneimittelgesetz). The Federal Office of Consumer Protection and Food Safety (German: Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, BVL) authorises on application of a pharmaceutical company. In the European Union, through Regulations and Directives, the European Commission created three admission procedures: the centralised procedure (CP), the decentralised procedure (DCP) and the mutual-recognition procedure (MRP). The CP authorises a veterinary medicinal product in all EU countries. It is handled by the European Medicines Agency (EMA) and the Committee for Medicinal Products for Veterinary Use (CVMP). The CP is mandatory for new active agents that have been produced by a certain biotechnological procedure. If the product is aimed at treating animals of the food industry, the CVMP will establish maximum residue limits for each active substance in a product. The MRP is used if a veterinary medicinal product is already nationally authorised in one or more member states and the authorisation holder seeks recognition in another member state. In the DCP a previous national authorisation is not required. The pharmaceutical company applies for a marketing authorisation in all selected member states with one serving as a reference member state. In accordance with the Regulation (EEC) No 2377/90, a marketing authorisation for a veterinary medicinal product

intended for one or more food-producing species cannot be applied until a valid application has been made for the establishment of maximum residue limits, at least six months should elapse between both. The 2001/82/EC additionally states that “where relevant, data on the potential emergence of resistant organisms of clinical relevance are necessary for veterinary medicinal products”. It further declares that “measures to limit resistance development from the intended use of the veterinary medicinal product shall be proposed by the applicant”.

Biocidal products are defined as “active substances and preparations containing one or more active substances, put up in the form in which they are supplied to the user, intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means” (98/8/EC, 1998).

As aforementioned, products that have lethal effects on external parasites including lice, fleas or ticks on humans and animals can be either authorised as biocidal or veterinary medicinal products depending on how they are used. If a product is used on the structures, where the animals are housed, kept or transported but not on the animal itself, it is classified as a biocidal product. If it is used on human beings or animals its classification depends on the intended use or demonstrated claim. Normally, however, such products are authorised as human or veterinary medicinal products with precise medicinal indications, including prevention or treatment of disease. In the absence of such a claim, they could be considered as biocidal products. Insecticides with lethal effects that are categorised as biocidal products belong to the product type 18 “Insecticides, acaricides and products to control other arthropods” of the main group 3 “Pest control product types” according to the biocidal product regulation (BPR, (EU) 528/2012). Products that only have a repellent activity, without any killing effect and without any medical claim, such as collars, neckties and ear marks, are, thus, regarded as biocidal products belonging to the product type 19 “repellents and attractants” of the main group 3 “Pest control product types” according to the biocidal product regulation (BPR, (EU) 528/2012).

The (EU) 528/2012 regulation declares that biocidal products can pose risks to humans, animals and the environment and, therefore, need to be regulated. Thus, one of the conditions for granting an authorisation for biocidal products is to ensure that they do not have an immediate or delayed effect themselves, or as a result of their residues, on the health of any human or animal, directly or through drinking water, food, feed, air, or through other indirect effects. Another condition is that the biocidal product should not have unacceptable effects as a product or as a result of its residues on the environment. Furthermore, when properly used for the purpose intended, biocidal products are expected to be sufficiently effective and to have no unacceptable effect on the target organisms like resistance or unnecessary suffering and pain. Moreover, the regulation (EU) 528/2012 explains that for authorisation maximum residue limits for food and feed should be established with respect to active ingredients contained in a

biocidal product to protect human and animal health. When these requirements are not fulfilled, biocidal products should not be authorised unless their authorisation is justified due to the non-symmetrical negative impact for society of not authorising them when compared to the hazards emerging from their use (528/2012, 2012). Based on those maximum residue levels of a substance in an organism which are established upon the acceptable daily intake of an animal, withdrawal periods are determined before a product is released to the market. During this withdrawal periods animal products like meat or milk are not allowed to be sold when treated with an insecticide. Questions concerning the legal limits of maximum residue levels of pesticides in food of plant or animal origin are controlled by the regulation (EG) 396/2005 (EC-Regulation, 2005). This limits the variety of products to a relatively small range.
Since 2006, there is an EU regulation (EC-Regulation, 1907/2006) which addresses the registration, evaluation, authorisation, and restriction of chemicals (REACH). It encompasses the production and use of chemical substances as well as their potential impacts on both human health and the environment. The regulation also established the European Chemicals Agency that manages the technical, scientific and administrative aspects of REACH. The competent authority for Biocidal products in Germany is the Federal Office for Chemicals (BfC) at the Federal Institute for Occupational Safety and Health (BAuA).

2.3.4.2 Insecticides

Historically, there are three generations of insecticides (Heimbucher, 1982). The first generation includes non-synthetic substances like inorganic arsenic and fluorine containing compounds, sulphur, some organic compounds and naturally occurring active substances out of plants. The second generation was developed in the 1930s with the discovery of chlorinated cyclic hydrocarbons and organophosphates and in the fifties of the 20th century when carbamates were started to be used. New classes like the synthetic pyrethroids and insect growth regulators represent the third generation of ectoparasiticides (Hatch Jr, 1911; Heimbucher, 1982).

2.3.4.2.1 Chlorinated cyclic hydrocarbons

Dichloro-diphenyl-trichlorethane (DDT) which was discovered in 1939 by the Swiss chemist Paul Müller was first employed as an insect control measure during World War II. Since then it has been used worldwide as a pesticide and insecticide. DDT inhibits the inactivation of the neuronal sodium channels and thereby provokes spasms and paralysis. Resistances could be observed over time (Davies et al., 2007; WHO, 1991). Due to the many kdr-gene based cross-resistances with synthetic pyrethroids, many Malaria transmitting mosquitoes of the

Anopheles species were cross resistant against pyrethroids as well as DDT. The same could be observed for *Aedes* species (Brengues et al., 2003; van den Berg, 2009). Although DDT possesses a high specificity to insects, as a result of its lipophilicity it accumulates in human and animal tissue and eventually in the food chain. It was also shown that DDT and some of its degradation products exhibit hormone-like characteristics (Bitman et al., 1968). Furthermore, it was found out that birds of prey laid eggs with a higher breakage frequency and a reduced eggshell thickness (Lundholm, 1987; Peakall et al., 1973; Ratcliffe, 1970) due to DDT intoxication. Exposure to DDT may also increase cancer risk in humans (Cohn et al., 2007; Garabrant et al., 1992). That is why DDT has been banned in most Western industrial states since the 1970s. Countries that signed the Stockholm Convention in 2004 can only produce and apply DDT against disease transmitting insects, like Malaria mosquitoes.

2.3.4.2.2 Organophosphates

The first organic phosphoric acid ester was synthetized in 1854. After that, highly toxic compounds have been used as weapons in the war. Later, organophosphates were employed against arthropods as contact and feed-through poisons, some volatile compounds were also used as respiratory poison (diazinon and phoxim). Their lipophilic properties facilitate their resorption through the skin and the digestive tract. Today, their use is exclusively external. Organophosphates irreversibly inhibit acetylcholinesterases and thereby provoke an increase of acetylcholine in neuromuscular synapses. This leads to a depolarising muscle relaxation which entails a spastic paralysis and ultimately death (Richter and Steuber, 2010). Warm-blooded animals are capable of detoxifying small amounts of organophosphates, and their persistence in the environment is relatively small. However, toxicity against bees (Schricker and Stephen, 1970; Zhu et al., 2014) and fish (Coppage and Matthews, 1974; Kavitha and Rao, 2008) is being investigated. Organophosphates that are frequently used as veterinary medicinal products are coumafos, which is still approved as veterinary medicinal products against varroosis in bees, fenthion (not further authorised in Germany), tetrachlorvinphos, diazinon, phoxim, and azamethiphos (in Germany as biocide only). Walsh et al. (2001) found that the thiophosphate ester azamethiphos was most potent compared to other organophosphates like dichlorvos and malaoxon. They supposed that this was a sign of the high affinity of the binding site of the AChE that appeared to outweigh possible mutational effects in the AChE genes. Apart from insects, azamethiphos is approved as a veterinary medicinal product (e.g. Salmosan® Vet, Norway) against sea lice in farmed salmons (Roth et al., 1996) or other fish (Trident®). However, it was reported that azamethiphos has neurotoxic effects on other marine animals like the marine mollusc *Mytilus edulis* in which it can also

modulate haemocyte functions. In genotoxicity studies with *Drosphila melanogaster,* diazinon, dichlorvos, methyl parathion and azamethiphos, in decreasing order, were observed to have genotoxic effects. Thus, although not yet proven, it is possible that they can have effects on human health and the environment (Çakir and Sarikaya, 2005).

2.3.4.2.3 Carbamates

Carbamates, which are employed systemically as indirect parasympathomimetics, are not sufficiently effective against insects. Only synthetically increased lipophilic carbamates can be used as pesticides. Similarly to the organophosphates, as contact and feed-through insecticides they reversibly inhibit the cholinesterases (Richter and Steuber, 2010). Their toxicity against warm-blooded animals and their environmental persistence is low. However, they are reported to be toxic against bees (Akca et al., 2009) and fish (Jabeen et al., 2015; Olson and Christensen, 1980). Since other insecticides are more advantageous, carbamates have been used less frequently over time (Beckmann and Haack, 2003). In the EU, bendiocarb is still allowed as a biocide (98/8/EC, 1998) but not approved yet as an active substance in a veterinary medicinal product. As veterinary medicinal products propoxur and neostigmin are available.

2.3.4.2.4 Pyrethrum

Pyrethrum are extracts out of chrysanthemum (Asteraceae) flowers which possess six active compounds: pyrethrine I and II, yasmoline I and II and cinerine I and II. As contact poisons, they have been used against insects for more than a hundred years. They feature a strong repellent and toxic effect on arthropods and a low toxicity on warm-blooded animals forming little residues (Richter and Steuber, 2010). When pyrethrum is being taken up by an arthropod through the prolonged opening of the sodium channels, first it induces excitation, then movement and coordination disorders, followed by paralysis or death. Pyrethrum is, however, rather unstable under UV light, so often their effect is short and affected parasites recover after a short time. Since 1973, chemical modifications, nevertheless, have helped to produce more stable derivatives, called synthetic pyrethroids (Davies et al., 2007; Heimbucher, 1982; Löscher et al., 2006). Insects are able to detoxify pyrethrines and pyrethroids by binding to the cytochrome P-450 dependent mixed function oxidases that can be blocked by piperonyl butoxide. Therefore, many pyrethrines or pyrethroid containing insecticides are fixed combined with the synergistic acting compound piperonyl butoxide (PBO) in order to increase the efficacy of the products. Thereby, the selective toxicity against parasites can be risen by a factor of 10 (Richter and Steuber, 2010). Resistance in insects caused by increased activity of the insect's

mixed function oxidase could be undermined by the combination with PBO in a product (Levot, 1994).

2.3.4.2.5 Pyrethroids

Pyrethroids are synthetic and more stable derivatives of the naturally occurring pyrethrum, chemically they are esters of the cyclopropanecarboxylic acid. They provide several advantages over other insecticide classes due to the rather small costs and the low toxicity in mammals.

Similar to the pyrethrines, the lipophilic pyrethroids passively pass through the insect's cuticula before they spread through their entire body. The prolonged inflow of sodium ions results in a continuous depolarisation. They then inhibit the inactivation of the neuronal sodium channels in a way that the channels stay open longer. The characteristic signs of an initial excitation and hyperlocomotion followed by a sublethal so-called "knock-down effect" and paralysis, which arthropods generally show after a contact with pyrethrum, are also shown when pyrethroids are applied. There are two types of pyrethroids: type I-pyrethroids (tetramethrin, permethrin, transfluthrin) without a substitution at the alpha-carbon site and type II-pyrethroids (cypermethrin, cyfluthrin, deltamethrin, flumethrin) with a cyano-group substitution (Richter and Steuber, 2010). Type I-pyrethroids attack at the peripheral nervous system, while type II-pyrethroids attack at the central nervous system (Miller, 1988). Pyrethroids are five to ten times more effective when absorbed topically than when ingested orally (Gunjima, 1992). Pyrethroids are highly toxic to cats, bees and fish. According to the summary of product characteristic of Deltanil® deltamethrin is excreted in faeces. Its excretion may take place over a period of 2 to 4 weeks. Faeces that contain deltamethrin excreted onto pasture by treated animals may reduce the abundance of dung feeding organisms. It states that "the risk to dung fauna can be reduced by avoiding too frequent and repeated use of deltamethrin (and other synthetic pyrethroids) in cattle, e.g. by using a single treatment per year on the same pasture". Deltamethrin, which belongs to the type II-pyrethroids, is one of the most frequently used insecticides in cattle and sheep livestock production systems (Jandowsky, 2009; Jandowsky et al., 2010). Its duration of effectiveness is between two weeks and five months depending on mode of application and dosage. Mostly, deltamethrin as veterinary medicinal product is marketed as pour-on preparations with withdrawal periods in cattle for edible tissues of 17 or 18 days and of zero hours for milk. In sheep it is one day for edible tissues and 12 hours for milk. Predominantly, it is used against sucking and biting lice, house and stable flies *(Haematobia irritans, Stomoxys calcitrans, Musca spp. and Hydrotaea irritans*) as well as Malaria mosquitoes. Regarding the latter particularly pyrethroid-treated bed nets can be used for personal protection to reduce malaria transmission in the home environment. Unfortunately,

78 % of 49 endemic malaria countries that monitored resistance, recorded resistance of malaria vectors against pyrethroids in 2014 which must be deemed alarming (Mnzava et al., 2015). There is, however, at present, no alternative available for the treatment of human bed nets and the WHO Pesticide Evaluation Scheme (WHOPES) is currently evaluating alternatives (WHO, 2016). Several studies, however, showed that insecticide treated nets can still be successfully used for the control of nuisance insects like tsetse, house and stable flies when putting around cattle surrounding fences (Bauer et al., 2006; Blank, 2008; Holzgrefe, 2013; Maia, 2009; Rohrmann, 2010; Zaspel, 2009). They were observed to be effective for five to six months (Arends, 2016; Holzgrefe, 2013). Additionally, it was suggested that pyrethroids can also be used on sheep against blue-tongue disease transmitting biting midges (*Ceratopogonidae*) (Weiher et al., 2014) but the indication is not yet approved in veterinary medicinal products.

2.3.4.2.6 Phenylpyrazoles

Phenylpyrazoles were introduced in the 1990s as a new class of pesticides. Fipronil represents a phenylpyrazole that is widely used on domestic animals such as dogs and cats in order to treat the infestation with fleas and ticks. It attacks the central nervous system of insects and acari by blocking the GABA-gated chloride channels and gluatamate-gated chloride channels inhibiting the transmission of nerval stimuli. Its insecticidal efficacy against *Stomoxys calcitrans* was evaluated by Fankhauser et al. (2015), who found that a topical spot-on formulation for dogs containing 6.76 % fipronil and 50.48 % permethrin in fixed combination was able to repel stable flies at a rate of 96.6 % for 28 days. However, in the EU, fipronil is not approved for food-producing animals. Since 2014, it is limited to the application on seeds growing in green houses. The treatment of maize and sunflower seeds will no longer be authorised (EU No 781/2013). Although fipronil is authorised in the EU as an active substance in products for plant protection, no such products containing fipronil are approved in Germany (BMEL, 2017). Fipronil, however, is authorised as a biocide against insects and acari (528/2012).

2.3.4.2.7 Insect Growth Regulators

Insect growth regulators (IGRs) are active agents that very selectively interfere with pre-adult development stages of arthropods and thereby impair their life cycles (Richter and Steuber, 2010). The insecticidal effect follows one of three mechanisms: 1. chitin synthesis inhibition (lufenuron, triflumuron, diflubenzuron), 2. inhibition of ecdysis and pupation by ecdysteroids (dicyclanil, diofenolan), 3. analogues of juvenile hormone (methoprene, pyriproxyfen) (Tunaz

and Uygun, 2004). Since mammals and birds lack the specific target sites, they can tolerate IGRs rather well. IGRs have no adulticidal activity, it is, therefore, recommended to start control measures with adulticidal treatment or by using a fixed combination of both IGR and adulticide.

Chitin synthesis inhibitors

The insect exoskeleton as well as the covering of the digestive tract, the reproductive duct and some glands are made of insect cuticle. It primarily consists of protein and chitin fractions and serves as a protective barrier between the animal and the environment. Most inhibitors of the chitin synthesis are the organic compounds of benzoyl phenylurea (triflumuron, lufenuron, diflubenzuron, fluazuron). All of these compounds interfere with the chitin polymerisation and the following incorporation of chitin chains into the cuticula. As a result, the cuticula gets malformed, ecdysis cannot be completed and the larvae die. The first benzoyl phenylurea which was introduced as a larvicide was diflubenzuron (Miyamoto et al., 1993). The target site of diflubenzuron is assumed to be a sulfonyl urea receptor (Matsumura, 2010; Nasonkin et al., 1999) which was identified in *Drosophila melanogaster*. Sulfonylurea receptors, which belong to one of the transporter family proteins, are ATP- and GTP-sensitive potassium channels that are responsible for the Ca^{2+} transport. When they get blocked by diflubenzuron the K^{+} influx into the cells is inhibited and the intracellular Ca^{2+} content is increased. As a result, the incorporation of N-acetylglucosamine into insect chitin is impeded (Matsumura, 2010). Generally, chitin synthesis inhibitors are poured on and mixed into manure and slurry pits. While normally inducing their larvicidal effect through surface contacts, Vazirianzadeh et al. (2007) described the successful use of cyromazine as a feed-through larvicide. Howard and Wall (1995) demonstrated that when adult flies ingested a sugar solution containing triflumuron, they produced eggs with a reduced hatching rate. However, oral applications via the gastrointestinal tract providing faeces of the treated host were also studied. Mascari et al. (2007) found novaluron, an insect growth regulator of the benzoylphenyl urea class, to inhibit the development of sand flies (*Phlebotomus paptasi* Scopoli) when their larvae fed faeces of hamsters that had been fed a diet containing novaluron. Wright et al. (1975) discovered that when feeding 1 mg/kg pellets to rhinoceros the hatch rate of the flies was reduced drastically. Pospischil et al. (1996b) showed that chitin synthesis inhibitors were effective against multi-resistant *Musca domestica* strains with resistances against pyrethroids, organophosphates and carbamates. Diflubenzuron as well as lufenuron had a measurable impact on the chitin synthesis of the trachea (Gangishetti et al., 2009). Since benzoyl phenylureas are not selective and affect all chitin forming organisms it is also harmful to bees (Amir and Peveling, 2004; Barker and Taber, 1977; El-Din et al., 1990), and crustaceans (Miura and Takahashi, 1974).

Triazines

Triazines are a group of chemical compounds that are based upon nitrogen-containing aromatic heterocycles. They are often used as a basis for various herbicides. The triazine derivative cyromazine is also employed as moulting process inhibitor. Cyromazine was established as a control method for *Musca domestica* and *Lucilia cuprina* in the 1980s (Magoc et al., 2005) but was also reported to be effective against *Stomoxys calcitrans* (Taylor et al., 2012a). It compromises their moulting and pupating process without affecting the chitin synthesis. Its specific mode of action, however, remains unknown (Magoc et al., 2005). Cyromazine was observed to be selective on dipteran species and is able to harm other insects only at higher concentrations (Hildebrand, 2017). While normally cyromazine is applied to manure or slurry pits, Miller et al. (1996) discovered concentrations above 0.5 mg/kg body weight to be effective when administered to diary calves. Nevertheless, any oral application resulted concurrently in residues in kidney fat, liver and occasionally in round muscles of the treated calves.

Juvenile hormone analogues

Synthetic juvenile hormone analogues imitate the hormonal activity of the natural juvenile hormone and thereby have an effect on its receptor. The insect juvenile hormone leads an essential role in the larval development and acts as an agonist of the moulting and pupation hormone ecdysone (Richter and Steuber, 2010). The peptides of the prothoracic tropic hormones from the brain induce the secretion of ecdysone from the prothoracic gland. When juvenile hormone levels are high, the epidermis is programed for a larval moult. If not, the epidermis is programed for metamorphosis (Tunaz and Uygun, 2004). Since the juvenile hormone is found to be absent in pupation and is, among others, necessary in adults for some reproductive functions, it was concluded that it suppresses pupation and induces vitellogenesis during the reproductive stage of the imago (Eto, 1990). Thus, any disturbance in the juvenile hormone balance is expected to cause a disorder in the development of the insect (Tunaz and Uygun, 2004). The instability and synthetic difficulties of the juvenile hormones themselves, however, were the reason to create selective juvenile hormone analogues (Eto, 1990). It has been shown that the activity of the juvenile hormones is dependent on the timing of application (Tunaz and Uygun, 2004). It was suggested that third instar larvae are only sensitive to juvenile hormones between its disappearance and the appearance of ecdysone (Miyamoto et al., 1993; Riddiford, 1976). When pupae are treated with juvenile hormones imagoes do not develop in a normal way (Tunaz and Uygun, 2004). Although the adult stages of most insects are insensitive to juvenile hormone analogues some are reported to become sterile after their application (Retnakaran and Percy, 1985).

Methoprene

Methroprene was the first compound introduced to the market. It is a terpenoid compound, which is compatible with mammals and produces very low residuals on plants or in soil due to its sensitivity to UV light. Therefore, it is often used for flee control in cats and dogs (Smith, 1995).

Methoprene was also tested against honey bees. When 250 µg of methoprene were applied on adult honey worker bees, they were induced to move from the broodnest to the food storage region prematurely where they showed a premature foraging behaviour (Robinson, 1985). It also activated the precocious production of two alarm pheromones. Workers treated with 2000 µg of methoprene died.

Pyriproxyfen

Pyriproxyfen is a pyridine based juvenile hormone analogue, which is more effective than methoprene. Additionally, pyriproxyfen is not likely to be inactivated by larval esterases (Richter and Steuber, 2010). Compared to methoprene it is stable to UV-light. In fleas it does not only inhibit the pre-adult development, it also interferes with the adult fleas' fertility (Richter and Steuber, 2010). Liu et al. (2012) demonstrated similar results in stable flies. The LD_{50} being 0.002 mg/kg for larvae, they reported that even the topical treatment of 1- and 3-day-old adult female stable flies had a bad effect on oviposition and egg hatching at a dose of 8 µg pyriproxyfen/fly. Bull and Meola (1993) calculated a LD_{50} of 128.3 mg/m^2. Ishaaya and Horowitz (1995) found a LD_{90} of 0.05 and 0.02 mg/kg for whiteflies and scale insects, respectively. While pyriproxyfen is hardly toxic to vertebrates, it is lethal to fish and crustaceans (Richter and Steuber, 2010). Pyriproxyfen, however, is sparingly soluble in water, it is highly soluble in organic solvents such as hexane and xylene (Sullivan and Goh, 2008). Since it was introduced into Brazilian drinking water in 2014 after the first Zika virus linked microcephaly outbreaks in neonates, the possibility that pyriproxyfen was responsible for the microcephaly has been discussed (Valentine et al., 2016). According to several WHO controlled studies, pyriproxyfen has now been ruled out as a likely aetiology (Valentine et al., 2016). Zhang et al. (1998) found that microsomes of pyriproxyfen resistant house flies had higher levels of total cytochrome P450s in both fat and gut body. Shah et al. (2015a) discovered that, after generating a high resistance against pyriproxyfen in *Musca domestica* after 30 generations of selection the resistance in the house fly was autosomally inherited, completely dominant and polygenic. The observed very low cross-resistance among pyriproxyfen and other insecticides tested leads to the suggestion to rotate with these insecticides in order to delay the pyriproxyfen resistance in flies.

2.4 Resistance development

Since several studies have shown that the persistent and non-strategic use of insecticides can enhance resistance development, it has become clear that resistance development is one of the central problems which modern pest control has to deal with (Unterstenhöfer, 1970).

2.4.1 Definition of insecticide resistance

According to the WHO (WHO, 1957), resistance is defined as the ability of an insect strain to tolerate doses of toxic substances that would cause the death of the majority of sensitive individuals of the same species.

The IRAC (Insecticide Resistance Action Committee) defines resistance in more detail as “a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species” (IRAC, 2017).

Resistance can be justifiably suspected when chemical control measures do not function adequately anymore although they had worked in the past (Beckmann and Haack, 2003). It is a significant genetic modification on a molecular or biochemical basis or in the behaviour of an insect as a result of selection by insecticides which limits the control measurements in the field (Robertson et al., 2007). An organism can show resistance towards one substance only (Künast and Güntzrodt, 1981), towards multiple substances of the same chemical group (Deplazes et al., 2012) or towards substances of different chemical groups. Resistance towards different chemical groups is called cross resistance (Schnieder, 2006) especially when the organism has not been selected by exposure to all of the insecticides. Generally, resistance towards several pesticides of different chemical classes is termed multi resistance. Resistance to the same chemical class with the same mode of action is called side resistance (Bates, 2012).

According to the World Health Organization which provided resistance definitions for malaria vectors, mosquitoes, a mortality rate below 98 % suggests a probable and one below 90 % indicates a complete resistance (WHO, 2016). Mortality or paralysis rates above 98 % reveal susceptibility. If the paralysis rate is between 90 % and 97 %, the occurrence of resistant genes in the field population must be verified by additional tests with the same population or by molecular assays. Resistance is confirmed if at least two of the tests result in mortality rates below 98 %. Given that at least 100 mosquitoes of each strain were tested, if the paralysis rates are below 90 %, further testing is not required. If not, it is necessary to prove samples of the population using multiples of the LD_{95}. Mortality rates of 98 to 100 % at the five-fold

concentration suggests a low resistance and it is not necessary to assay higher concentrations. Paralysis of below 98 % at the five-fold concentration, however, requires further testing at the tenfold concentration. A mortality rate between 98 and 100 % at the tenfold concentration affirms a moderate resistance and mortality rates below 98 % confirms a high resistance intensity. In this study every strain was tested with the discriminating dose (i.e. the LD_{95}), a four-fold of the LD_{95} and a 16-fold of it.

2.4.2 Resistance mechanisms

Natural selection by an insecticide supports the initially very rare but naturally occurring insects with resistance genes to survive and to pass the resistance trait on to their progeny (IRAC, 2017). “Under permanent selection pressure, resistant insects outnumber susceptible ones and the insecticide is no longer effective. The speed with which resistance develops depends on several factors, including how fast the insects reproduce, the migration and host range of the pest, the availability of nearby susceptible populations, the persistence and specificity of the crop protection product, and the rate, timing and number of applications made” (IRAC, 2017).

Miller (1988) categorised all occurring resistance mechanisms in four groups:

1. *Behavioural resistance,* where insect behaviour is modified and thus, the insect no longer comes into contact with the insecticide.
2. *Penetration resistance,* where the design of the insect’s exoskeleton is altered in ways that inhibit insecticide penetration.
3. *Site-insensitivity,* where the chemical site of action for the insecticide becomes modified to have reduced sensitivity to the active form of the insecticide.
4. *Metabolic resistance,* where the insect’s metabolism becomes modified in ways that detoxify the insecticide, or impede the metabolism to convert it into its toxic form. In the case of pyrethroids most of these metabolic resistance mechanisms include multifunction oxidases, glutathione-S-transferase, and esterases.
    a) Oxidases are enzymes which catalyse a variety of different reactions. Cytochrome P450 monooxygenases are involved in many cases of insecticides resistance (Bergé et al., 1998). Oxidases generally metabolize water-insoluble compounds the way they can be excreted more easily (Guengerich, 1991).
    b) Glutathione-S-transferases comprise a family of isoenzymes that are able to catalyse attachments of the reduced form of glutathione to xenobiotic substances for detoxification (Scott et al., 1990) by conjugating the thiol group from glutathione

to compounds that have an electrophilic centre (Low et al., 2007). Those conjugates can be eliminated from the organism by excretion or in vacuoles. The insect specific epsilon and delta glutathione-S-transferases are capable of detoxifying organophosphates (Huang et al., 1998; Lewis and Sawicki, 1971; Oppenoorth et al., 1979) and organochlorines (Clark and Shamaan, 1984; Tang and Tu, 1994) by conjugation. Glutathione-S-transferases even assign resistance to pyrethroids by reducing the oxidative damage that they cause to lipids (Vontas et al., 2001).

c) Esterases are hydrolase enzymes that split esters into an acid and an alcohol. Oppenoorth and Van Asperen (1960) found that genetically altered ali-esterases are able to break down organophosphates which under normal circumstances inhibit esterases.

Generally, it is assumed that site-resistance and metabolic resistance play the main role in resistance development (Georghiou, 1994). Resistance is based on the primary selection of genetically resistant individuals that through natural mutation already existed among the population before insecticides had been employed (Georghiou, 1994). Georghiou and Taylor (1977b) stated that some factors like the initial frequency of resistant alleles, reproductive potentials, migration rates, dominance of alleles that concede resistance, and the presence of refugia are not human-controlled. The long-term use of insecticides, however, the prolonged application of the same active ingredient or insecticides having the same mode of action over time, underdosing, the employment of antagonistic insecticides as well as the non-strategic use of insecticides are observed to enhance resistances (Richter and Steuber, 2010). Georgihou and Taylor (1977a, b) assumed that insecticide resistance evolved most slowly when the population was diluted with immigrants, when population density was suppressed by selection and when susceptible individuals had a reproductive advantage over the resistant correspondent. Furthermore, lower insecticide dose levels, higher selection thresholds, varying selection schedules and the presence of refugia were reported to slow down insecticide resistance development. Since those operational factors can be controlled, the risk of resistance progress could be reduced by modifying them (Georghiou, 1994).

2.4.3 Resistance marker genes

In comparison to *Drosophila melanogaster* and *Musca domestica*, little is known about the genetic resistance in stable flies. In house flies, distinctive resistances are controlled by a variety of resistance genes: knock-down resistance (kdr) (Busvine, 1951; Farnham et al., 1987; Ingles et al., 1996; Rinkevich et al., 2012; Tsukamoto et al., 1965), reduced-penetration resistance (pen) (Farnham, 1973), cytochrome-p450-monoxygenase mediated resistance (Kulkarni and Hodgson, 1980), acetylcholinesterase resistance (AChE-R) (Walsh et al., 2001), and larvicide resistance genes (Shen and Plapp, 1990). It can be assumed that resistance in the closely related stable fly is controlled by similar resistance genes as in *Musca domestica* and other fly species (Ingles et al., 1996; Pitzer et al., 2010; Soderlund and Bloomquist, 1990). Based on Feyereisen (1995), on a molecular level, these mechanisms of resistance are realized by: “point mutations in the ion channel portion of a GABA receptor subunit (cyclodiene insecticides); point mutations in the vicinity of the acetylcholinesterase (AChE) active site (organophosphorus and carbamate insecticide resistance); amplification of esterase genes (organophosphorus and carbamate insecticides); mutations linked genetically to a sodium channel gene (DDT and pyrethroid insecticides); and yet uncharacterized mutations leading to the up-regulation of detoxification enzymes, such as cytochrome P450 and glutathione S-transferases (many classes of insecticides)”.

Kdr resistance

Kdr resistance was first described by Busvine (1951) who found that its resistance is located in a recessive factor of chromosome III. Kdr resistance includes kdr and super-kdr resistance. Since the voltage-sensitive sodium channel (Vssc) is the main target site of DDT and pyrethroids, the phenomenon known as knock-down resistance (kdr) is characterized by nerve insensitivity to these compounds (Williamson et al., 1993). It was shown that resistance rendered by kdr/super-kdr mechanism maps to a house fly sodium channel gene referred to as *Msc* (Williamson et al., 1993). Huang et al. (2004) discovered that the frequency of kdr mutations was strongly correlated with reduced mortality rates of various house fly strains when treated with pyrethrum and bioresmethrin. Depending on the active ingredient, strains with super-kdr 3D were described to show a 38- to 64-fold resistance whereas super-kdr A2 and kdr-carrying strains displayed an 11- to 20-fold resistance (Farnham et al., 1987). Vais et al. (2001) found that the incorporation of super-kdr mutation into the *Drosophila* sodium channel increased channel inactivation and reduced deltamethrin sensitivity by over a 100-fold. Based on cross-resistance patterns, the absence of synergism of esterase and oxidase inhibitors, and verified electrophysiological evidence of reduced neuronal sensitivity, it was

implied that kdr-linked reduced neuronal sensitivity corresponds to an important mechanism of pyrethroid resistance in a number of insect taxa (Soderlund and Bloomquist, 1990). Kdr and super-kdr mutations could be attributed to leucine-phenylalanine replacements in the hydrophobic IIS6 transmembrane segment, while super-kdr mutations appeared to be linked to an additional methionine to threonine replacement within the intracellular IIS4-S5 loop. Neither of those mutations could be found in pyrethroid-sensitive strains (Williamson et al., 1996). To date, 120 haplotypes of the kdr nucleotide sequence have been described in flies (Rinkevich et al., 2012).

In 2011, Olafson et al. (2011) identified a point mutation resulting in a leucine to histidine amino acid change in stable flies. Since this location coincided with that observed for knockdown resistance mutations in other insects the allele was then termed kdr-his. Using a molecular assay, the frequency of the mutation was subsequently determined from five field-collected stable fly colonies. The accumulation of the mutation ranged from 0.46 to 0.78, supporting further evaluations on the prevalence of the allele.

Reduced-penetration resistance (pen)

Mutation of the pen resistance factor gene, which is localised on chromosome III as well is characterized by reducing the penetration rate of insecticide through the cuticle. By itself, it only results in minor resistance but may increase the effect of other factors (Farnham, 1973).

Cytochrome-p450-monoxygenase mediated resistance

Since cytochrome-p450-monoxygenase metabolizes a variety of organic compounds that otherwise would accumulate to toxic concentration, its low sensitivity is likely to induce cross resistances in other non-related insecticide classes (Kulkarni and Hodgson, 1980).

Acetylcholinesterase resistance (AChE-R)

Acetylcholinesterase is a serine esterase in the α/β hydrolase that completes nerve impulses by degrading the neurotransmitter acetylcholine. It is the target of organophosphate and carbamate compounds that phosphorylate or carbamylate the active site of serine to block the hydrolysis of acetylcholine. This eventually leads to the death of the insect. Mutations in the AChE gene, which in *Musca domestica* is mapped to chromosome II were found to lead to an insensitivity against acetylcholinesterase in several insects. In the house fly five mutations (Val-180 → Leu, Gly-262 → Ala, Gly-262 → Val, Phe-327 → Tyr and Gly-365 → Ala) could be observed (Mutero et al., 1994), these occurred in isolation or in combination (Walsh et al., 2001). Villatte et al. (2000), however, suggested that more than seven mutations might be involved in the organophosphate and carbamate resistance.

Rdl resistance

The gene for resistance to dieldrin operates the gamma aminobutyric acid receptor. Cross-resistance has been observed towards fipronil (Ffrench-Constant et al., 2000).

Larvicide resistance genes relevant to stable flies

Shen and Plapp (1990) found that in *Musca domestica* the gene that conferred resistance to cyromazine and diflubenzuron, a benzoylurea insecticide, was on chromosome V. Stable fly resistance to cyromazine and diflubenzuron, however, appeared to be different from other types of insecticide resistance. Douris et al. (2016) found that the resistance mutation (I1042M) in the chitin synthase 1 (*CHS1*) gene of benzoylurea resistant cabbage moths (*Plutella xylostella*), insects, was at the same position as the I1017F mutation reported in spider mites, acari, that confers etoxazole (an acaricide that targets nymphs, larvae and eggs) resistance. Zhang et al. (1998) confirmed that microsomal cytochrome P450 monooxygenases play an essential part in the pyriproxyfen resistance of the house fly. Furthermore, they suggested that the fat body must be as important as the gut for the metabolism of pyriproxyfen in resistant house fly larvae.

2.4.4 Occurrence of insecticide resistances in *Stomoxys calcitrans*

As early as in 1976, the “WHO expert committee on Insecticides” reported on resistances in vectors and reservoirs of disease to pesticides. Over 100 vectors were identified as being resistant against one or more insecticides (WHO, 1976).

The “arthropode pesticide resistance database” http://www.pesticideresistance.org which is maintained by Michigan State University, publishes data on arthropod resistance cases from 1914 to the present. The “Insecticide Resistance Action Committee” (IRAC) which was formed in 1984 works as a specialized technical group of the industry association “CropLife”, which is an international trade association of agribusiness companies founded in 2001, and provides “a coordinated industry response to prevent or delay the development of resistance in insect and mite pests” (IRAC, 2017). IRAC consists of employees from the agrochemical and public health companies normally associated with “CropLife” through membership in the relevant national associations.

Many research studies worldwide have focused on the resistance development in house flies (Abbas et al., 2014; Acevedo et al., 2009; Akiner and Çaglar, 2012; Bell et al., 2010; Cetin et al., 2009; Harris et al., 1976; Jandowsky, 2009; Jandowsky et al., 2010; Kaufman et al., 2001b; Khan et al., 2015; Kristensen, 2008; Kristensen et al., 2001; Pap and Farkas, 1994; Pinto and Prado, 2001; Pospischil et al., 1996a; Pospischil et al., 1996b; Seraydar and Kaufman, 2015)

and found increasing resistances against all insecticide groups. Thus, in 1999, Keiding (1999) gave an overview over the global resistance status in field populations of *Musca domestica* since the 1970s and discovered worldwide resistances against DDT although this active ingredient had not been applied in several years. Additionally, he observed pyrethroid and organophosphate resistances globally. Resistances against the insect growth regulators cyromazine and diflubenzuron were already known in a few places in the Netherlands, the USA and Japan.

Resistances in stable flies have also been investigated for a variety of insecticides throughout the world albeit to a lesser extent than in house flies. Insecticide resistances have been demonstrated previously in *Stomoxys calcitrans*, but in many cases against insecticides that are not used any more, such as organochlorines and DDT (Pitzer et al., 2010; Sømme, 1962; Stenersen, 1966).

In 1958, Sømme (1958) observed a rise in tolerance by five or six times in field collected stable fly strains when exposing them to DDT or lindane, an organochlorine insecticide. In the laboratory they then generated even higher resistance levels within only two generations.

In 1962, Sømme (1962) studied the resistance to chlorinated-hydrocarbon insecticides in six stable fly field strains in Norway. Due to laboratory selection, two strains became resistant against DDT and most of the strains were resistant against chlordane. Resistance could, therefore, be caused via selection. Strains with high chlordane resistance were also more resistant to dieldrin, an organochlorine insecticide, which indicated cross resistance.

Right after that, Stenersen and Sømme (1963) showed by selection that compared to sensitive strains stable fly field strains became 25 times more resistant to DDT, 110 times more resistant to methoxy-DDT, and even more resistant to DDD (dichlorodiphenyldichloroethane) although stable flies possess very little dechlorinase and do not detoxicate DDT. Genetical studies revealed that resistance to these insecticides is mainly caused by a recessive gene, nonetheless, less important genes are probably also present. Stenersen (1966) also found that selection with Dilan, an insecticide derived from the nitroparaffins, not only conferred DDT-resistance on insects, but that a selection with DDT also conferred resistance to Dilan. Through gas-liquid chromatography, Mount et al. (1966) had discovered that the metabolism or the excretion of dieldrin was more rapid in resistant flies than in susceptible ones. As a further proof that DDT-resistance is not a result of increased detoxication, it was shown that adding the synergist “WARF Antiresistant” - an inhibitor of the DDT-dehydrochlorinase in insects - to DDT, the resistance of the Dilan and DTT resistant stable fly strains did not decrease (Stenersen, 1966).

In 1964, Harris (1964) studied the susceptibility of stable flies against 15 insecticides and found that the organophosphorous insecticides were more effective than the chlorinated

hydrocarbons. He additionally discovered that the response of the flies was more variable after the exposure to chlorinated hydrocarbons than to the organophosphates.

In 1986, Hogsette and Ruff (1986) demonstrated in Florida, USA, that ear tags impregnated with the synthetic pyrethroid flucythrinate and ear tapes containing the synthetic pyrethroid permethrin were effective against stable flies for ten weeks.

In 1994, Cilek and Greene (1994) detected insecticide resistance against two organophosphates, dichlorvos and stirofos, and a pyrethroid, permethrin, in stable fly field strains collected from eight cattle feedlots in south-western Kansas, USA. The prevalence of resistance ranged from 2 to 100 % and was generally of the following decreasing order in each population: dichlorvos, stirofos, permethrin. Resistance was also found in feedlots where insecticide use was non-existent or did not exceed one application per year. It was assumed to have resulted from the intermingling of resistant flies from nearby feedlots with local ones.

When comparing three exposure techniques for the analysis of stable fly susceptibility in southeastern Nebraska, USA, Marçon et al. (1997) found that the topical application was better suited for this species than treated filter papers and treated petri glass dishes. However, the magnitude of the paired comparisons of field and susceptible stable fly strains after topical treatment with permethrin or methoxychlor was not bigger than the significant differences between one and another within the sensitive laboratory strain. In 2005 it was reported that the stable fly strains tested were still susceptible to synthetic pyrethroids (Cruz-Vázquez et al., 2005; Muraleedharan, 2005). In Bangalore, India, Muraleedharan (2005) discovered that a single application of 0.2 % of the synthetic pyrethroids esfenvalerate and deltamethrin was 100 % effective against stable flies during a 21-day period of observation. Cruz-Vázquez et al. (2005) reported stable fly populations collected from sixteen dairy farms in Aguascalientes, Mexico, to be susceptible to permethrin when exposing them to two discriminating doses. Stable flies were subjected to glass vials that contained the LD_{50} and LD_{99} (0.0014 µg/cm^2 and 0.0026 µg/cm^2, respectively) and were observed to be susceptible when compared to a sensitive reference strain. Yet, this was the first study in Mexico on the susceptibility of stable flies to any insecticide.

In 2010, Pitzer et al. (2010) demonstrated resistance to permethrin in field-collected stable flies from geographically separated horse farms in Florida. Three *Stomoxys* field strains displayed a maximum of 57 % and 21 % survival to permethrin residues of 3 times and 10 times the LD_{99} of a susceptible strain, respectively. Even stable flies which had not had contact with insecticides expressed a 20 % survival when treated with the 3x-LD_{99}. A stable fly strain that previously had shown a low resistance level, had a 15-fold increased resistance after five generations permethrin selection in the laboratory.

The research group around Salem et al. (2012a) was the first one to study stable fly susceptibility to insecticides in Europe. In France, they evaluated resistance against the

pyrethroids cypermethrin, deltamethrin, fenvalerate, λ-cyhalothrin and the organophosphate phoxim in two stable fly field strains after a one-hour exposure to an impregnated filter paper. One of the field strains originated from an organic farm where insecticides had not been used for over ten years and the other field strain was collected at a farm where insecticides had frequently been applied. The LD_{50} and LD_{90} were observed to be higher in blood-engorged stable flies than in non-blood-engorged flies. Except for phoxim, which had not been used on both farms in several years, the LD_{90} values obtained for the organic farm strain were 1 to 4 times lower than the recommended doses for all tested pyrethroids. For the conventional farm strain the LD_{90} values were between 7.1 and 22.6 times over the recommended doses for the pyrethroids employed indicating a correlation between the use of insecticides and resistance development.

2.4.5 Methods for detection of insecticide resistances

2.4.5.1 *In-vivo* methods

Resistance testing by *in-vivo* methods can be performed either in the field or in the laboratory. When testing in the field a complete description of all factors that could have an influence on execution, comparability, and significance is necessary. Specifications on hygiene management, the use of former control measures, the season or temperatures should be taken into consideration (Hildebrand, 2017). The European Chemicals Agency (ECHA) is regularly controlling active ingredients and their product concentrations. When testing in the laboratory, first a representative number of individuals has to be collected before being bred in the laboratory under parameters, like light, temperature and humidity, that are as constant as possible (Keiding, 1999). Tests are then conducted with the F1 or following generations. Normally, field populations are then being compared to a sensitive reference strain which often originates from a laboratory WHO strain. Natural variations in sensitivity within those of sensitive reference strains (Schaub et al., 2002) should be taken into account.

2.4.5.1.1 Test of adults

Among the tests for adult insects there are “self-dosing” tests in which insects have the possibility to avoid insecticide contact and “forced exposure” tests in which the substance is directly applied to every single insect.

“Self-dosing” tests with which feed-through insecticides are often tested can be differentiated into “choice” and “non-choice” feeding tests. “Choice” tests examine above all the

attractiveness of a product. In “non-choice” assays, the tested product is the only food source available in the way that insects cannot avoid ingesting them. “Choice self-dosing” tests, however, cannot differentiate between the refusal of the product intake and a resistance. Additionally, feed-through tests are not suited in stable flies since they almost exclusively feed on blood sources.

In “forced exposure” tests insects cannot avoid the contact with the insecticide. Most of those tests are performed with the method of topical application which is a WHO approved standard method. On that account, pure substances are being dissolved in oil, acetone or an oil-acetone-mixture. Then between 0.3 and 1 µl of this insecticide solution is topically applied on the dorsal surface of the insect’s thorax. Another method to force a contact between the animal and the insecticide is the use of a treated vial or impregnated filter paper to which the insects are exposed. The control group is only treated with the solvent. The CDC bottle bioassay similarly measures the time it takes an insecticide to penetrate a vector. Information derived from this bioassay may provide initial evidence that an insecticide is losing its effectiveness. It can be performed on vector populations collected from the field or on those reared in an insectary from larval field collections (CDC, 2010). The bottles are coated with a diagnostic dose of the insecticide. It is recommended that a minimum of 100 insects, divided among four replicate bottles, are tested for an insecticide at a given concentration. Insects are then transferred to the bottles and mortality is noted every 15 minutes up to two hours (CDC, 2010). The Flybox® test used in this study can also be considered a “forced exposure” test since the flies are transferred into a box coated with an insecticide impregnated net. Due to the darkness of the inner side of the box, flies do not have a choice other than sitting down and getting into contact with the respective insecticide. Before those “forced exposure” tests, a lethal dose (LD) has to be determined with the aid of a sensitive reference strain. Normally, the LD_{50} or LD_{90} is being used in order to compare mortalities between the test and the reference population. Marçon et al. (1997) found the topical application as a test method most sensitive for stable flies when comparing it to impregnated filter papers and treated glass petri dishes.

2.4.5.1.2 Test of larvae

Larvicide tests are normally being conducted by placing insect eggs or different larval stages on a rearing medium which has been dispersed evenly with the larvicide under investigation (Jandowsky, 2009; Jandowsky et al., 2010). Various concentrations are produced and tested, often around the dosage recommended by the manufacturer. Eggs are normally situated on a serial dilution of rearing media mixed with larvicide in order to control the hatching rate more easily. Depending on the outside temperature, after 3 to 6 weeks the emerging imagoes of the

test strains are compared to the ones of the sensitive reference strain (Kristensen and Jespersen, 2003). Cerf and Georghiou (1974) tested third instar larvae of house flies by the topical application of insect growth regulators.

2.4.5.2 *In-vitro* methods

In-vitro methods have important advantages compared to *in-vivo* methods. They do not only detect resistance but also provide information on the probable mechanisms involved, they are faster, and permit the analysis of a single insect (Brown and Brogdon, 1987). In *in-vitro* methods, enzymes as well as genetic changes such as mutations being suspected to play a role in the development of insecticide resistance are tried to be detected. In particular, the polymerase chain reaction (PCR) is being applied (Walsh et al., 2001). Additionally, spectrophometry or electropherograms for the detection of increased glutathione S-transferase activity are being used (Brown and Brogdon, 1987). The activity and inhibition of the DDT dehydrochlorinase can be uncovered by gas-liquid chromatography (Oppenoorth, 1965).

2.4.6 Strategies for the delay of resistance development

As Busvine (1957) already stated in 1957, resistances appear to be growing faster than the ability to deal with them. As far back as then, an urgent need for researching whether resistance can develop in any insect to any insecticide that is extensively in use and for long periods was proposed (Busvine, 1957). Later, the correlation between the use of insecticides and the levels of resistance could be confirmed (Scott et al., 2000). The Food and Agriculture Organization of the United Nations (FAO) states in Article 3.10 of its “International Code of Conduct on the Distribution and Use of Pesticides”: “It is recognized that the development of resistance of pests to pesticides can be a major problem. Therefore, governments, industry, national institutions, international organizations and public-sector groups should collaborate in developing strategies which will prolong the useful life of valuable pesticides and reduce the adverse effects of the development of resistant species.”

Resistance development depends on the frequency of naturally occurring genes for resistance and the severity of selection determined by the size of the population exposed to the insecticide and the proportion killed (Busvine, 1957). Busvine (1957) suggested that since resistance appeared to develop more rapidly in response to high dosages the lowest level sufficient to prevent transmission of disease should be applied in most cases. He recommended that good alternative insecticides should be reserved for the use in epidemics. He pointed out that the

rotation of insecticides might not have the expected practical value since it would take a long time for resistance to disappear. In *Musca domestica* a DDT resistance might last for about ten years and the effectiveness of DDT use afterwards would be limited to a two-year period (Busvine, 1957). Kočišová et al. (2002), however, who also stated that the alteration of insecticides would only prolong the successful application of some insecticides, valued this as a practicable option for future control measurements.

Busvine (1957) expected increased dosages and the use of alternative insecticides not to be efficient against high-level resistances and supposed that the future of insect control lay in the use of control measures other than insecticides. Nevertheless, he assumed that if the selection by insecticides would cease in a population, that mainly consists of resistant and hybrid individuals, in the end the population would be made up of resistant individuals only due to the possible dominance of resistant ones over susceptible ones.

Hammock and Soderlund (1986) stated that a key strategy for integrated pest management (IPM) is to use insecticides only when damage is expected to exceed clearly defined economic thresholds. This should decrease the selection pressure imposed by the extensive use of insecticides which generally leads to resistance development and preserves natural enemies of the target insect. At the same time this would naturally reduce environmental contamination as well as the exposure of farm workers and consumers. Effective pest control requires also the development of new insecticides with novel modes of action and negative cross-resistances to existing ones (Voss, 1988) although this will only slow down resistance development and prolong the usefulness of available chemicals (Hammock and Soderlund, 1986). The finding of new molecular and biochemical targets in insects and mites as wells as extensive research efforts in biochemistry, physiology, genetics and insect behaviour are expected to improve existing monitoring measures. Since the risk of resistance development is not equally high in all arthropod life stages, control methods should always be directed at the life stage in which resistance is least likely to prosper (Voss, 1988). Hoffmann (1987b) emphasized to also focus on waste hygiene in order to reduce the need for insecticide use, and Foil and Younger (2006) found that treated targets could be a feasible addition in stable fly control programs. Biological control measures like a mass release of the pupal parasitoid *Spalangia cameroni* is also reported to be effective against stable flies (Skovgård, 2004), especially when new flies do not immigrate and temperatures are not too high (Birkemoe et al., 2009). Additionally, education of those who sell, recommend and use insecticides is also an important step in minimizing the application of chemical control measures by raising awareness (Voss, 1988).

According to Hoffmann (1987a) and Roush (1993), resistance development can be slowed down by using different active ingredients at the same time, by avoiding the application of sublethal doses, by consideration of non-persistent substances (Hoffmann, 1987a; Scott et al.,

1990), by using primarily non-chemical control measures and by using insecticides as a last resort and restricting their application to seasons in which the population density is highest. The dose of an insecticide should be intended to kill heterozygotes in populations that are still not fully resistant (Roush, 1993). An unreasonable increase in insecticide concentration and application frequency, however, is not suited to reduce resistance development since it can lead to intoxications in humans and livestock (Hoffmann, 1987a). Furthermore, it is not legally allowed. Additionally, the advantage to a high dose approach is very small when the resistance allele frequency is more than 10^3. Instead of rotating two different biocidal insecticides, which are used in the surroundings of livestock, they can also be combined and applied at the same time (Roush, 1993). The main reason for that is a concept known as “redundant killing”: an individual is very unlikely to carry both resistance alleles of two separate loci (Roush, 1993). Therefore, if both active ingredients are applied in sufficiently high dosages that would kill susceptible insects by themselves, those pesticides are redundant in terminating fully susceptible individuals. Their effectiveness, however, is weakened if one of the ingredients has a significantly longer persistent activity (Roush, 1993). The one with the longer persistent activity only benefits from redundant killing when the other is present (Roush, 1993), otherwise it can enhance resistance development by favouring heterozygote resistant individuals. However, not all insecticide combinations result in synergism, some have a significantly antagonistic effect (Abbas et al., 2014).

Although the concept of refugia has not been fully verified by experiments to flies (Heath and Levot, 2015) , it could also be applied to stable flies. It states that if parts of a pest population are removed from exposure to an insecticide, genes that confer resistance reach levels low enough to be of no concern (Goss and McKenzie, 1996). Heath and Levot (2015) comment that alleles that are responsible for resistance would be disadvantageous for survival in the absence of the insecticide. Thus, periods during which animals are not chemically treated could play a major role in the reduction of insecticide resistance development.

Georghiou (1994) categorized approaches to resistance management in three groups: management by moderation, management by saturation and management by multiple attacks. Thus, a low selection pressure supplemented by a strong non-chemical component, the addition of attractants or unspecific synergists like PBO in order to increase the insecticide intake and the use of biocidal combinations, rotations or chemicals with multi-site action were suggested, supporting recommendations of other authors.

In conclusion, there are many very similar approaches for reducing resistance development. Nonetheless, according to the WHO the use of insecticides can be summarized under the motto: as much as necessary and as little as possible (WHO, 1991).

2.5 Alternative control strategies

Apart from chemical control measures there are, as aforementioned, mechanical, biological and physical control methods (2.3.1.–2.3.3). Other than those, due to the little threat they pose to the environment and human health, botanical pesticides are considered to be attractive alternatives to synthetic chemical insecticides. Pyrethrum (dried flowers of *Chrysanthemum spp*.) as well as neem (*Azadirachta indica* seed powder extract) are well established on the market, and insecticides based on plant essential oils are also sometimes commercially used (El-Wakeil, 2013). The use of plant derivatives dates back at least 2000 years in ancient Egypt, China, Greece, and India (Thacker, 2002; Ware, 1983). Apart from plant derived toxins, certain essential oils are reported to be able to repel not only certain arthropods but also to have contact and fumigant pesticidal effects against some insects (Grundy and Still, 1985; Isman, 2000). Other oils like dillapiole and parsley seed oil were found to serve as potential pyrethrum synergists against house flies (Joffe et al., 2012). Essential oils from *Minthostachus verticillata, Hedeoma multiflora* and *Artemisia annua* were shown to be potent insecticides to *Musca domestica* themselves. (4R)(+)-pulegone, an organic compound which is often used as flavouring agent in perfumery due to its peppermint-like odour, was identified to be the main ingredient in those plant oils. Those oils are observed to act as a natural insecticide against house flies (Palacios et al., 2009). Khater et al. (2009) reported essential oils of camphor (*Cinnamona camphora*), onion (*Allium cepa*), peppermint (*Mentha piperita*), chamomile (*Matricaria chamomilla*) and rosemary (*Rosmarinus officinalis*) to show significant repellent properties against *Stomoxys calcitrans.* Hieu et al. (2010) tested 21 essential oils against *Stomoxys calcitrans.* Patchouli (0.5 mg/cm^2) was found to be the most effective. Compared to the commonly used repellent N,N-diethyl-3-methylbenzamide (DEET), however, it was observed to be less active. Very strong repellence was reported for the essential oils of clove bud, lovage root and clove leaf, whereas only strong repellence was obtained from thyme white. (Zhu et al., 2014); Zhu et al. (2011) found *Nepeta cataria* (catnip) oil (dosage 20 mg) to show the highest toxicity against stable flies when using it as a fumigant. It had the quickest knock-down time (7 min) and the shortest lethal time (19 minutes). When applying catnip oil topically, its lowest lethal dose was 12.5 µg/fly, 50 µg/fly resulted in a 100 % mortality. It also affected the stable flies' blood feeding behaviour negatively. Zhu et al. (2014) also demonstrated that catnip oil encapsulated in gelatine caused a 98 % inhibition rate in stable fly larval growth and female oviposition when larval or oviposition media were treated with 0.5 g of it. The site of action for essential oils in insects appears to be the octopaminergic nervous system (Enan, 2005). Since mammalian animals lack octopamine receptors, essential oils would cause no harm to them.

Today, however, the employment of botanical pesticides is limited due to their lower activity and long-term residue persistence compared to synthetic chemical insecticides. In view of the almost unstoppable resistance development, the use of those natural insecticides should be considered as a viable option in integrated pest management.

Education aiming at raising awareness for the development of insecticide resistance as well as intensified governmental support of organic vegetable or livestock farms would support the reduction of insecticides in use.

3 Materials and Methods

3.1 Study design

A previous study on insecticide resistances performed on dairy farms in the Federal State of Brandenburg in 2008 (Jandowsky, 2009; Jandowsky et al., 2010) indicated severe resistances in house flies (*Musca domestica*) against some of the most frequently used active ingredients. The objective of this study was to estimate the occurrence of stable flies (*Stomoxys calcitrans*) on dairy farms in Brandenburg and the use of different insecticides. At the same time it aspired to assess insecticide resistances in *Stomoxys calcitrans*. Moreover, it seeked to propose best-bet strategies for on-farm pest control aiming to minimize the use of insecticides and thus, resistance development.

The study was based on the distinctions made above between *in-vivo* methods performed in the field or in the laboratory, between tests of imagoes and tests of larvae as well as between "self-dosing" and "forced exposure" tests (2.4.5.1.). Following those distinctions, the study was designed and carried out in four steps:

1) At first, a questionnaire analysis was conducted on 52 dairy farms in order to appraise the stable fly abundance and the use of insecticides in Brandenburg.
2) 40 dairy farms were subsequently selected for an on-farm field study using the FlyBox® method (as a "forced exposure" device) in order to evaluate the susceptibility of the stable flies to a deltamethrin impregnated polyester fabric.
3) In order to contrast the results of the previous step in the laboratory with another forced exposure test, *Stomoxys* populations from 10 farms were caught and colonies established in the laboratory. The susceptibility of the emerging offspring was tested against the most frequently used insecticides with current methods under controlled conditions. In a first step, the LD_{95} of deltamethrin and azamethiphos were calculated with sensitive laboratory strains of *S. calcitrans*. Then the toxicity was assessed by topical application of the LD_{95} and multiples of it.
4) Finally, the larvicidal effects of two insect growth regulators, cyromazine and pyriproxyfen, were evaluated in the laboratory at different concentrations based on the manufacturers' recommendation.

In order to keep the age of the tested stable flies constant for better comparability, deltamethrin was tested on 5- to 10-days old flies of the F1-generation, azamethiphos on same-aged flies of the F_2-generation and cyromazine and pyriproxyfen on eggs of the F_2 and F_3-generation, respectively. All of the tested flies were blood-engorged in order to avoid variations in susceptibility since blood-engorged and non-engorged flies are reported to have LDs that can

vary by a factor up to 20 (Salem et al., 2012a). At the same time, this provided an impression of the inheritability of resistance.
The different test methods and insecticides used are summarized in the following table.

Table 1 Active ingredients and test methods used during the project, May – December 2015

Trade name	***Active Ingredient***	***Insecticide Class***	***Test method***
PermaNet®[1]	Deltamethrin	synthetic pyrethroid	FlyBox®-Test
Deltamethrin PESTANAL®[2]	Deltamethrin	synthetic pyrethroid	Topical application
AzamethiphosPESTANAL®[2]	Azamethiphos	organophosphate	Topical application
Neporex 2SG®	Cyromazine	triazine	Larvicide test
Archer®	Pyriproxyfen	pyridine-based	Larvicide test

[1]PermaNet® by Vestergaard-Frandsen, Lausanne, Switzerland, with a deltamethrin concentration of 55 mg/m^2 (see 3.6.1.)
[2]Pure substances of Sigma Aldrich Laborchemikalien GmbH, Seelze (see 3.6.1.)

3.2 Questionnaire analysis

During the months of June and July 2015 a questionnaire survey (see Annex) was performed on 52 dairy farms in the Federal State of Brandenburg. Altogether, 78 dairy farms had been selected. Local veterinarians had recommended 18 of them. The remaining 60 matched the 60 farms Jandowsky (Jandowsky, 2009; Jandowsky et al., 2010) had used in her study in 2008. They had originated from a list of the 150 best dairy farms (according to milk yield and quality) in Brandenburg registered by the Landeskontrollverband Berlin-Brandenburg eV in 2006. When farmers accepted to collaborate, they were included into the study on the condition that they had at least 50 dairy cows. However, five of those 60 dairy farms did not rear dairy cattle anymore.

In order to generate an adequate response rate the questionnaire survey was carried out by phone. Fifty-two farms agreed to participate resulting in a response rate of 71 %. The 52 dairy farms were located in the Brandenburg counties of Barnim, Dahme-Spreewald, Havelland, Märkisch-Oderland, Ostprignitz-Ruppin, Oberhavel, Oder-Spree, Potsdam-Mittelmark, Teltow-Fläming and Uckermark.

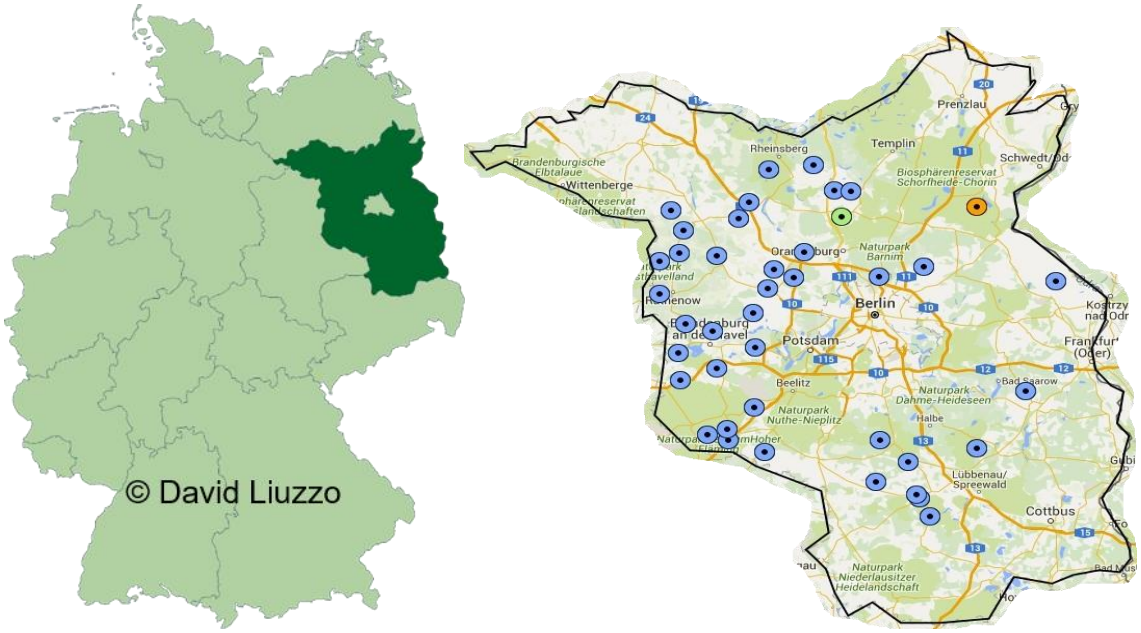


Figure 2 Left: The federal state of Brandenburg (dark) in Germany © David Liuzzo; right: questionnaire analysis, spatial distribution of the 52 dairy farms interviewed, © Google Maps, Brandenburg, June 2015

Data regarding the size of herd, the animal husbandry management system, the subjective appraisal of fly abundance, the type of control measure, the effectiveness of those control measures and the frequency of insecticide applications were documented. All information was anonymized.

3.3 Field study

3.3.1 Selection of test farms

Based on the mainly telephone-based questionnaire analysis, 40 farms out of the 52 farms interviewed were selected for an on-farm cross-sectional survey using the FlyBox® method in order to evaluate the susceptibility of stable flies to a deltamethrin (55 mg/m^2) impregnated polyester fabric. The 40 farms selected for the study were those that claimed to have a moderate or high fly abundance. They were located in the counties of Barnim, Dahme-Spreewald, Havelland, Märkisch-Oderland, Ostprignitz-Ruppin, Oberhavel, Oder-Spree, Potsdam-Mittelmark, Teltow-Fläming. The four farms interviewed in the counties of Uckermark were not included into the Flybox® test study due to the large distance. Dairy farms in Elbe-Elster, Oder-Spreewald-Lausitz, Prignitz and Spree-Neiße were not included into the study at all due to lack of contact information. In order to enable anonymization, each farm was labelled with the abbreviation of the county and a number for identification.

Figure 3 Spatial distribution of the 40 dairy farms selected for an on-farm cross-sectional survey, blue = conventional farms, green = organic farms, orange = Demeter farms, Brandenburg, © Google Maps, June – August 2015

3.3.2 Reference strains

The *Stomoxys calcitrans* strain of the Federal Environment Agency in Berlin (UBA) served as a reference for the Flybox® test of deltamethrin. It had originated from the stable fly strain which had been isolated by Novartis Animal Health Inc. CRA St. Aubin at the Centre de recherche animale, St. Aubin, Switzerland, in the 20th century, where it had been reared and used as a sensitive strain for testing (Table 2). In 2015, it was transferred to the UBA.

In 1999, Moyses and Gfeller had tested fifteen standard chemicals including deltamethrin and azamethiphos by topical application on the same strain at Novartis Animal Health Inc. in order to determine discriminating doses. The LD_{95} of deltamethrin found by Moyses and Gfeller (1999) was 2.34 ng/fly. Since this value was close to the LD_{95} (2.5 ng/fly) of the pyrethroid λ-cyhalothrin of the sensitive *Musca domestica* strain WHO (Jandowsky, 2009), the UBA strain was considered to be sensitive to deltamethrin as well.

In the laboratory tests, the *Stomoxys calcitrans* strain of MSD Animal Health Innovation GmbH Schwabenheim (MSD) was considered as reference strain. It had been isolated in the 20th century by Hoechst AG in Frankfurt, Germany, and then transferred to MSD Animal Health in the 1990s where it was continued to be reared. Since then, it had served as a model organism for testing new substances like deltamethrin and diazinon on fly activity (personal communication with Dr H. Williams, MSD). It had shown a paralysis rate of 96.6 to 100 % in contact with deltamethrin on paper in concentrations of 78.8 mg/m^2, 39.3 mg/m^2, and 19.6 mg/m^2 after an exposure time of 6 hours. At 9.8 mg/m^2 the mortality rate was calculated to be 89.7 % (personal communication H. Williams, MSD). The active ingredient was diluted in a non-acetone containing solvent.

In 2012, Salem et al. (2012a) had performed 1-hour contact tests with deltamethrin diluted in acetone on filter paper. He determined the LD_{90} for an insecticide exposed field strain to be of 264.3 mg/m^2 and for a sensitive strain isolated from an organic farm in 2010 to be of 28.1 mg/m^2. Although there is a difference in use of solvent and in time of exposure in both studies biasing the comparability, concentrations for accomplishing mortality rates above 90 % are of the same magnitude. Additionally, due to the non-acetone containing solvent the time to reach full susceptibility with the MSD strain is expected to be longer than tests based on acetone (personal communication with Dr H. Williams, MSD). Furthermore, the MSD strain which was exposed for 6 hours still showed full susceptibility when the concentration was 8.4 mg lower (19.6 mg/m^2) than in Salem's tests (28.1 mg/m^2) and a susceptibility of 89.7 % when the

concentration was 18.3 mg lower (9.8 mg/m^2). Thus, comparing both values, Salem's and the ones obtained by MSD, the MSD strain can be regarded as sensitive against the pyrethroid deltamethrin, too.

Table 2 Stomoxys calcitrans reference strains used in field and laboratory tests, Berlin, June 2015 – January 2016

Name	Origin	Sensitive to	Reference
Stomoxys calcitrans strain UBA	Isolated in the 20th century by Novartis Animal Health Inc., Switzerland.	Deltamethrin Azamethiphos	Moyses and Gfeller (1999)
Stomoxys calcitrans strain MSD	Isolated in the 1990s by Hoechst AG in Frankfurt, Germany.	Deltamethrin Diazinon	Personal communication H. Williams, MSD

At this point, there were no other laboratory stable fly strains available.

3.3.3 Capture of test flies

The stable flies were caught with a special scoop net that at the end had an evagination sewed on which facilitated the transfer of the flies into a test tube. With the assistance of the test tube, flies could be released into the FlyBox® or into a cage.

3.3.4 Flybox® Test

The Flybox® test method was developed by Dr Burkhard Bauer (Jandowsky, 2009; Jandowsky et al., 2010). It was performed according to indications of Jandowsky and Hildebrand (Hildebrand, 2017; Jandowsky, 2009; Jandowsky et al., 2010). The FlyBox® consists of a folded cardboard box of 5 cm x 18 cm x 8 cm (Co. FAPACK, Berlin). A small flap at one end of the box allows transferring insects with the aid of a test tube inside the box. When opening the other end of the box, insects can be released. The FlyBox® was coated with a deltamethrin impregnated polyester PermaNet® bed net of the company Vestergaard-Frandsen (Lausanne/Switzerland) with a concentration of 55 mg/m^2. Indications concerning the concentrations of deltamethrin used as a contact insecticide against stable flies vary. Jandowsky (2009) used a net impregnated with 100 mg deltamethrin/m^2 and Hildebrand one with 280 mg deltamethrin/m^2 against *Musca domestica*. Following the literature (Cruz-Vázquez et al., 2005; Robert and Carnevale, 1991; Salem et al., 2012a), however, it was decided to choose a lower concentration when working with *Stomoxys calcitrans*. The concentration of

55 mg/m^2 was chosen because of its commercial availability. Due to the sensitivity of deltamethrin to UV radiation, the bed net contained an UV protection factor, which had already been added during the production process. The mesh size was 25 per cm^2.

The impregnated polyester fabric was cut down to the size of the FlyBox® and attached to the box with staples.

Since the stable fly abundance was not equally high on each farm, a minimum number of 10 stable flies per test was determined. In most cases (42.5 %) 40 to 90 flies were used per test, on 35 % of the farms 10 to 15 flies were used and on 22.5 % 15 to 40. The stable flies were transferred to the FlyBox® in a test tube. In the complete darkness of the closed FlyBox®, the flies sat down upon the impregnated bed net and thus were exposed to the contact insecticide deltamethrin. After an exposure time of 10 or 30 seconds the flies were released into an observation cage that was equipped with a water-soaked cotton pad for hydration. The paralysis rate of the caged stable flies was determined after 1, 5, 10, 15, 60 minutes as well as after 6 and 24 hours. The *S. calcitrans* strain UBA of the Federal Environment Agency (table 2) was used as a deltamethrin sensitive reference strain. The effectiveness of the net was verified before and after the field tests with it.

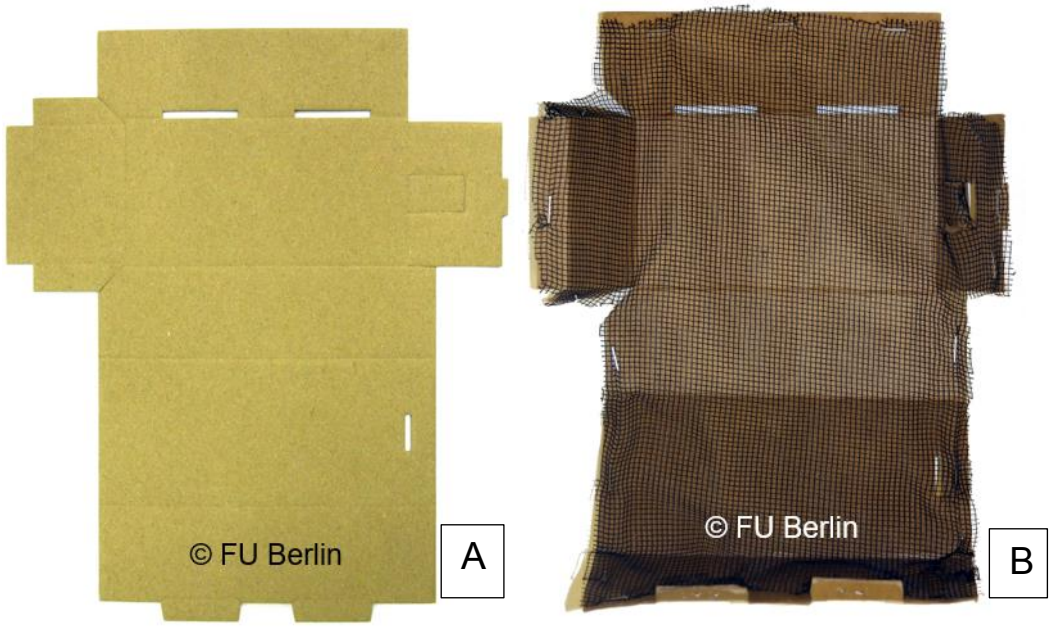


Figure 4 Folded box for FlyBox® test performance according to Jandowsky et al. (2010). A) without deltamethrin impregnated bed net, B) coated with deltamethrin impregnated bed net

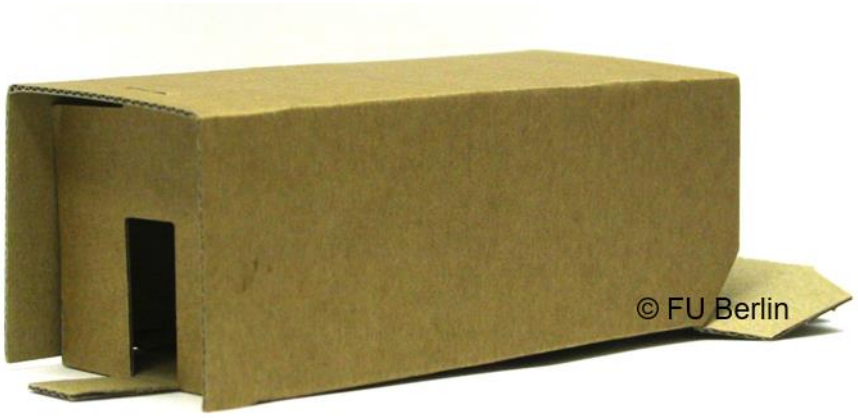


Figure 5 Folded FlyBox®

The observation cages were constructed by the Firm ATH Service GmbH, Dormagen, and were composed of stainless steel. They were made up of a bottom panel (14 cm x 30 cm) and two wire bows (Ø = 28 cm). In order to prevent the flies from escaping and in order to allow observation at the same time, the cages were coated with transparent gauze.

3.4 Laboratory study

3.4.1 Selection of test farms

For further tests, stable flies were caught and colonies established in the laboratory. On that account, in September 2015 *Stomoxys* populations from 10 farms were selected based on the results of the questionnaire analysis and the FlyBox® tests. Seven of the selected dairy farms had claimed to have many flies and to apply insecticides regularly. Furthermore, their stable fly populations had shown resistances to deltamethrin in the FlyBox® test. One farm belonged to the Demeter Wirtschaftverbund that does not allow the application of insecticides, and two of the selected farms had not made use of insecticides in the past three years. However, those populations that had not employed insecticides in the past years displayed resistances to deltamethrin in the Flybox® test that were as high as those of the populations where insecticides had frequently been used.

Figure 6 Spatial distribution of the ten farms from which ten stable fly field strains were isolated, © Google Maps, September 2015

3.4.2 Rearing of stable fly populations

From September 2015 to January 2016, the *Stomoxys calcitrans* populations were reared in the laboratories of the Freie Universität Berlin, Institute for Parasitology and Tropical Veterinary Medicine, Germany. With the same dip net that had been used in the field tests, 500 to 1000 stable flies per farm were caught.

3.4.2.1 Laboratory conditions and adult stable fly feeding

The colonies were maintained at 22 to 25 °C, around 50 % relative humidity under a 12-hour light cycle. Adult stable flies were kept in the same cages that previously had served as observation cages during the field tests.

They were fed daily with citrated bovine blood collected from a slaughterhouse in the vicinity of Berlin. It was gathered weekly in 1 l autoclaved glass bottles containing 102.4 ml sodium citrate (3.13 %) preventing coagulation. Blood was stored for up to a week at 4 °C.

Before feeding, the blood was heated in a water bath (40 °C). It was then poured onto fresh cotton pads which were placed on the mull surrounding the cages. Stable flies fed by piercing the blood-soaked cotton pads through the mull. The volume of blood administered was 60 ml per 1000 stable flies daily, as recommended by MSD Animal Health Innovation GmbH Schwabenheim (Gros, 2015, personal communication).

Moreover, the stable flies also had *ad libitum* access to a 10 % sugar water solution, which was provided to them in plastic bottles in a horizontal position sealed with soaked cotton pads.

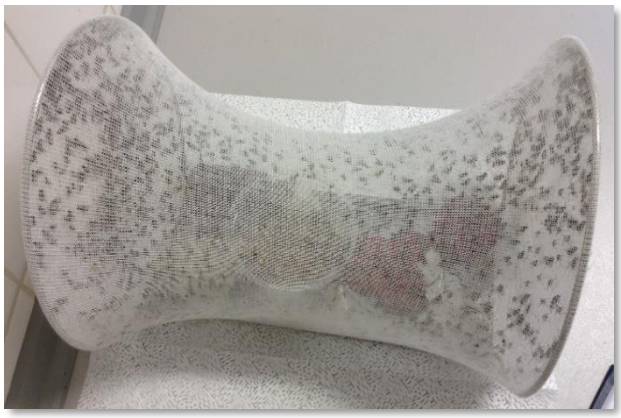


Figure 7 Adult stable fly cage surrounded with mull, provided with water and a 500-ml plastic cup for oviposition, rearing of stable fly populations, September 2015 – January 2016

3.4.2.2 Rearing medium

The rearing medium which was used for breeding the larvae as well as for the larvicide tests was prepared according to the medium used by MSD Animal Health Innovation GmbH Schwabenheim and Farm Hygiene Products Laboratory Novartis Animal Health Incorporated (Gfeller, 2014). It consisted of 1.5 kg of lucerne meal, 1.5 kg of shredded wheat bran, 84 g of dry yeast, and 208 g of sugar. To this mixture 7 l of tap water were added. It was stirred daily and fermented for three days. As a protection against invading flies and in order to prevent undesired oviposition the medium was covered with gauze and towels.

3.4.2.3 Larval rearing

A plastic cup of 500 ml half filled with medium was prepared and placed into the adult fly cage for three days in order to induce oviposition. Eggs could also be collected from the blood soaked cotton pads on which the stable flies fed. Both, larvae and eggs, were transferred into a 2-L plastic container half filled with medium which was stirred daily. Fresh medium was added every second day according to the amount of larvae and the level of decay. Ten to 20 days later the first pupae appeared. Then the whole container was placed in a conically shaped cage which had been constructed based on the hatching cages of MSD Animal Health Innovation GmbH Schwabenheim (Williams, 2015, personal communication). The lower part of the cage was obscured stimulating the recently hatched stable flies to proceed to the luminous upper part of the cage, a demountable Roubaud cage. The upper part was then

removed and the imagoes were transferred into the adult observation cage described in 3.4.2.1. by a test tube. There they were kept until further use. Each cage was marked with the abbreviation of the rural district and the number assigned to the farm the stable fly population came from.

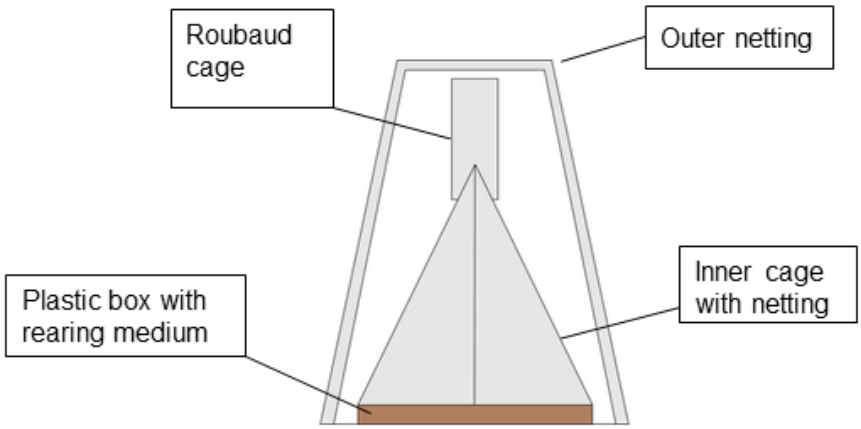


Figure 8 Hatching cage constructed based on the MSD Animal Health hatching cages

3.4.3 Topical Application

The effects of the adulticides deltamethrin and azamethiphos were determined by topical application. Hence a technique which was established by Moyses and Gfeller (2001) and modified by Jandowsky (Jandowsky, 2009; Jandowsky et al., 2010) at the Institute for Parasitology and Tropical Veterinary Medicine was used.

3.4.3.1 Experimental setup

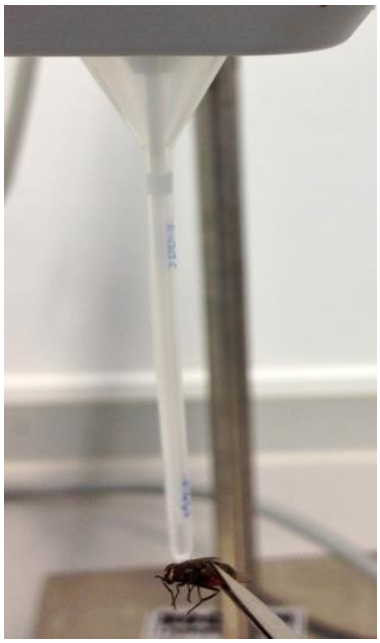
Employing the electronic dispensing system EDOS 5222 (Eppendorf company Germany GmbH, Wesseling – Berzdorf) stable flies were topically treated by applying 1 µl of insecticide diluted in acetone on the dorsal surface of the thorax. Flies treated with solvent only served as control. In comparison to the FlyBox® test the exact, forced contact with the insecticide ensured a sufficient absorption of active ingredient. To enable working with both hands, the doser of the system was attached to a tripod and controlled by a foot pedal.

3.4.3.2 Test execution

When sclerotized, five- to ten-day-old stable flies were removed from the breeding cages by test tubes and immobilized for 5 minutes by being placed on ice. Each experimental and control

group comprised 10, of which five were female and five male. Accordingly, flies were counted and separated by sex on petri dishes which were also cooled down on ice. After that, stable flies were treated topically with 1 µl of insecticide-acetone-solution and were then transferred into a 250-ml-plastic-cup for observation. It contained a 10-%-water-sugar-solution, which the flies could suck out of small test tubes sealed with dental swabs. There the paralysis rates of the flies were determined after 24 and 48 hours.

A



B

Figure 9 A: electronic dispensing system EDOS 5222 (Eppendorf company Germany GmbH, Wesseling – Berzdorf), B: Topical application on stable fly (*Stomoxys calcitrans*) with electronic dispensing system EDOS 5222), October - November 2015

3.4.3.3 Test of acetone and DMSO as carrier solutions

To prove the harmlessness of acetone as a solvent and control, its effects were compared to those of dimethyl sulfoxide (DMSO), a frequently used carrier solution in entomology. Accordingly, 36 field stable flies from one dairy farm were topically treated with 1 µl acetone and 51 stable flies of the same farm were topically treated with 1 µl 0.05 % DMSO acetone solution. They were then kept in groups of no more than ten flies in a 500-ml-plastic cup provided with water which they could pick up from a water filled test tube tapped with a soaked dental plug.

3.4.3.4 Determination of the LD_{95} of azamethiphos and deltamethrin

In order to detect resistance the paralysis rates of the field strains were compared to the LD_{95} of the sensitive laboratory stable fly strain MSD.

The LD_{95} was newly defined through a dose-range-finding test against both drugs with the established laboratory strain of MSD Animal Health Innovation GmbH Schwabenheim, which had been used as a sensitive strain at the company. The LD_{95} values of the technical report of Moyses and Gfeller (1999) were used as a reference. Around those values a serial dilution was prepared in which the concentrations differed by a factor of ten. In order to ascertain the LD_{95} as precisely as possible serial dilutions around the potential LD_{95} concentrations were set up.

Since 4.1 ng/µl of azamethiphos was the LD_{95} determined in the technical report by Moyses and Gfeller (1999), the serial dilution for the evaluation of the LD_{95} of azamethiphos ranged from 0.0041 ng/µl to 41 ng/µl. After finding out that the paralysis rates varied between 0.041 and 41 ng/µl, finer serial dilutions between those values were prepared.

Due to the calculated LD_{95} value of 2.34 ng/µl of deltamethrin in the technical report (Moyses, Gfeller 1999), the serial dilution for the determination of the LD_{95} of deltamethrin was prepared from 0.00234 ng/µl to 234 ng/µl. The paralysis rates scattered and differed heavily between 0.0234 and 2.34 ng/µl. Therefore, between those two concentrations more serial dilutions were made.

The generated data were then evaluated with the statistics software GraphPad Prism® Graph Pad Software Incorporated California. First the data were logarithmically transformed. Then they were analysed by a dose/effect logarithmic regression model. The coefficient of determination R^2 was used for indicating the proportion of the variance in the dependent variable that is predictable from the independent variable. It provided a measure of how well observed outcomes, such as the paralysis rates, are replicated by the logarithmic regression model based on the proportion of total variation of outcomes explained by the model. The coefficient of determination ranges from 0 to 1 in which 1 represents a perfect correlation where the dependent variable, the paralysis rates, can be fully explained by the regression model.

Once the LD_{95} of both, deltamethrin and azamethiphos, were determined, all ten stable fly field strains were topically treated with the LD_{95}, 2,34 ng/µl and 4,92 ng/µl respectively, a four-fold of the LD_{95}, 9,36 ng/µl and 19,7 ng/µl respectively, and 16-times the LD_{95}, 37,5 ng/µl and 78,6 ng/µl respectively. Each concentration was tested three times with ten stable flies at a time.

3.4.4 Larvicide tests

The sensitivity of the stable fly test populations to the insect growth regulators, the triazine derivative cyromazine and the pyridine-based pyriproxyfen, was tested by larvicide tests. The serial dilution of the test concentrations was based on the dose recommended by the manufacturer. For cyromazine the recommended dose was 5 µg/g rearing medium and for

pyriproxyfen 0.027 µg/g rearing medium. Serial dilutions were prepared according to similar studies on *Musca domestica* and *Stomoxys calcitrans* (Jandowsky, 2009; Jandowsky et al., 2010; Leon;, 2012). Four cyromazine concentrations which differed by a factor of four (1.25 µg/g, 5 µg/g, 20 µg/g, 80 µg/g) and five pyriproxyfen concentrations which differed by factors of two, five and ten (0.005 µg/g, 0.01 µg/g, 0.05 µg/g, 0.1 µg/g, 1 µg/g) were prepared.

To verify the effectiveness of both larvicides, *Stomoxys calcitrans* eggs were transferred to a plastic cup filled with 75 g rearing medium spiked with insect growth regulator. On that account each evening or morning plastic cups filled with medium were transferred to the adult flies for three to twelve hours in order to induce oviposition. In addition, eggs were also collected from the blood soaked cotton pads on which the stable flies fed. With forceps the freshly laid eggs were then carefully transferred to a black filter paper in a petri dish where they were counted with the aid of a binocular loupe. For each concentration 30 to 50 *Stomoxys* eggs were put onto a 1.5 x 1.5 cm cut filter paper and subsequently placed into one of the plastic cups containing the treated larval medium. The larval medium which was used in the tests was prepared according to the same protocol which had been used for the rearing medium (3.4.2.2.). It was also stirred daily and fermented for three days. Following that, it was treated with the respective larvicide solution at a ratio of 1:10. Each concentration was tested three times. The eggs of the control group were transferred onto an untreated larval medium that was only mixed with water. The hatching rates of the field strains were then compared to the MSD strain.

Before and alongside own larvicide tests of the field strains the effectivity of the larvicides had been tested for the sensitive lab strain of MSD Animal Health Innovation GmbH Schwabenheim. Two days later the hatching rate of the stable flies was determined under the binocular loupe. Then the larval medium was covered with sawdust to keep it from drying out. As an indicator of resistance after three to five weeks the number of developed imagoes was counted and the efficacy of the larvicides was evaluated.

Figure 10 Possible results of larvicide tests, A: 100 % larval development inhibition rate (no flies developed), B: high larval development inhibition rate (only few flies developed), C: low larval development inhibition rate (a high number of developed flies occurs)

3.4.4.1 Cyromazine

The preparation of the serial dilution of cyromazine used in the larvicidal test was as follows:

- The commercial product Neporex 2SG® of the company Novartis Animal Health Inc. was taken.
- The aim was to obtain a serial dilution of 1.25, 5, 20 and 80 µg of cyromazine per g of larval medium around the dosage of 5 µg/g recommended by the manufacturer.
- In order to achieve such a serial dilution in the larval medium and since the Neporex 2SG® product had to be dissolved in water, first a serial dilution of 12.5 µg, 50 µg, 200 µg and 800 µg per ml of water was prepared.
- The values for the serial dilution in water were ten times higher than the ones needed in the larval medium.
- 1 g Neporex 2 SG® contains 20 mg Cyromazine.
- In order to produce the serial dilution in water, first a concentration of 800 µg of cyromazine per ml of water was produced.
- 800 µg of Cyromazine correspond to 0.8 mg of Cyromazine.
- Since 20 mg Cyromazine are in 1 g of Neporex 2SG®, in order to obtain 0.8 mg Cyromazine per ml of water, 0.04 g of Neporex 2SG® had to be dissolved in 1 ml water.
- In order to measure a larger amount for more accuracy, 8 g of Neporex 2SG® were dissolved in 200 ml of water.
- To sum it up, 8 g of Neporex 2SG® per ml of water correspond to 0.04 g Neporex 2SG® per ml of water corresponding to 0.8 mg Cyromazine or 800 µg Cyromazine per ml of water.
- The stock solution of 800 µg Cyromazine per ml of water was then diluted with water at a ratio of 1:4 respectively according to the concentration steps.

- Thus, the needed serial dilution of 12.5 µg/ml, 50 µg/ml, 200 µg/ml and 800 µg/ml was prepared.
- To obtain the final concentrations of 1.25, 5, 20 and 80 µg/g, at a ratio of 1:10 30 ml of each concentration step was mixed with 270 g of larval medium.
- For each concentration, three 500-ml-plastic cups were stocked with 75 g of larval medium.
- Each concentration was tested three times.

3.4.4.2 Pyriproxyfen

The preparation of the serial dilution used in the larvicidal test of pyriproxyfen was as follows:

- The sensitivity of pyriproxyfen was tested with the commercial product Archer® (1.3 % pyriproxyfen) of the company Syngenta Crop Protection AG.
- According to the manufacturer 1 fluid ounce Archer® /gallons of water or 1 fluid ounce Archer®/1500 square feet should be employed, to sufficiently inhibit the development of the stable flies.
- One fluid ounce /gallon equals 28 ml Archer® / 3.8 L of water.
- Due to the fact that Archer® contains 1.3 % of pyriproxyfen (1.3 g pyriproxyfen/ 100 ml water) 28 ml of Archer® contain 0.364 g of pyriproxyfen.
- When those 28 ml of Archer® are mixed with 3.8 L of water, the solution consists of 0.01 % pyriproxyfen.
- When applying Archer® directly to the manure 1 fluid ounce /1500 square feet should be applied according to the manufacturer.
- For the larvicidal tests this recommendation was followed.
- One fluid ounce / 1500 square feet correspond to 28 ml of Archer® per 135 m^2 or 0.207 ml of Archer® per m^2.
- As Archer® contains 1.3 % of pyriproxyfen (1.3 g pyriproxyfen/ 100 ml of water) 0.207 ml Archer® contain 2.69 mg pyriproxyfen/ m^2.
- The manufacturer thus recommends the use of 2.69 mg of pyriproxyfen per m^2.
- Since the product was going to be added to the larval medium, this value had to be converted into volume values.
- Considering that the amount of larval medium employed in the tests was not higher than 10 cm, the active ingredient did not have to penetrate the larval medium for more than that.

- Thus, for converting the values calculated above into volume amounts, a cylinder with the surface area of 78.85 cm^2, a height of 10 cm and, therefore, a capacity of 788.5 cm^3 (788.5 ml) was calculated.
- The amount corresponded to the cylinder that was formed by the larval medium in the plastic cups used in the tests.
- Subsequently the measurement of 2.69 mg pyriproxyfen/m^2 calculated above could be converted into mg/ml.
- Thus, 0.021 mg of pyriproxyfen should to be applied to a hypothetical cylinder with a surface area of 78.85 cm^2 and a capacity of 788.5 ml of water, corresponding to 0.027 µg pyriproxyfen per 1 ml rearing medium.
- The calculation is almost in line with the 0.028 µg/ml used in a technical report by Novartis Animal Health Inc. (Gfeller and Breuer, 2012) which was based on the label concentration of Pyri-Shield®.
- The serial dilution of 0.005 µg/ml, 0.01 µg/ml, 0.05 µg/ml, 0.1 µg/ml and 1 µg/ml around the manufacturer's recommended dosage of 0.027 µg/g which had to be prepared, was based on several previous studies (Liu et al., 2012).
- Then, 999.23 ml of water were mixed with 770 µl of Archer® corresponding to 0.77 ml Archer®/ 1000 ml solution. One ml stock solution contained 10 µg pyriproxyfen.
- The stock solution was then diluted accordingly to get the serial dilution of 10 µg/ml, 1 µg/ml, 0.5 µg/ml, 0.1 µg/ml, and 0.05 µg/ml.
- Finally, the aspired concentrations of 1 µg/ml, 0.1 µg/ml, 0.05 µg/ml, 0.01 µg/ml and 0.005 µg/ml were obtained by mixing 30 ml of each concentration with 270 g of larval medium at a ratio of 1:10.
- Each concentration was tested three times.

3.5 Data analysis

3.5.1 Evaluation of resistance

In addition to the control groups of each stable fly field strain, each test was performed with a sensitive laboratory strain. The FlyBox® test was performed with the strain of the Federal Environmental Agency (UBA), the topical application of deltamethrin and azamethiphos as well as the larvicide tests of cyromazine and pyriproxyfen were performed with both laboratory strains, the one of MSD Animal (MSD) and the one of the Federal Environment Agency Berlin (UBA). Thus, the mortality rates of the field strains could be statistically compared to the ones of the sensitive strains.

The paralysis rates of the field strains were corrected by Abbott's formula (Abbott, 1925) considering the natural mortality rates of the control groups, respectively:

$$\text{Mortality} = \frac{(\text{Mortality (\%) of test group} - \text{Mortality (\%) of control group}) \text{ x}100}{100 - \text{Mortality (\%) of control group}}$$

The results were evaluated and categorized according to the definition of resistance established for vectors of Malaria by the WHO (2016).

Table 3 Resistance definition according to (WHO, 2016) at the LD_{95} on Malaria vectors transferred to stable flies

Mortality of the tested flies after 24 hours	Degree of susceptibility
98 – 100 %	Susceptible
90 – 97 %	probably resistant, further testing required
< 90 %	Resistant

3.5.2 Statistical evaluation

The data was statistically analysed with the software SPSS version 22, IBM. Results with a p-value below 0.05 were classified as statistically significant.
In order to verify if two categories are stochastically independent, the chi-squared test was used. It is a statistical hypothesis test wherein a statistically significant result means that the variables are not combined randomly but there is a dependency between them. In that case, the null hypothesis, which states that there are no dependencies, has to be rejected.

In order to evaluate the categorical results of the questionnaire analysis, the chi-squared test was applied first. When more than 25 % of the cells had expected counts less than five, Fisher's Exact Test was employed. In case of a statistically significant result, with a p-value below 0.05, further testing was performed. As such, it was calculated if it is more probable to expect a fly problem when performing a certain dung management or a certain control measurement.

When testing the independence of two categories such as resistance development and the employment or non-employment of insecticides the chi-squared or Fisher's Exact test was used as well. In this manner the connection between resistance development and the use of pyrethroids, the frequency of application or rotation of insecticides could be examined.
Since normal distributions could not be assumed, when comparing the paralysis rates of the field strains to that of a sensitive laboratory strain, the Mann-Whitney-U-test was employed. It

is a non-parametric test, in which two independent samples are compared. When calculating if there were significant differences between the tested active ingredients deltamethrin and azamethiphos or between the topical application and the FlyBox® test, the Mann-Whitney-U-test was also used.

In order to evaluate the possibility of agreement of two tests or observations, for example the FlyBox® test and the topical application, Cohen's kappa was employed. Cohen's kappa is used as a coefficient to measure an inter-rater agreement for categorical items. Cohen's kappa can be interpreted as follows: values ≤ 0 as indicating no agreement and 0.01–0.20 as none to slight, 0.21–0.40 as fair, 0.41– 0.60 as moderate, 0.61–0.80 as substantial, and 0.81–1.00 as almost perfect agreement.

For the larvicide testing a statistical evaluation was not possible since cyromazine as well as pyriproxyfen caused an inhibition rate of 100 % in all tested field strains.

3.6 Materials used

The following materials were used:

3.6.1 Insecticides

Pure substances:

Deltamethrin PESTANAL®	product no.: 45423, batch no.: SZBF090XV	Sigma Aldrich Laborchemikalien GmbH, Seelze
Azamethiphos PESTANAL®	product no.: 45331, batch no.: SZBD338XV	Sigma Aldrich Laborchemikalien GmbH, Seelze

Commercial products:

PermaNet® bed fabrics with deltamethrin concentration of 55 mg/m^2	batch no.: 52918-63-5	Vestergaard-Frandsen, Lausanne Switzerland
Neporex® 2SG Cyromazine 20 mg/g	product no.: 603296, batch no.: 178948	Sigma Aldrich Laborchemikalien, Seelze
Archer® Pyriproxifen	product no.: 1008269, batch no.: MGK13G05001	Syngenta® Crop Protection AG, Basel

3.6.2 Disposable items

FlyBox® Faltschachtel, FlyBox® foldable box	FAPACK, Berlin
Rotilabo®-Reagenzröhrchen, test tubes Ø 16x160 mm	Carl Roth GmbH & Co. KG, Karlsruhe
LABOCAP-Kappen ohne Griff silber, test tub caps without handle silver	Carl Roth GmbH & Co. KG, Karlsruhe
Rotilabo®-Reagenzgläser, test tubes, Ø11x70mm	Carl Roth GmbH & Co. KG, Karlsruhe
Rotilabo®-Fertigstopfen aus Zellwatte, cellucotton plugs,12/8mm	Carl Roth GmbH & Co. KG, Karlsruhe
Rotilabo® Einmal-Wägeschalen Blau, weighing dish blue, 20ml	Carl Roth GmbH & Co. KG, Karlsruhe
Verpackungsbecher rund, plastic cups round, 500ml	Plastikbecher.de GmbH, Giengen
PP-Deckel natur, pp-plastic cup lids nature, Ø 101,0mm	Plastikbecher.de GmbH, Giengen

20ml-Einmalspritzen, disposable syringes 20 ml	Henry Schein Inc., Melville, USA
Hypodermic Needles	Henry Schein Inc., Melville, USA
tg® Schlauchverband Gr. 5, tube bandage size 5	Lohmann&Rauscher International GmbH & Co. KG, Neuwied
Cotton wool	Handelsmarken GmbH, Offenburg
Celluron Zahnwatterollen, Absorbent tooth cotton	Paul Hartmann AG, Heidenheim
Gloves	Rotiprotect®- Latex
Combitips advanced 0.1ml	Eppendorf AG, Hamburg
Eppendorf tubes	Eppendorf AG, Hamburg
Petri dishes, Ø 90mm	Carl Roth GmbH & Co. KG, Karlsruhe
Zucker, Sugar	EDEKA Zentrale GmbH & Co. KG
Weizenkleie, wheat bran	J. Ruckdeschel & Söhne, Berlin
Hefe Pulver, yeast powder	EDEKA Zentrale GmbH & Co. KG
Lucerne pellets, Luzerne-Pellets 10 kg	AniForte Equi Flora
Filter Papier rund, filter paper round	Macherey-Nagel GmbH & Co. KG, Berlin
Sägespäne, saw dust, Goldspan	J. Ruckdeschel & Söhne, Berlin

3.6.3 Reusable items

V2A-Käfige, V2A cages, 10x5x15cm	Metallbau Thorsten Eigner, Emmelsbüll-Horsbüll
Edelstahlkäfige, stainless steel cages	ATH GmbH Service, Dormagen
Pinzetten, forceps	Fiebig Lehrmittel, Berlin
Kunststoffeimer rund mit Deckel, plastic buckets round with cover, 200 x 310 mm Vol.15 l	Auer GmbH, Amerang
Kunststoffeimer rund mit Deckel, plastic buckets round with cover, 200 x 139 mm Vol. 3 l	Auer GmbH, Amerang
Wasserzerstäuber, spray bottle	Toom Baumarkt, Berlin
Insektenfangnetz, insect net	Fiebig Lehrmittel, Berlin
Zentrifugenröhrchen 45ml, centrifuge tubes 45 ml	VWR International GmbH, Darmstadt
Edding 3000	Edding Vertrieb GmbH, Wunstorf
Combitips plus Standard 0,1 ml	Eppendorf Vertrieb Deutschland GmbH, Wesseling Berzdorf

3.6.4 Laboratory equipment

EDOS 5222 elektr. Dosiersystem, electronic dosage system	Eppendorf Vertrieb Deutschland GmbH, Wesseling-Berzdorf
Laborwaage, laboratory scale, Sartorius portable PT120	Sartorius AG, Göttingen

Halbmikroanalysenwaage „Discovery“ DV215CDM, Kapazität 81 g/210 g, Ablesbarkeit 0,01 mg/0,1 mg	Ohaus, Nänikon, CH
Stereo-Mikroskop STEMI DV4	Carl Zeiss AG, Oberkochen

3.6.5 Computer programs

Microsoft Office Word 2015	Windows
Microsoft Excel 2015	Windows
SPSS Statistics 24	IBM
Endnote X8	Thomson Reuters
LaTeX 2_ε	Leslie Lamport
batchgeo	BatchGeo LLC

4 Results

4.1 Questionnaire analysis

Out of the 78 dairy farms contacted, 52 agreed to partake in the questionnaire.

4.1.1 Stable fly occurrence

Sixty per cent of the dairy farms had classified their fly occurrence in general as moderate and 13 % as high. Twenty-seven per cent had claimed their fly occurrence as low. However, 37 out of 52 farmers (71.2 %) could not distinguish between house (*Musca domestica*) and stable fly (*Stomoxys calcitrans*). Of the 15 (28.8 %) farm owners who claimed to be able to tell the difference between the two fly species, only 3 (5.8 %) considered their stable fly occurrence as moderate and 3 (5.8 %) as high. This could be confirmed for all six of them by own assessment when the farms were visited. The remaining nine dairy farmers (17 %) claimed to have a low stable fly occurrence.
When visiting those farms in order to perform the FlyBox® test this appraisal resulted to be wrong since most of the flies seen were stable flies.
Furthermore, stable flies could be found on all 40 farms included in the on-farm Flybox® test. Before starting with the Flybox® test stable flies were collected for 60 or 90 minutes with a scoop net and transferred into a Roubaud cage. For each test an equal number of stable flies was then used. At least ten *Stomoxys* had to be included into each test. If there were only ten to 15 flies available for each of the tests, the stable fly occurrence on those dairy farms was classified as low. The indicator for a moderate stable fly occurrence was the use of only 15 to 40 stable flies in all three tests. More than 40 stable flies in each test meant a high stable fly abundance. Thus, from the 40 dairy farms visited 14 dairy farms (35 %) could be classified as farms with a low, 16 (40 %) as farms with a moderate and 10 (25 %) as farms with a high stable fly affluence. This classification did not correspond with the appraisal of the farmers, which generally estimated their fly abundance to be lower. Twelve farms were not visited and could, therefore, not be assessed (3.3.1.).

4.1.2 Insecticides in use

Most dairy farmers interviewed employed different control measures at the same time. Out of all the 52 dairy farms included in the questionnaire analysis, 78.9 % used insecticides against

flies, 34.6 % applied physical control methods such as adhesive tapes or calcium hydroxide. Since ventilators helped to cool down the temperature in all farms visited, they were not counted as a type of physical control measure although the wind produced by them also helped to keep the flies away. However, 11.5 % of the farmers claimed to have not used any sort of control measure in the past 10 years. None of the interviewees made use of one of the classic biological control measures such as the black dump fly (*Hydrotaea aenescens) or parasitic wasps (Spalangia spp.* or *Muscidifurax raptorellus*). Swallows (Hirundinidae) were accidentally present on all dairy farms and thus, in a certain way served as biological control measures. Nevertheless, they were not counted as such in the questionnaire analysis.

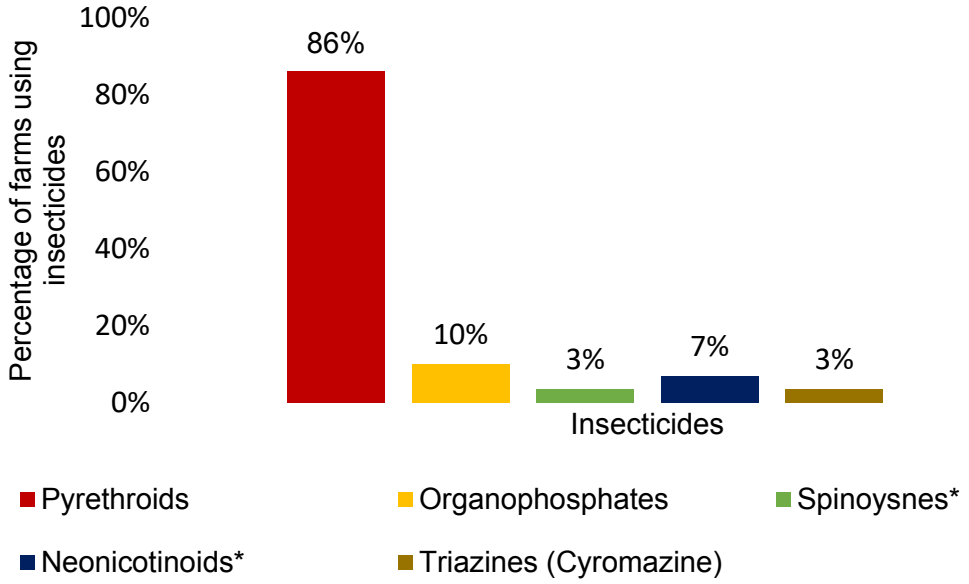
The collection of data turned out to be quite problematic since most of the employees did not have an overview over the applied insecticides, nor did they know the exact time-span of use. Only 30 out of 52 (57.7 %) dairy farmers interviewed were able to name the insecticides that had been used three years ago, 29 out of 52 (55.8 %) were able to name the products currently in use.

Table 4 Active ingredients currently in use on 29 of the 52 dairy farms interviewed, some farms employed more than one insecticide at the same time, Brandenburg, 2015

Insecticide class	Active ingredient	N/29	%
Pyrethroids	Deltamethrin	13	44.8
	Permethrin	6	20.7
	Cypermethrin	7	24.1
	Cyfluthrin	6	20.7
Triazines	Cyromazine	1	3.4
Neonicotinoids	Imidacloprid	1	3.4
Organophosphates	Azamethiphos	3	10.3
Spinosynes	Spinosad	1	3.4

The predominant insecticides in use were pyrethroids. Twenty-five of the 29 dairy farms (86 %) that actually knew what kind of products they had employed, claimed to have applied an active ingredient out of this insecticide class (Graphic 1). According to the questionnaire analysis the application of the larvicide cyromazine was limited to one out of 29 farms (3 %). Organophosphates were used by three of the 29 farms (10 %). Spinosynes and neonicotinoids, both feed-through insecticides, were utilized by one (3 %) and two (7 %) of the farms,

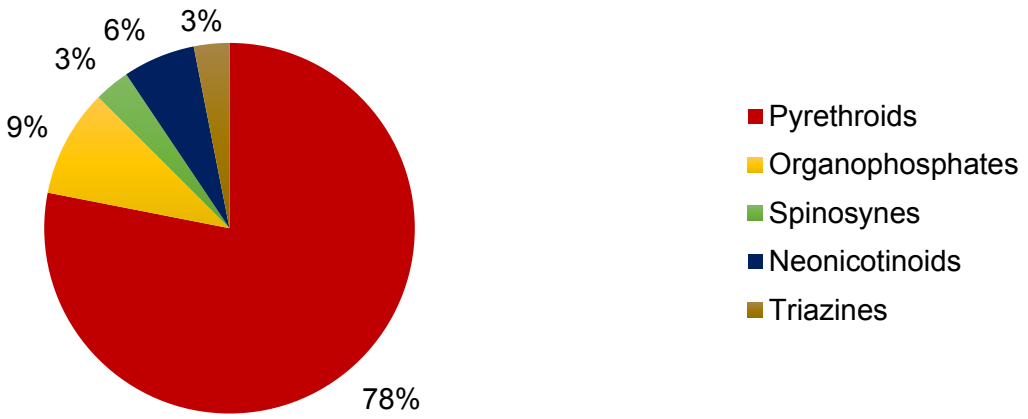
respectively, although feed-through insecticides are not effective against *Stomoxys calcitrans* since both male and female feed blood.



Graphic 1 : Classes of insecticides in use (in %) on the 29 out of the 52 dairy farms interviewed that could name their insecticides, some of them applied more than one insecticide at the same time, Brandenburg, June – August 2015; *feed-through insecticides = not effective against *Stomoxys calcitrans*

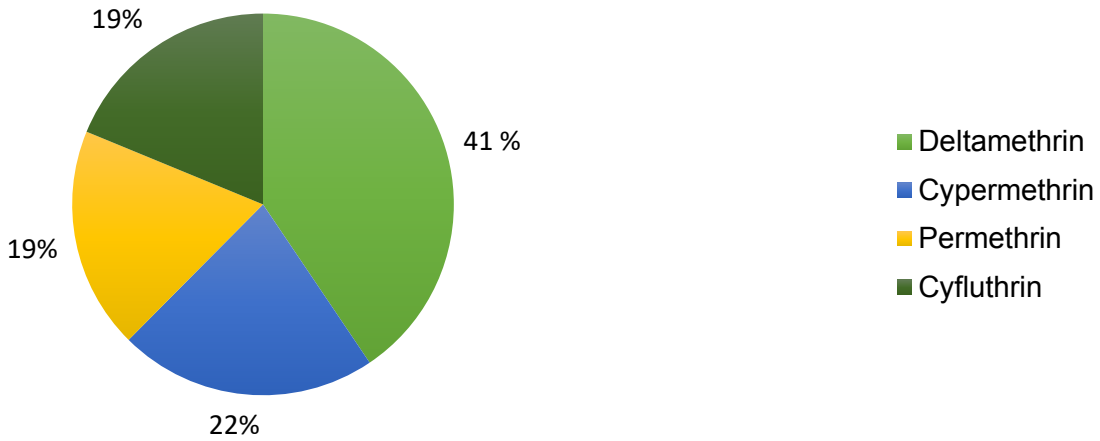
Thirty-one per cent of the 29 farmers claimed to have used two or more chemical products against flies at the same time. Thirty-eight per cent had switched the product in the past 3 years. In 30 % of those cases, they had switched the product within the same class of insecticides.

Since many farmers applied more than one active ingredient at the same time, comparing the insecticide classes in use with the total number of insecticides applied by the farmers and not with the total number of farmers, revealed slightly different percentages (Graphic 2).



Graphic 2 Classes of insecticides in use (in %) on 29 out of the 52 dairy farms interviewed that could name their insecticides compared to the total number of insecticides applied by the farmers, Brandenburg, June – August 2015

Within the insecticide class of the pyrethroids, deltamethrin was the most frequently used pyrethroid at a percentage of 41 %.

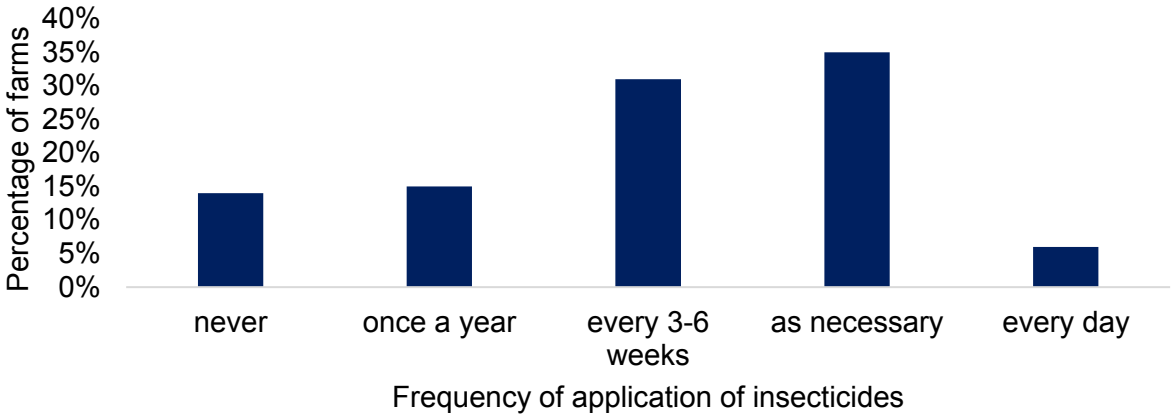


Graphic 3 Pyrethroids in use (in %) on 29 out of the 52 dairy farms interviewed that could name their insecticides, compared to the total number of pyrethroids applied by the farmers, Brandenburg, June – August 2015

Only three out of the 52 farms interviewed (5.8 %) had employed a combination of an adulticide and a larvicide. The usage of insecticides is limited to the months of June to October. Forty-two per cent of the farmers estimated the greatest fly abundance to be during July, whereas 48 % claimed to observe the largest fly occurrence during August. Ten % stated that September is the month with the worst nuisance caused by flies.

Thirty-one per cent of the dairy farmers claimed to make use of insecticides three to five times a summer, 34 % applied insecticides whenever needed and 6 % of the farmers used

insecticides daily (Graphic 4). However, 14 % stated to never employ insecticides and 15 % only applied them once a summer.



Graphic 4 Frequency of application of insecticides on 52 dairy farms interviewed, Brandenburg, June – August 2015

Eleven farmers (21 %) were unsatisfied with their type of control measure, 10 of them had already noticed a loss of efficacy of their chemical control measure. Twenty-five (48 %) of the farmers were rather satisfied and 16 (31 %) were very satisfied with their current type of control measure.

4.1.3 Size of herds

At the time of the survey the 52 dairy farms included in the questionnaire analysis housed between 50 and 1700 dairy cattle cows and had an average size of herd of 425 milking cows (SD: +/- 358,18). Three of the dairy farms (6 %) included in the questionnaire analysis consisted of 50 dairy cows, 11 (21 %) had between 50 and 200 dairy cows, 24 (46 %) kept between 200 to 500 dairy cows, 12 (23 %) owned 500 to 1000 and two farms held over a 1000 dairy cows.

4.1.4 Methods of rearing

Forty-nine of the 52 dairy farms interviewed were conventional farms, the remaining three were organic farms. Two of the three organic farms were part of the Demeter® organisation and had never applied any insecticides.

Seventy-nine per cent of the milk cows on the dairy farms were kept in free stall housing systems with open cubicles. Nineteen per cent in deep litter stables and one of the 52 dairy farms still practised the tethered housing system.

4.2 Field study

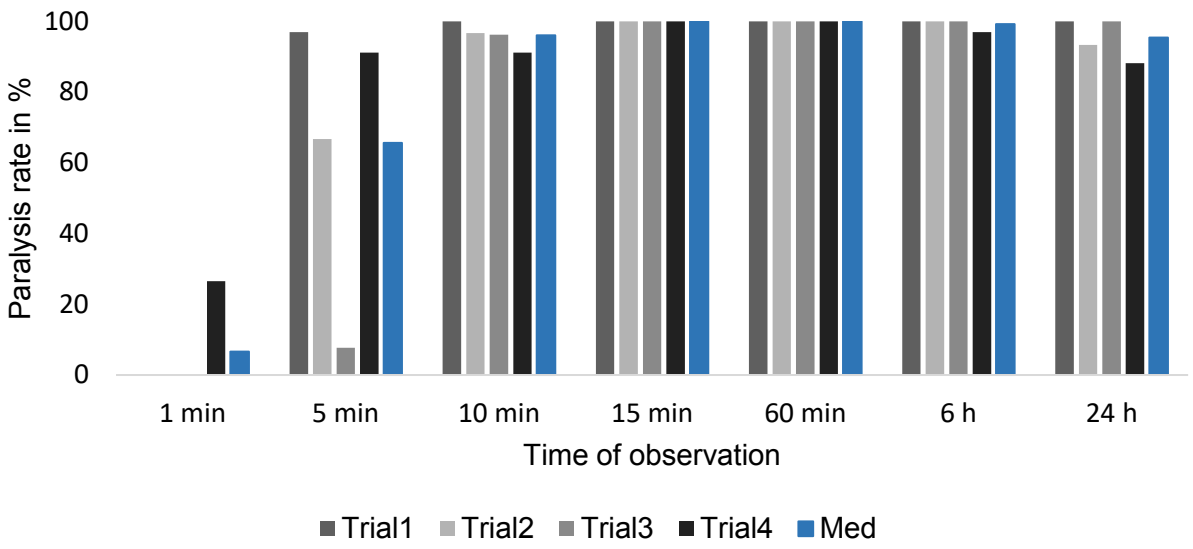
4.2.1 FlyBox® test

4.2.1.1. Results of *Stomoxys calcitrans* reference strain

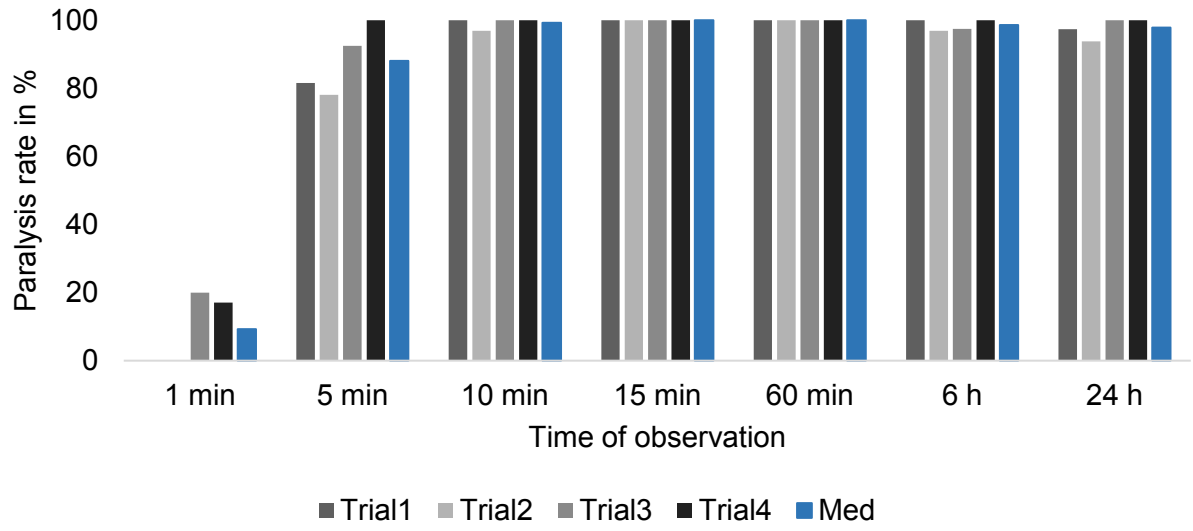
Before testing the 40 field strains, first the laboratory strain UBA of the Federal Environmental Agency in Berlin was tested with the FlyBox® test. For each of the two times of exposure, ten and 30 seconds, four tests were performed. In order to verify the effectiveness of the impregnated bed net, which was attached to the FlyBox®, the test was repeated after 2 months. No loss of activity could be detected. The stable flies showed first signs of paralysis already after a few minutes of observation.

Following a time of exposure of ten seconds and five minutes of observation the medium paralysis rate of the UBA strain was 66 ± 40.8 % and after 10 minutes it was 96 ± 3.6 % (Graphic 5). A paralysis rate of 100 ± 0 % was reached after 15 minutes. Due to the reversibility of the knock-down effect of deltamethrin in which sodium channels of the nervous system get blocked, as time passed some stable flies were not paralysed anymore. After six hours the medium paralysis rate was 99 ± 1.5 % and after 24 hours it had decreased to 95 ± 5.7 %. Very similar results were observed when exposing stable flies to the FlyBox® for 30 seconds (Graphic 6). After a time of observation of 5 minutes 88 ± 10.1 % of the stable flies were paralysed. After 10 minutes 99 ± 1.6 % and, again, after 15 minutes 100 ± 0 % were paralysed. After 24 hours still 98 ± 2.9 % of the flies had not recovered.

According to WHO (2013), flies are considered dead when they are paralysed after six hours of observation. Thus, the impregnated deltamethrin net and the time of exposure of 10 and 30 seconds could be judged as sufficient.



Graphic 5 Results of 4 FlyBox® tests with a deltamethrin (55 mg/m2) bed net after 10 seconds of exposure according to Abbott (1925), grey ("Trial") = paralysis rates of the UBA strains, blue ("Med") = mean paralysis rates of the *Stomoxys calcitrans* susceptible reference strain UBA



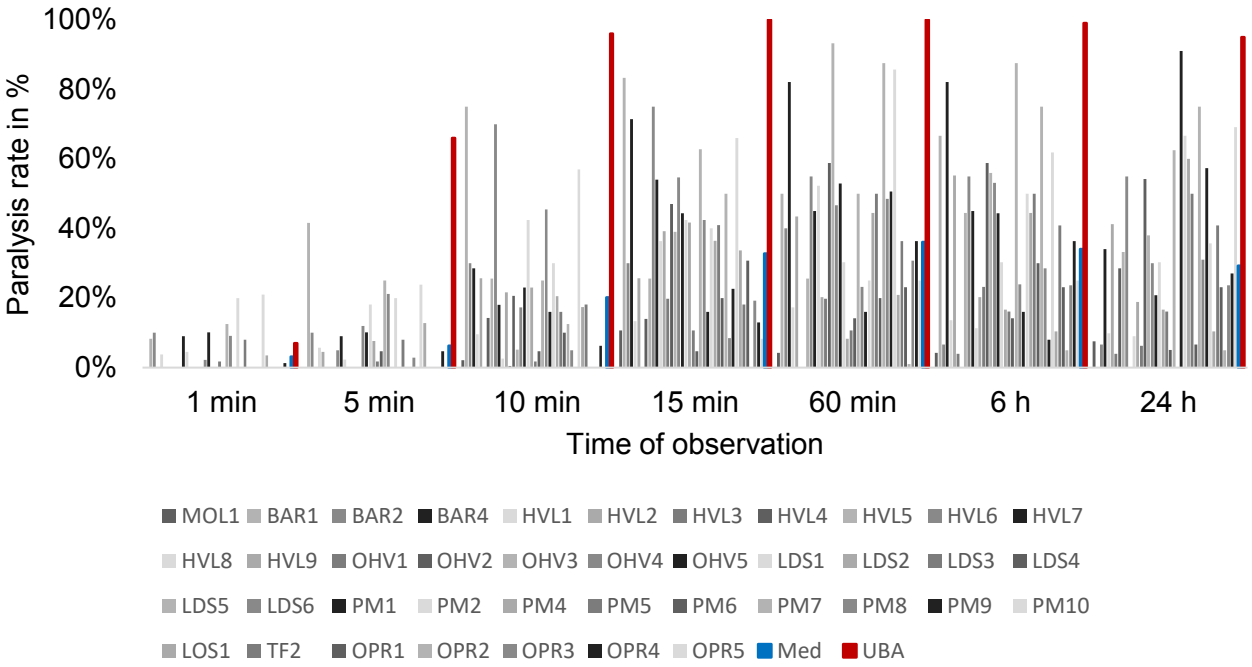
Graphic 6 Results of 4 FlyBox® tests with a deltamethrin (55 mg/m2) bed net after 30 seconds of exposure according to Abbott (1925), grey ("Trial") = paralysis rates of the UBA strains, blue ("Med") = mean paralysis rates of the *Stomoxys calcitrans* deltamethrin susceptible reference strain UBA

4.2.1.2. Results of field strains

The results differ strikingly from those for the sensitive Federal Environmental Agency strain (UBA). After ten seconds of exposure and 15 minutes of observation, the field stable fly strains displayed a paralysis rate of 32.8 ± 21.1 % on average. Moreover, after one hour the paralysis

rate was at 39.4 ± 24 %. After six hours, it was at 37.2 ± 23 % and after 24 hours, it was at 36.7 ± 23.7 %. None of the tested field strains was susceptible to the deltamethrin impregnated bed net (Graphic 7).

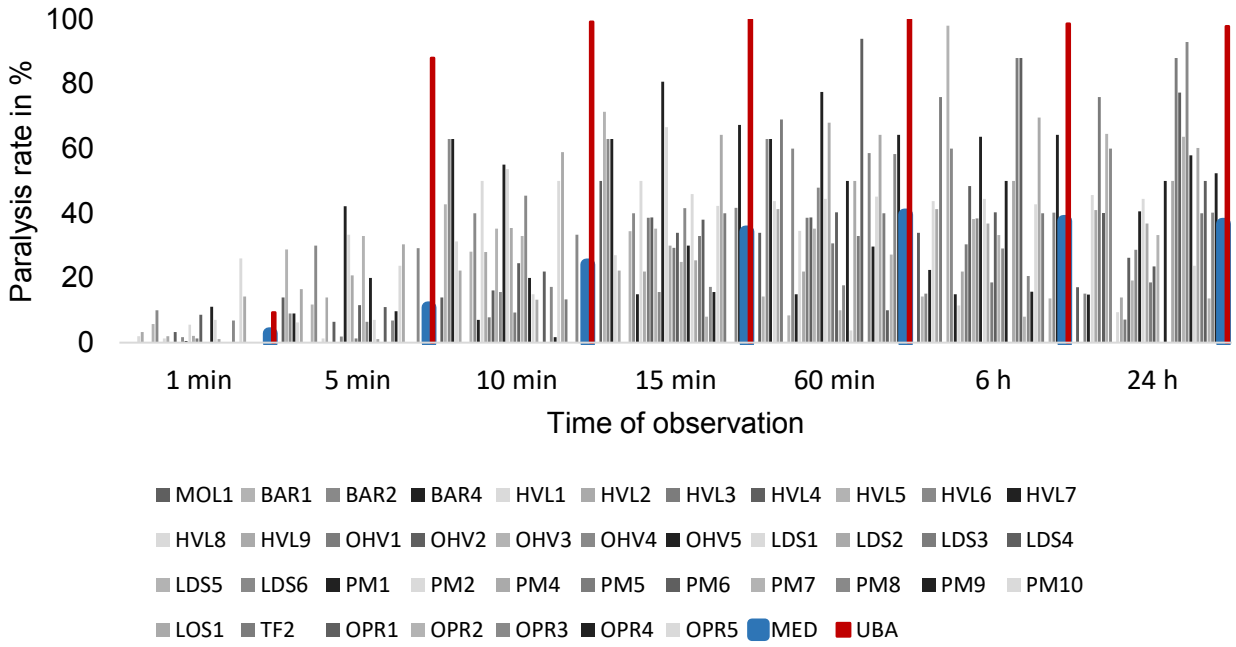
Thus, according to WHO (2016), after six hours of observation 100 % of the strains could be classified as resistant at a paralysis rate below 90 %. Forty-nine per cent even showed a mortality rate below 40 %.



Graphic 7 Results of the FlyBox® tests with a deltamethrin (55 mg/m^2) bed net after 10 seconds of exposure according to Abbott (1925), grey = paralysis rates of the field strains, blue ("Med") = mean paralysis rates of the 40 field strains, red ("UBA") = mean paralysis rates of the sensitive UBA strain

Very similar results could be observed after a time of exposure of 30 seconds (Graphic 8). After an observation time of 15 minutes, a mean paralysis rate of 34.4 ± 20.8 % was calculated. After one hour, it was at 39.6 ± 22.4 %, after six hours at 37.6 ± 24.5 % and after 24 hours at 36.7 ± 24.6 %. After 6 hours, none of the tested field strains was highly susceptible to the deltamethrin impregnated bed net. According to (WHO, 2016), only 2.5 % (one out of 40) were rated as probably resistant at a paralysis rate between 90 and 98 % and 97.5 % were classified as resistant since their paralysis rate was below 90 %. Fifty-five percent even showed a mortality below 40 %.

Remarkably, on the seven visited farms that had claimed to have used no insecticides in the past ten years, the stable fly strains were found to be 'resistant' according to both of the Flybox® tests.



Graphic 8 Results of the FlyBox® test with a deltamethrin (55 mg/m2) bed net after 30 seconds of exposure according to Abbott (1925), grey = paralysis rates of the 40 field strains, blue ("MED") = mean paralysis rates of the field strains, red ("UBA") = mean paralysis rates of the sensitive UBA strain

4.3 Laboratory study

For confirmatory evaluation and further testing, ten stable fly field strains were caught and colonies established in the laboratory (see 3.4.1. selection of test farms).

4.3.1 Topical Application

4.3.1.1 Test of acetone and DMSO as carrier solutions

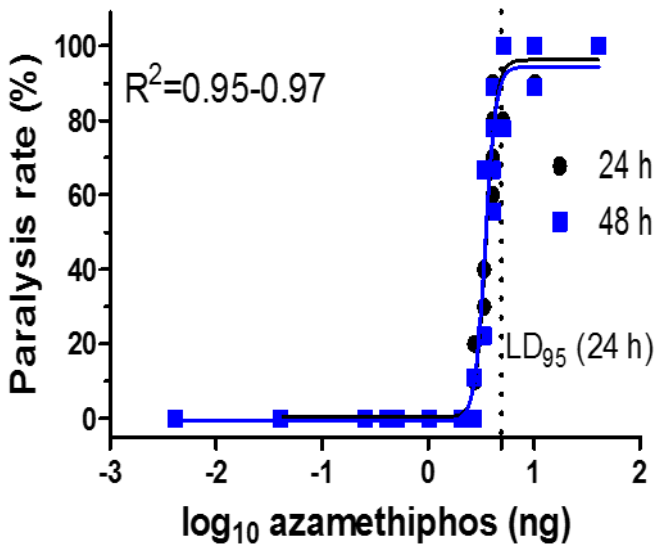
Thirty-six field stable flies were topically treated with 1 µl acetone and 51 stable flies of the same dairy farm were topically treated with 1 µl 0.05 % DMSO acetone solution in order to evaluate which were more suited as a carrier as well as a control solution.

There were no significant differences in the paralysis rates observed (Mann-Whitney-U-Test, $p = 0.571$). Since the time of reaction of stable flies treated with DMSO 0.05 % was longer

when moving the observation cup, they seemed to be less vital. Thus and considering that acetone is used as a standard solvent in the topical application test method, the use of DMSO was rejected in this study.

4.3.1.2 Determination of the LD_{95} of azamethiphos

The dose-effect logarithmic regression model revealed that the lethal dose at which 95 % of the sensitive stable flies (MSD strain) die after an observation time of 24 hours was 4.92 ng/µl and after 48 hours 4.86 ng/µl (Graphic 9). The paralysis rates did not differ much and the recovery rate of the flies was negligible. Hence, R^2 for the times of observation after 24 and 48 hours was at 0.97 and 0.95, respectively. The closer the R^2 value is to 1, the better the calculated graph can be explained by the increasing concentrations of the active ingredient. The R^2 values can, therefore, be rated as good. Thus, the doses for the topical application of azamethiphos were based on 4.92 ng/µl. This value is close to the LD_{95} value of 4.1 ng/µl determined by Moyses and Gfeller (1999).

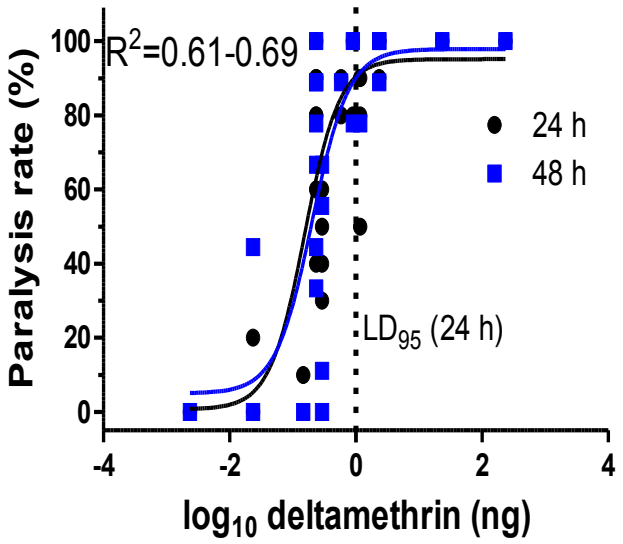


Graphic 9 Determination of the LD_{95} after topical application of azamethiphos with the azamethiphos sensitive reference stable fly strain MSD at 24 hours with an R2 value of 0.95 to 0.97

4.3.1.3 Determination of the LD_{95} of deltamethrin

When determining the LD_{95} of deltamethrin with the deltamethrin sensitive MSD strain, the paralysis rates differed widely between 0.234 and 2.34 ng/µl. Furthermore, after 48 hours of observation 5 % of the sensitive stable flies recovered from the knockdown effect of the

pyrethroid deltamethrin, which prevents the sodium channels from closing. Both led to the fact that the coefficient of determination values R^2 for an observation time of 24 and 48 hours were 0.61 and 0.69, respectively. The determined R^2 values can, therefore, be classified as moderately good. After correcting the paralysis rates according to Abbott (Abbott, 1925), the LD_{95} was at 1.00 ng/µl for an observation time of 24 hours and at 1.45 ng/µl for an observation time of 48 hours (Graphic 10).

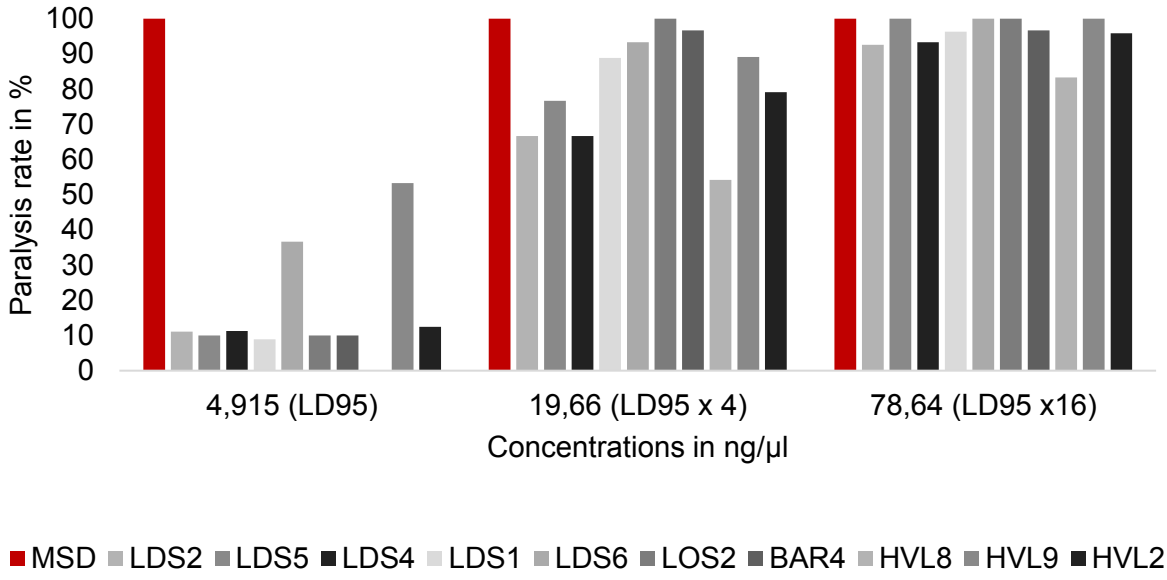


Graphic 10 Determination of the LD_{95} after topical application of deltamethrin with the sensitive reference strain MSD at 24 hours with an R2 value of 0.61 to 0.69

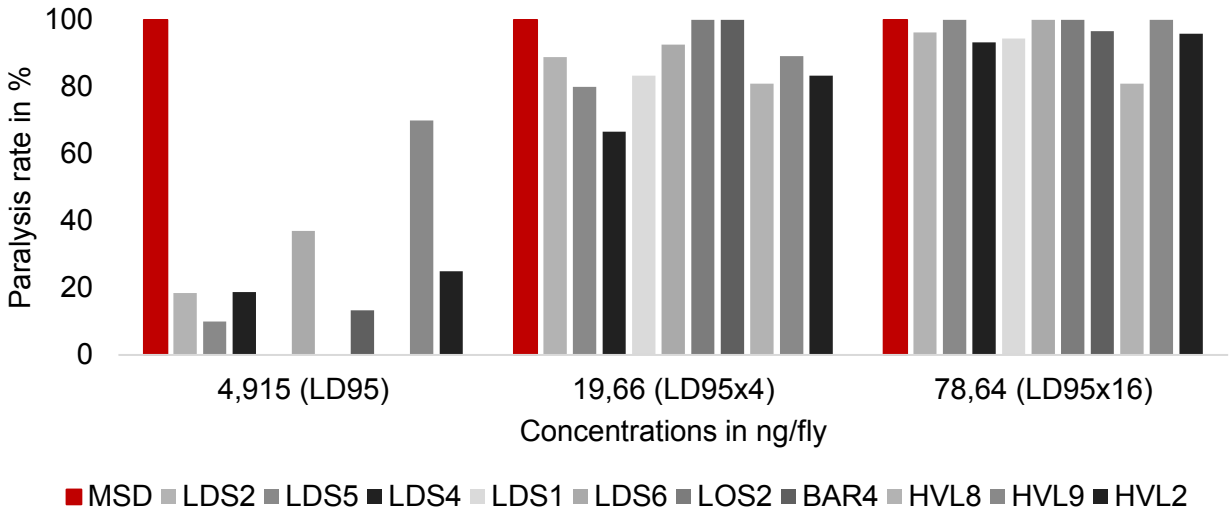
Since the paralysis rates fluctuated very strongly, significantly delaying the final determination of the LD_{95} value, for time reasons the LD_{95} value of 2.34 ng/µl determined by Moyses and Gfeller (1999) was used in the laboratory tests with the field strains. However, the LD_{95} value of 1 ng/µl, which was determined with the MSD strain, can be considered to be approximately of the same magnitude. When the determination of the LD_{95} of deltamethrin was repeated later with another sensitive stable fly laboratory strain of the Federal Environment Agency (UBA), the paralysis rates varied as strongly as those established by the MSD strain. However, the LD_{95} value was 2.36 ng/µl for an observation time of 24 hours and at 6.65 ng/µl for an observation time of 48 hours. On one side, this underlines the strong paralysis fluctuations that are caused by this type of active ingredient (synthetic pyrethroids), on the other side, it illustrates the natural variability among fly populations reared in laboratories.

4.3.1.4 Results of the topical application of azamethiphos

The topical application of 4.92 ng of the pure substance azamethiphos dissolved in 1 µl of acetone on the ten stable fly field populations resulted in a mean paralysis rate of 16.37 ± 15.9 % after 24 hours and 19.26 ± 21.4 % after 48 hours. This can be classified as highly resistant according to the WHO (2016). A four-fold increase in the initial azamethiphos concentration to 19.66 ng/µl, resulted in a mean paralysis rate of 81.13 ± 15.0 % after 24 hours and 86.49 ± 10.0 % after 48 hours confirming the classification as highly resistant. In the highest concentration tested (a 16-fold of the LD_{95}), a mean paralysis rate of 95.8 ± 5.2 % was observed after 24 and 48 hours. Since the value is below 98 %, a high resistance is confirmed according to the WHO (2016).



Graphic 11 Laboratory results: results of the topical application of azamethiphos after 24 hours corrected according to Abbott (1925), comparison of the results of the sensitive stable fly MSD strain (red) with those of the 10 field populations (grey), October – November 2015



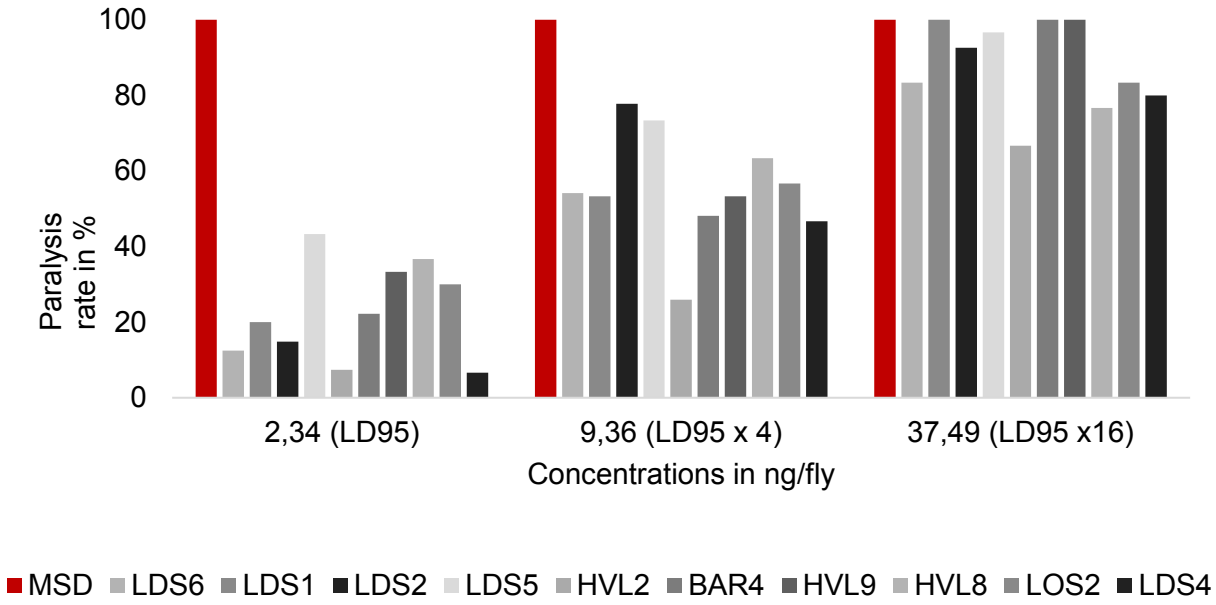
Graphic 12 Laboratory results: results of the topical application of azamethiphos after 48 hours corrected according to Abbott (1925), comparison of the results of the sensitive stable fly MSD strain (red) with those of the 10 field populations (grey), October – November 2015

Looking at each one of the ten populations it is evident that 24 as well as 48 hours after the topical application of the LD_{95} of 4.92 ng/µl the paralysis rates were in the WHO range of resistance. In all of the 10 field strains paralysis rates below 90 % were observed 24 and 48 hours following topical application of azamethiphos at the LD_{95} of 4.92 ng/fly. Accordingly (WHO, 2016), it could be concluded that these populations were resistant to the tested insecticide at the LD_{95}. Nine out of the 10 populations (90 %) even showed a paralysis rate below 40 % at the LD_{95} after both times of observation. After 24 hours of observation, the four-fold increase to 19.66 ng/µl resulted in 70 % of the populations having to be classified as resistant. Twenty per cent were rated as probably resistant. One of the ten populations (10 %) showed a paralysis rate of 100 %. After 48 hours at 19.66 ng/µl, 20 % of the populations turned out to be resistant. Ten per cent were probably resistant and 70 % sensitive to this concentration. At a 16-fold increase of the LD_{95} at both observation times, 40 % of the populations displayed a paralysis rate of 100 % and 60 % showed mortality rates below 98 % indicating a high resistance of the populations (WHO, 2016).

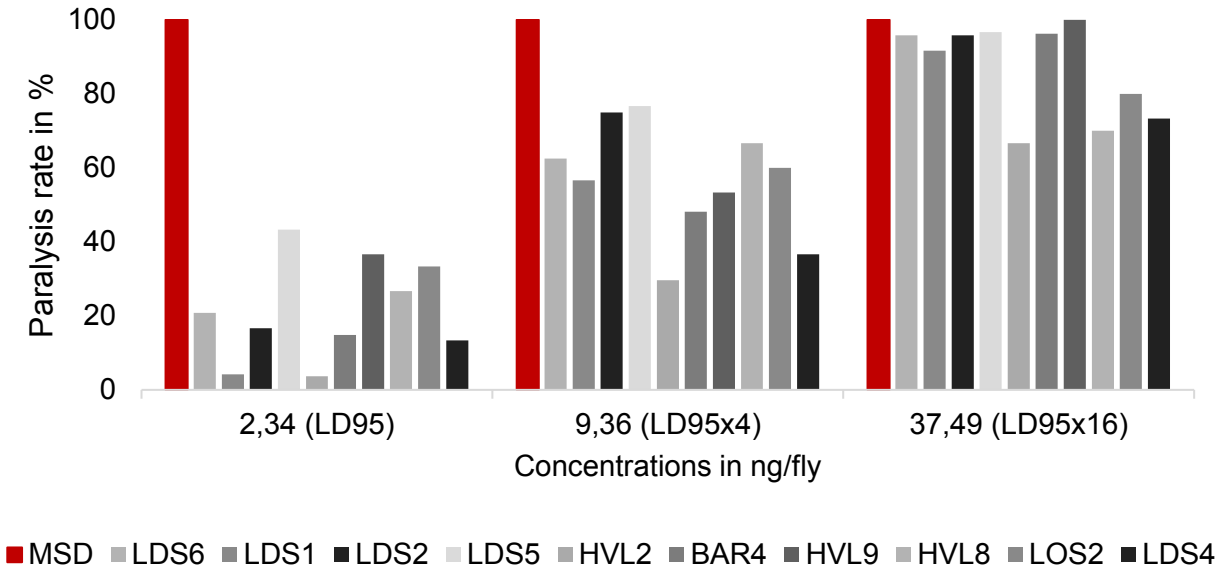
4.3.1.5 Results of the topical application of deltamethrin

The topical application of 2.34 ng of pure deltamethrin dissolved in 1 µl of acetone (LD_{95}) led to a mean paralysis rate of 22.7 ± 12.7 % after 24 hours of observation and 21.3 ± 13.4 % after 48 hours. A four-fold increase of the LD_{95} to 9.36 ng/µl resulted in a mean paralysis rate of 55.3 ± 14.5 % after 24 hours of observation and 53.2 ± 15.3 % after 48 hours of observation.

The topical application with a 16-fold augmentation of the LD_{95} to 37.49 ng/µl increased the mean paralysis rates to 87.9 ± 11.6 % ng/µl after 24 hours and 86.6 % ± 12.8 after 48 hours. According to WHO (2016), this indicates a high resistance in all populations.



Graphic 13 Laboratory results: results of the topical application of deltamethrin after 24 hours corrected according to Abbott (1925), comparison of the results of the sensitive stable fly MSD strain (red) with those of the 10 field populations (grey), October 2015



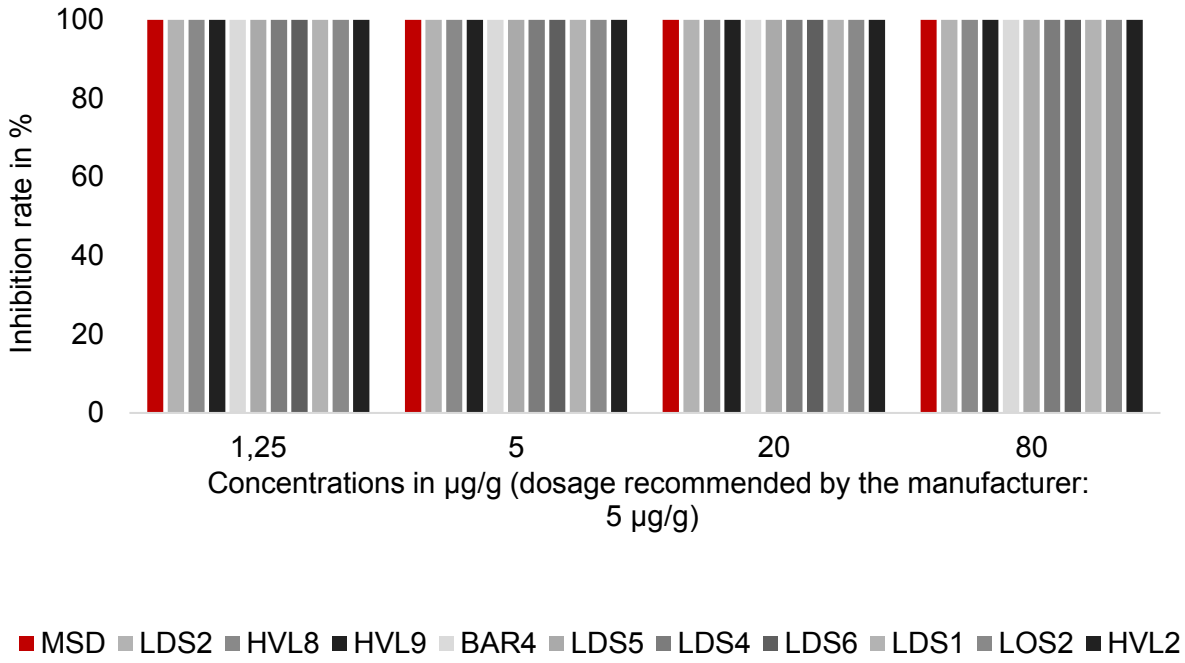
Graphic 14 Laboratory results: results of the topical application of deltamethrin after 48 hours corrected according to Abbott (1925), comparison of the results of the sensitive stable fly MSD strain (red) with those of the 10 field populations (grey), October 2015

Due to the paralysis rates of all ten tested field populations being below 90 % at both times of observation resistance against deltamethrin at the LD_{95} was obvious (WHO, 2016). A paralysis rate below 40 % was calculated in 90 % of the field populations even after both times of observation. Furthermore, the tested field strains turned out to be resistant at the four-fold increase of the initial concentration after 24 and 48 hours of observation. At the 16-fold increase of the LD_{95} a paralysis rate of 98 to 100 % was observed in 30 % of the field populations at 24 hours but 70 % showed high resistance with paralysis rates below 98 %. After 48 hours of observation 90 % could be classified as resistant, 10 % showed paralysis rates of 100 % (WHO, 2016). These findings support the previous results of resistance against deltamethrin in the FlyBox® test.

4.3.2 Larvicide tests

4.3.2.1 Cyromazine

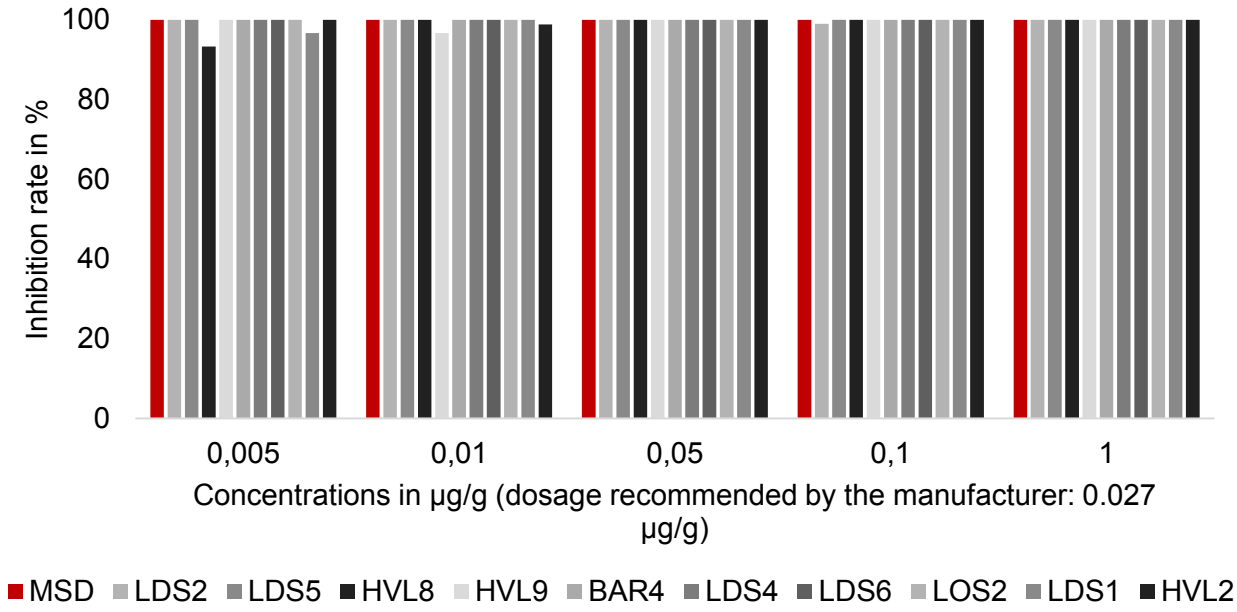
When treating the rearing medium with cyromazine, none of the four tested concentrations resulted in hatching of stable fly imagoes. The inhibition rate of cyromazine was 100 ± 0.7 % at, below and above the dosage recommended by the manufacturer of 5 µg/g.



Graphic 15 Laboratory results: results of the larvicide testing of cyromazine corrected according to Abbott (1925), comparison of the results of the sensitive stable fly MSD strain (red) with those of the 10 field populations (grey), November 2015

4.3.2.2 Pyriproxyfen

The inhibition rate of two of the ten tested field populations (20 %) was calculated to be 93.3 and 96.7 %, respectively, at a concentration of 0.005 mg/g pyriproxyfen. The inhibition rate of the remaining test populations was 100 ± 0 %. At a dosage of 0.01 µg/g the inhibition rate of one population was 96.7 %. Concentrations above the recommended dosage of 0.027 µg/g caused very high inhibition rates. It was 99 % for one and 100 ± 0 % for the remaining field populations at a concentration of 0.1 µg pyriproxyfen/g rearing medium.



Graphic 16 Laboratory results: results of the topical application of pyriproxyfen corrected according to Abbott (1925), comparison of the results of the sensitive stable fly MSD strain (red) with those of the 10 field populations (grey), December 2015

4.3.3 Statistical evaluation

The questionnaire analysis yielded exploitable results.

At first it had seemed that the dairy farmers were less sensitive towards the fly occurrence on their farms. However, the chi-squared test revealed that the farmers' observations concerning the fly abundance on their farms did not differ significantly from own observations (X^2, $p = 0.099$). Nevertheless, the p-value was not much higher than the 0.05-threshold. When comparing dairy cattle housing systems, the chi-squared test could not detect any statistically significant difference between the fly occurrence in the open cubicle-type stables with slatted floor and the deep litter stables (X^2, $p = 0.152$). Even, when contrasting the fly abundance in stables based on the predominant flooring conditions, the chi-squared test revealed no significant difference between stables where there was litter in some areas and stables where there was litter only in the calving and in the calf rearing areas (X^2, $p = 0.294$). Accordingly, neither did the dung storage arrangement appear to be significantly related to the degree of stable fly occurrence (X^2, $p = 0.473$). Thus, whether the dung was stored outside or inside the stables did not have an impact on the number of flies in and around livestock.

Only three of the visited farms were organic farms, so it is not possible to make sound statistical statements. Nevertheless, there did not seem to exist any difference between the resistance development on organic or conventional farms (Fisher's exact test, $p > 0.999$).

Furthermore, the resistance development assessed with the FlyBox® test was neither statistically related to the type of dairy cattle housing system (Fisher's exact test, $p > 0.999$), nor to the predominant flooring conditions (Fisher's exact test, $p > 0.999$), nor to the dung storage arrangement (FET, $p > 0.999$). Additionally, the type of dairy cattle housing system (Fisher's exact test, $p = 0.120$), nor the flooring conditions (Fisher's exact test, $p > 0.999$), nor the dung storage arrangement (Fisher's exact test, $p = 0.476$) did have any impact on the use of pyrethroids.

Since all of the 40 dairy farm stable fly populations tested in the field by the FlyBox® test were resistant, it is hardly surprising that the loss of effectivity noted by the farmers was not significantly related to the actual measured loss (Fisher's exact test, $p = 0.200$).

Only eight of the 40 (20 %) field tested dairy farms had noticed a decrease in efficacy. Additionally, the farm where the Flybox® test had resulted in a probable resistance had claimed to have noticed a drop of efficacy. There was no significant connection between the frequency of insecticide usage and the loss of efficacy noted by the farmers (FET, $p = 0.578$) or the actual measured efficacy (FET, $p > 0.999$). Furthermore, the change of insecticides in the last three years did neither have an impact on the loss of efficacy noticed by the famers (X^2, $p = 0.448$), nor did it have one on the actual resistance development (FET, $p = 0.325$). When comparing the resistance development to the use of pyrethroids, the probability to have a Flybox® test resistant stable fly population was not related to the use of pyrethroids according to the farmers (FET, $p > 0.999$).

In addition, the size of herd was neither significantly related to the resistance development (FET, $p = 0.475$), nor to the use of chemical control measures (FET, $p > 0.999$), nor to the frequency of insecticide application (FET, $p = 0.533$), nor to the type of physical control measurement (X^2, $p = 0.345$).

In all tests of the study, the difference between the paralysis rates of the sensitive laboratory strain and the field strains was statistically significant. At all exposition times as well as observation times, the sensitive laboratory strains were significantly more susceptible to the tested insecticides than the field strains (Mann-Whitney-U test, $p < 0.001$).

In the FlyBox® test the paralysis rates after 10 or 30 seconds exposition time did not differ significantly from each other (Mann-Whitney-U test, $p = 0.617$). Neither did the paralysis rates of the FlyBox® test in comparison to the topical application of deltamethrin (Mann-Whitney-U test, $p = 0.529$). When treating the ten stable fly populations that were involved in the FlyBox® test as well as in the topical application as dependent samples, no significant difference could be observed either (Wilcoxon Sign test, $p = 0.333$). In addition to that, Cohen's kappa coefficient showed that both tests revealed a substantial agreement ($k = 0.615$, $p = 0.035$), suggesting that both tests often measure the same results.

In conclusion, both tests, the Flybox® test and the topical application, revealed mortality rates that could be rated as resistant.

Furthermore, comparing the paralysis rates of the topical application of deltamethrin after 24 and 48 hours observation time, no significant differences were noted (Mann-Whitney-U test, $p = 0.853$). The same was observed for azamethiphos at both time points post application (Mann-Whitney-U test, $p = 0.631$). The efficacy of deltamethrin and azamethiphos did also not differ significantly from each other after an observation period of 24 hours (Mann-Whitney-U test, $p = 0.165$) nor after an observation period of 48 hours (Mann-Whitney-U test, $p = 0.436$).

For the larvicide tests, a statistical analysis was not possible since both active substances, cyromazine and pyriproxyfen, resulted in inhibition rates of 100 % in all stable fly populations tested. Results were unambiguous.

5 Discussion

The aim of this thesis was to evaluate current practices of insecticide use in a questionnaire survey considering the federal state of Brandenburg as a showcase in Germany. Furthermore, it intended to assess the occurrence and distribution of insecticide resistance in stable flies on dairy farms allowing to suggest integrated pest management strategies for on-farm pest control aiming to minimize both the use of insecticides and the risk of resistance development.

5.1 Questionnaire analysis

The study design followed the hypothesis that, according to earlier surveys (Hildebrand, 2017; Jandowsky, 2009; Jandowsky et al., 2010), a frequent and non-strategic use of chemical control measures may lead to the development of insecticide resistance.

For the questionnaire analysis, 18 farms were recommended by local veterinarians and 60 farms originated from a list of the best dairy farms in Brandenburg according to milk yield and quality. Out of the 78 dairy farms contacted, 52 agreed to participate, resulting in a response rate of 71 %. This rate can be regarded as good, especially in the light of the currently strained political situation of the dairy industry.

Nevertheless, similar to Jandowsky (Jandowsky, 2009; Jandowsky et al., 2010), the collection of data with the aid of a questionnaire analysis turned out to be quite difficult since only 55.8 % of the farmers or their employees had an exact idea of which biocides or insecticides had been used and during which kind of period. Since it is vital for an active ingredient to be applied at the lethal dosage in order to prevent the development of resistances, it would have been of interest which products and which dosages had been used. Thirty-one per cent of the 29 farmers that actually knew which products they employed indicated to have used two or more chemical products against flies at the same time. Thirty-eight per cent had changed the product in the past 3 years. Thirty per cent of them had replaced the product within the same class of insecticides. The preferred insecticides in use were synthetic pyrethroids. Twenty-five of the 29 dairy farms (86 %) were currently employing one. According to the questionnaire analysis, in 2015 only one out of 29 farms (3 %) applied cyromazine in combination with a feed-through neonicotinoid, which is ineffective against blood feeding stable flies. Three of the 29 farms (10 %) of the farms used organophosphates. The combined use of larvicide (the insect growth regulator cyromazine) and adulticide (deltamethrin) against stable flies was only performed once three years ago.

Furthermore, 71.2 % of the farmers were not able to differentiate between house and stable flies. This, however, could be remedied when visiting 40 (76.9 %) of the 52 dairy farms

interviewed. Probably due to habituation, the farmers had estimated their (stable) fly abundance generally to be lower. This corresponds to the fact that despite all tested stable fly populations being resistant, the loss of efficacy noted by the farmers was not significantly related to the actually measured loss (4.3.3.). Additionally, the farms where the Flybox® test had indicated a probable resistance claimed to have noticed a drop of efficacy. There was not even a significant connection between the frequency of insecticide usage and the loss of efficacy observed by the farmers or the actually measured efficacy (4.3.3.). Furthermore, there was no significant correlation between the change of insecticides in the last three years and the loss of efficacy as noticed by the famers, nor was there one to the actual resistance development (4.3.3.).

When comparing the stable fly abundance in different conditions of dairy cattle housing, flooring systems or dung storage arrangements, no significant differences were observed. This may indicate that, regardless of the housing conditions, flooring systems or dung storage arrangement, any management system can promote the reproduction of *Stomoxys calcitrans.* On the other hand, all systems visited were relatively similar: they were never completely litterless and the slurry was never shut away entirely. In every cow housing system in Germany, calves and parturient cows are always kept on litter or straw, which after use is never completely stored away from the flies. Thus, in each cattle housing system the propagation of them is always possible.

Out of the 52 dairy farms interviewed, 78.9 % applied insecticides against flies. Thirty-three per cent of them claimed to use more than one control measure at a time. This is often a combination of mechanical cleaning and a physical control measure like sticky or UV traps. Mechanical cleaning, however, is normally not sufficient for eliminating every fly egg in every corner (Kaufman et al., 2005). Weidhaas and Haile (1978) stated that in order to reduce flies with traps permanently it is necessary to achieve daily reductions of the populations by 24 to 58 % in order to reach a 50 to 90 % decrease in a closed population. Since, however, cow housing systems are not fully closed, it is almost impossible to achieve such a reduction by traps only.

The herd size was neither significantly related to the resistance development nor to the use of chemical control measures nor to the frequency of insecticide application, nor to the type of physical control measurement. In conclusion, size is not a factor that could skew the results when dairy farms with herd sizes above 50 dairy cows are compared.

When contrasting the resistance development to the employment of pyrethroids, the probability to have a Flybox® test resistant stable fly population was not related to the use of pyrethroids as indicated by the farmers. Only three of the visited farms were organic farms, so it is not possible to make final statistical statements concerning them. Nevertheless, there did not seem to exist a difference between the resistance degrees when comparing organic with

conventional farms. This statement, however, requires more investigation since the data base concerning organic farms was too small to be meaningful.

Nonetheless, resistance in house or stable flies apparently does not entail disadvantages such as shorter life span or a decreased fertility. Thus, once resistance has been established in a population, it will persist for a long time. Additionally, contamination of the farm premises by resistant strains through dung, wind drift or even active movements cannot be ruled out.

The results of the questionnaire analysis imply that resistance development is possible in every kind of dairy farming and in consonance with every fly controlling behaviour.

As the questionnaire analysis revealed, most farmers and their employees did not know what kind of insecticides they used and how often they applied them. Thirty-one per cent of the farmers estimated to have employed insecticides three to five times a summer, 34 % applied insecticides whenever needed and 6 % of them used them daily. This, however, is not in accordance with the information leaflets of the veterinary medicinal products they had indicated to have used. Normally, pyrethroid-containing pour-on products should only be applied once a summer. If needed, they can be applied every six to ten weeks. Wall sprays often state in their user instructions that their usage should be repeated only once after two to three days. When visiting the 40 dairy farms for the Flybox® test, however, two farmers claimed to use those fumigants every day. In addition, when applying wall sprays, they never wore skin or mouth protection during the application which indicates that they were not aware of the instructions on the safety data sheets of the insecticide.

In conclusion, farmers as well as veterinarians need to be more sensitised to the correct use of insecticides and to the concept of integrated pest management (IPM). IPM is defined as a "system that, in the context of the associated environment and the population dynamics of the pest species, utilizes all suitable techniques and methods in as compatible a manner as possible and maintains the pest populations at levels below those causing economic injury" (FAO, 1967).

Lowering the selection pressure by combining chemical measurements with non-chemical ones, the use of attractants or synergists in order to increase the contact time with insecticides even in resistant individuals and the employment of multi-directional insecticide attacks through rotation and mixture is expected to delay resistance development (Georghiou, 1994). Crespo et al. (1998) found that in large house fly populations a combination of cultural and chemical control followed by biological control measures is most effective for a successful fly reduction and for maintaining them at low levels over time. This could be used as an indicator for how to manage stable flies as well. Furthermore, the release of sterile male stable flies (Patterson et al., 1981) or susceptible flies (Imai, 1987) are expected to reduce fly populations.

For these reasons, chemical insect control should never be the only control measurement used against flies. It should always be used only as a last resort when every other method has been already exploited and only in adequate proportions.

5.2 Field study

Following the studies by Jandowsky (2009) and Jandowsky et al. (2010) and Hildebrand (2017) on house flies, field tests using the FlyBox® test were performed in order to get a first estimation concerning the susceptibility of the stable fly farm populations to deltamethrin. The practicability of this test was extensively proven by Hildebrand (2017) and Jandowsky (2009). However, caution must be paid when transferring the flies into and out of the box in order not to harm them and, thus, bias the results. In addition to that, monitoring the conditions with a control group has shown to be essential to the FlyBox® test evaluation. After that, Abbott's formula (Abbott, 1925) is applied in order to distinguish the effects of insecticide treatment from those caused by natural factors (Fleming and Retnakaran, 1985). The inner FlyBox® was coated with a commercial bed net fabric impregnated with 55 mg deltamethrin/m^2. Information regarding the discriminating dose of stable flies to deltamethrin as a contact insecticide vary in the literature. Cruz-Vázquez et al. (2005) successfully used permethrin in a concentration of 0.026 mg/m^2 (LD_{99}) on a glass vial for a two-hour-contact against stable flies. Salem et al. (2012a) calculated a LD_{90} of 264.3 mg/m^2 for a resistant field population and 28.1 mg/m^2 for a sensitive stable fly population after contact with deltamethrin treated filter papers for 1 hour. Jandowsky (2009) used a net saturated with 100 mg deltamethrin/m^2 and Hildebrand one with 280 mg deltamethrin/m^2 against *Musca domestica*. Robert and Carnevale (1991) found a deltamethrin bed net concentration of 25 mg/m^2 to be effective against *Anopheles mosquitoes*. Thus, the choice of a lower bed net concentration appeared to be indicated (Salem et al., 2012a). When testing a 55 mg/m^2 bed net against the laboratory stable (UBA) and house fly (WHO) strains the concentration was proven to be effective against both species. Nevertheless, for future testing the net concentration of deltamethrin should be standardized in order to obtain comparable results. Furthermore, compared to the WHO *Musca domestica* strain there is no universal stable fly reference strain available as a gold standard. As the UBA strain was tested to be fully susceptible in laboratory tests, it could serve as a reference strain in the future.

The bed net in the FlyBox® was not changed during the three months of testing. However, it was not exposed to light and was tested on a sensitive laboratory stable fly strain right before and right after testing in the field as well as six months and one and a half year later. Schreck et al. (1978) found that *Aedes aegypti* and *Aedes americanum* were still susceptible to a

permethrin treated cloth after 33 to 50 rinses of water, two soapy washes or one month exposure to outdoor climate. Arends (2016), Holzgrefe (2013) and Rohrmann (2010) observed deltamethrin nets used in the field around livestock to be robust and suitable for at least five to six months. Thus, it can be assumed that pyrethroid impregnated bed nets are usable in the FlyBox® field assay for a long period of time.

All of the 40 tested populations (100 %) were classified to be resistant in the Flybox® test. After ten seconds of exposure and 15 minutes of observation, the stable fly strains trapped in the field showed a mean paralysis rate of only 32.8 %. After six and 24 hours, it was slightly increased to 37.2 % and 36.7%, respectively. It can be concluded that none of the tested field strains were fully susceptible to the deltamethrin impregnated bed net. According to WHO (2013), after six hours of observation 100 % of the strains were proven to be resistant at a paralysis rate below 90 %. Forty-nine per cent even showed a mortality rate below 40 %. Those values can be regarded as a rather high degree of resistance. While our stable fly populations reach a paralysis rate of 36.7 % on average at 24 hours, Jandowsky (2009) had observed a mean paralysis rate of more than 50 % and Hildebrand (2017) described a mean paralysis rate of 53 % in house fly field populations in Germany. Even after increasing the time of exposure to 30 seconds, the paralysis rate was still 36.7 % on average after 24 hours of observation, confirming that the resistant status of the fly population was not challenged by increasing the contact times.

Similar to *Musca domestica* (Kristensen et al., 2001; Pap and Farkas, 1994; Pinto and Prado, 2001) resistances in *Stomoxys calcitrans* against synthetic pyrethroids are found throughout the whole world. Cilek and Greene (1994) observed stable flies to be resistant against the pyrethroid permethrin in Kansas, USA. In Florida, Pitzer et al. (2010) found stable flies from geographically separated horse farms to be resistant against the LD_{99}, a three-fold and a tenfold of the LD_{99} of permethrin. Notably, even strains that had not been in contact with insecticides showed a 20 % survival rate when exposed to the three-fold of the LD_{99}. In France, Salem et al. (2012a) were the first research team that evaluated insecticide resistance against stable flies in Europe. They tested one field strain from an organic farm and one from a conventional farm against the synthetic pyrethroids cypermethrin, deltamethrin, fenvalerate, λ-cyhalothrin and the organophosphate phoxim after one hour of exposure to an impregnated filter paper. With the exception of phoxim, the LD_{90} values obtained for the organic farm stable flies were 1 to 4 times lower than the recommended doses for the pyrethroids tested. Particularly for the flies originating from the conventional farm, the LD_{90} values were between 7.1 and 22.6 times above the recommended doses for the pyrethroids applied suggesting once more a correlation between the use of insecticides and the development of resistance.

As the questionnaire analysis revealed, 86 % of the farmers had employed an insecticide of the chemical class of pyrethroids with deltamethrin being the most frequently used active

ingredient within this group (41 %). Hence, it is not surprising that 100 % of the 40 tested farms resulted to be resistant in the Flybox® test. As other studies demonstrated, side resistance within the synthetic pyrethroids exists (DeVries and Georghiou, 1980; Jandowsky, 2009; Jandowsky et al., 2010). They are all of the same insecticide class and have the same mode of action. Therefore, it can certainly be assumed that other pyrethroids are also not effective anymore.

Ten farms had stated that they had not used insecticides in the past ten years. Nevertheless, the stable fly strains from seven farms were still classified as 'resistant' according to the Flybox® test. This indicates that resistance development is not a localised problem, rather it can be spread from the adjoining agricultural crop land and conventional animal husbandry to organic farms. Resistant flies from neighbouring livestock can also be transferred from one farm to another by flight and wind drift.

5.3 Laboratory study

In the laboratory, ten field strains were reared in order to verify both the results of the Flybox® test by topical application and to test active substances from other classes of insecticides. Investigations were conducted with the F1, F2 and F3 generation of the reared population in order to get flies of comparable age and to check whether resistance can be passed genetically to the subsequent generation.

5.3.1 Determination of the LD_{95}

Since there was no current reliable data available concerning the LD_{95} of stable flies to the insecticides tested by topical application, the lethal dose was determined by a dose-range-finding procedure on susceptible laboratory reference strains with serial dilutions. In 1999, Moyses and Gfeller had determined the LD_{95} of deltamethrin and azamethiphos for the UBA strain with only 20 flies (Gfeller, 1999). Due to this small amount of data, however, the LD_{95} of deltamethrin was never established in laboratories. Therefore, tests to determine the LD_{95} of deltamethrin were conducted with a sensitive stable fly laboratory strain from the German federal environment agency (UBA) and a sensitive laboratory strain kindly provided by the company Merck Sharp & Dohme (MSD).

When performing own tests on the MSD strain for azamethiphos, the LD_{95} value was unambiguously 4.92 ng/µl, which was very close to the value determined by Moyses and Gfeller (1999) of 4.1 ng/µl. The identification of the LD_{95} of deltamethrin with the MSD strain, however, turned out to be rather difficult. The paralysis rates varied by a power of ten between

0.234 ng/µl and 2.34 ng/µl. In addition, 5 % of the sensitive laboratory flies recovered from the knockdown effect after 48 hours of observation. Unlike azamethiphos, which inhibits the cholinesterase activity in the synapses of the neurons, deltamethrin hinders the sodium channels of the neurons from closing. This initially leads to agitation followed by paralysis. The latter is, however, known to be potentially reversible (Sfara et al., 2006). Therefore, this might be a reason for the difficulties to determine an accurate LD_{95} for deltamethrin.

Derived from the first data set, the LD_{95} was 1.00 ng/µl at 24 hours and 1.45 ng/µl at 48 hours for the MSD strain. However, this determination was considerably delayed due to the strong variation of the paralysis rates. Therefore, the LD_{95} value of 2.34 ng/µl determined by Moyses and Gfeller (1999) was used in the laboratory tests. Since both LD_{95} values (1.00 vs. 2.34 ng/µl) do not differ substantially, the use of the LD_{95} value of 2.34 ng/µl calculated by Moyses and Gfeller (1999) is not expected to have had a negative influence on the study results.

The determination of the LD_{95} of deltamethrin was later repeated with the laboratory reference strain from the UBA. Again, the paralysis rates varied as already observed with the MSD reference strain. For the UBA reference strain a LD_{95} value of 2.36 ng/µl at 24 hours and a LD_{95} of 6.65 ng/µl at 48 hours was calculated. The somewhat differing susceptibility against insecticides within two laboratory reference strains can, however, be attributed to the occurrence of natural variations (Kristensen et al., 2001; Schaub et al., 2002; Scott et al., 2000) and appears to be common within stable fly populations independently from previous selection pressure through insecticides (Marcon et al., 1997).

In the literature, there are data concerning other active substances or other test methods. For permethrin, Pitzer et al. (2010) had calculated a LD_{90} of 60.3 ng per gram per stable fly for the topical application. Lotmar (1949) reported that the mean weight of non-engorged flies is 0.008 g whereas that of blood-engorged stable flies is 0.0216 g. This could largely be confirmed when weighing engorged females showing a weight of 0.0235 ± 0.0049 g. Males, in contrast, weighed 0.0115 ± 0.0018 g after feeding only. Thus, as we used males as well as females, a mean weight of 0.0175 g was drawn for calculation. Therefore, following Pitzer et al. (2010), when 60.3 ng permethrin per gram of stable fly weight is required, 1.05 ng/fly can be calculated as LD_{90}. Since deltamethrin is four times more effective than permethrin according to Salem et al. (2012a), a LD_{90} of 0.26 ng/fly can be roughly estimated. Nonetheless, as aforementioned this concentration was observed to be too low when testing it on the sensitive laboratory strain of the UBA. This, confirms again the importance of establishing a LD_{95} based on a defined reference strain.

In contrast to a sensitive *Musca domestica* strain called WHO there is, at present, no worldwide reference strain available for stable flies. When performing own tests for the evaluation of the LD_{95} on both the UBA and MSD strain, the LD_{95} of deltamethrin and azamethiphos were nearly in the same range as those determined by Moyses and Gfeller (1999). Since in this study, LD_{95}

values have been evaluated for both the UBA and MSD stable fly strains, both could serve as susceptible reference strains for insecticide testing in the future.

In future studies it would be more compelling to calculate resistance factors for each field population tested. This would make the data even more comparable throughout the world. The resistance factor (RF or resistance ratio: RR) is defined as the ratio of either the LD_{50} of a population relative to the calculated LD_{50} of a known reference, usually susceptible population (Heath and Levot, 2015). In addition to the LD_{50} other LD values are also used in the scientific literature (FAO, 2004). Thus, a resistance factor distinctly exceeding the RR = 1 would suggest a low efficacy of the insecticide in the field. It should be noted in this context, however, that the determination of a resistance factor is not always practicable in extensive field surveys. It requires enormous commitment of time, housing, equipment and mobilization as well as human efforts in order to rear the adequate number of flies needed for the subsequent evaluation of the LD_{95} for each field population of interest.

5.3.2 Topical application

In the laboratory, in contrast to the Flybox® test, the contact with the insecticide was more exact and, therefore, is judged to be more comparable. When comparing three exposure techniques for the analysis of stable fly susceptibility, Marçon et al. (1997) found topical application to be most sensitive for this species in comparison with treated filter papers and residues on petri glass dishes.

For the topical application test, insecticides were dissolved in acetone in order to achieve the correct serial dilution. Due to its good tolerance in insects and its high evaporation time, acetone is presently considered the state of the art dissolvent (Gfeller, 1999; Jandowsky, 2009). Nevertheless, we tried to replace it by DMSO, which often serves as dissolvent in parasitology studies. However, since there was no significant difference in survival noted and stable flies treated with DMSO seemed to act a little bit less vivid, acetone appeared to be the better choice for this study.

Three concentrations were tested topically on stable flies: the LD_{95}, the four-fold and the 16-fold of the LD_{95}. The choice of those concentration steps was based on earlier studies in our institute (Hildebrand, 2017; Jandowsky, 2009) and is largely in agreement with the concentration steps suggested by the WHO, which recommends five- and tenfold concentration steps of the LD_{99} for testing resistance in mosquitoes. By this method, one is able to deduce the LD_{99} of the field strains since “although these higher concentrations for each insecticide will not correspond to their recommended field application rates, they will yield relevant information about the intensity of resistance, or the ’strength’ of expression of the

resistance phenotype(s) in question" (WHO, 2016). At the LD_{95} of both insecticides tested, deltamethrin and azamethiphos, each of the tested field population was highly resistant with efficacy rates below 90 %. Since, however, only 30 flies of each strain were tested at this concentration, further testing was deemed necessary. When tested, therefore, with the four- or sixteen-fold of the LD_{95} of both adulticides the mean percentage efficacy of the stable fly field strains were again below 90 % and thus, could be finally classified as resistant. At a four-fold of the LD_{95} of azamethiphos still 70 % of the ten tested populations were classified as resistant at 24 hours post application. At a 16-fold of the LD_{95} 60 % of the populations showed paralysis rates below 90 % indicating high resistance levels of those populations. This must be considered as remarkable since only 10 % of the 52 farmers interviewed claimed to have used organophosphates such as azamethiphos. Of the ten farms from which stable fly strains were established in the laboratory, none had remembered the use of organophosphates. This on the one hand, can be attributed to the lack of knowledge of the farmers interviewed, but it might also be attributed to mechanisms of cross-resistance to other more frequently used insecticides on the other hand (Bisset et al., 1997; Devonshire and Moores, 1982; Liu and Yue, 2000). Thus, further testing is advisable in future studies.

When testing deltamethrin by topical application, all of the ten populations tested at four-fold the LD_{95} resulted in paralysis rates below 90 % and were, therefore, classified as resistant. At a 16-fold of the LD_{95} still 70 % of the strains showed paralysis rates below 90 %, thus confirming the results of the Flybox® test. The results were largely in accordance with the extensive use of synthetic pyrethroids on the ten farms selected. Six of them (60 %) had responded to have used synthetic pyrethroids, one of them (10 %) even deltamethrin. Two of them, however, had never used chemical control measures (20 %) and another two did not know what kind of insecticides they had applied. However, it can be assumed that more dairy farms than recorded had made use of pyrethroids. Furthermore, side resistances among pyrethroids have been observed before (Chandre et al., 1999; DeVries and Georghiou, 1980). That is why all of the ten stable fly strains were resistant to deltamethrin although probably not all of them have had contact with it. The fact that resistance was also found in the two stable fly populations of the dairy farms which had emphasized to never have used insecticides is remarkable and can be explained by the entry of resistant flies from other neighbouring farms or agricultural crop land where pesticides had been used, by wind drift, dung or active movement. This again shows that farmers need to be more sensitised and that the use of insecticides that consistently have been proven not to be effective anymore should be more regulated across the European Union (Agency, 2017).

5.3.3 Larvicide tests

The results of the larvicide tests were very different from those of the adulticides. When treating the rearing medium with the insect growth regulator (IGR) cyromazine, the development of stable fly imagoes was completely inhibited at the four concentrations tested. In conclusion, the inhibition rate of cyromazine was 100 % at, below and above the dosage of 5 µg/g recommended by the manufacturer. This was in accordance with the assumed correlation between resistance and the use of insecticides. According to the questionnaire analysis, only one of the ten tested dairy farms had used cyromazine. Jandowsky (2009) who had tested the efficacy of cyromazine in dairy farms in Brandenburg six years earlier and Hildebrand (2017) who had tested it on pig farms in the federal state Schleswig-Holstein, Germany, reported quite similar results. However, several other authors found house flies to be resistant against cyromazine. Nevertheless, these resistances occurred primarily in countries where cyromazine is used as an animal feed supplement on chicken farms (Acevedo et al., 2009; Bell et al., 2010; Pinto and Prado, 2001). The constant exposure of the flies to such IGR may promote the selection of resistant populations and is likely to be responsible for the decreasing efficacy of cyromazine in those countries.

The dosage of pyriproxyfen recommended by the manufacturer was 0.027 µg/g. At a concentration of already 0.005 mg/g, the inhibition rate of two of the ten tested field populations (20 %) was 93.3 and 96.7 %, respectively, whereas the inhibition rate of the remaining test populations was 100 %. A dosage of 0.01 µg/g caused a growth inhibition of 96.7 % in only one population. Concentrations above the recommended dosage of 0.027 µg/g caused very high inhibition rates. For one population inhibition of 99 % was demonstrated at a concentration of 0.1 µg/g whereas the remaining field strains showed 100 % inhibition of hatching. Since pyriproxyfen is presently not available as an insecticide for livestock in Germany, results confirmed the expected good efficacy of the IGR. Other authors (Pospischil et al., 1996b; Shah et al., 2015a), however, did observe resistances in house flies against pyriproxyfen. Among them Pospischil et al. (1996b) described a resistance factor of 53 in a *Musca domestica* strain in Germany that neither had had contact to IGRs.

Nevertheless, resistances against IGRs can also be generated by selection as demonstrated by Keiding (1999) and Shah et al. (2015b) in laboratory tests. Therefore, IGRs as well as adulticides should be employed only after evaluating resistance status and only according to a well-designed rotation plan.

5.4 Conclusions

The results of the study showed a high prevalence of resistance in *Stomoxys calcitrans* to deltamethrin and azamethiphos on dairy farms in the Federal State of Brandenburg in Germany when compared with the LD_{95} of sensitive reference strains (UBA, MSD). Surprisingly, no strong differences in the resistance pattern could be observed among the populations investigated. Stable fly populations from farms on which insecticides had presumably never or at least not for the last three years been used showed resistances that were as high as those of strains from farms that regularly resorted to chemical fly control. On the one hand, this suggests that resistance is transmitted from one generation to the next, i.e. that resistance is genetically fixed and that it has been in existence for a longer time. On the other hand, it can be assumed that crop production farms on which insecticides are frequently applied and which often are close to dairy farms might have a relevant influence on the selection of resistance in stable fly populations.

Insect growth regulators like cyromazine and pyriproxyfen, however, appeared to be still highly effective in *Stomoxys calcitrans* in Brandenburg. Yet, any incorrect use of ectoparasiticides or pesticides can lead to a strong selection pressure and, thus, considering the high reproduction rate of insects, to the development of resistance within a few generations.

The study showed that there is an urgent need to conduct field surveys nationwide and in different livestock production systems in order to draw general conclusions concerning the respective resistance status in Germany. Based on such surveys, it would become possible to individually adapt control measures for each dairy farm. This as well as resistance tests in other livestock production systems would sensitise farmers and veterinarians on resistance selection and the impact of it on the environment. In any case, both farmers and veterinarians need to be both sensitised and trained about the correct use of insecticides, and the knowledge about the consequences of a non-strategic use of insecticides ought to be enhanced. Furthermore, considering the paucity of effective insecticides, advice should be offered with regard to alternatives. Accordingly, all dairy farmers who have participated in this survey have been informed about outcomes and conclusions through an information brochure. On this basis, instructions for a better integrated pest management could be elaborated in order to ensure the employment of insecticides as a last resort only within a given cascade of possible control measures. Best-bet strategies and continuous pharmacovigilance could prove essential in an improved management of the risk of insecticide resistance development. Thus, insecticides should only be applied after evaluating the resistance status of an insecticide used as either veterinary medicinal product or biocide and only if they were judged effective. In case they are applied, veterinarians as well as farmers need to be sensitised in order not to exceed

or undercut the adequate dosages of an insecticide and in order to plan regular rotations. Furthermore, the combined use of larvicides and adulticides is expected to produce better results since they attack the targeted pest at different developmental stages. However, primarily, one should resort to physical and biological control measures. An improved dung management as well as better hygiene should always be a first step in order to reduce the currently demand-based use of adulticides. Furthermore, the advancement of genetic markers for the detection of insecticide resistance should be encouraged in order to facilitate further testing particularly under field conditions.

In conclusion, the use of insecticides and, therefore, their resistance development should never be encouraged where the application of chemical control measures is not necessary or not effective anymore. Neither the environment nor the consumer should be exposed to insecticides that are applied without justification or without being effective. It is necessary for the EU to encourage the examination of the efficacy of insecticides. Based on that, the legal regulation of some of those purposeless insecticides should be reconsidered on a regular basis in order to protect human health and prevent negative impacts on honey bees, hymenoptera as well as other non-target insects. The study showed that the use of insecticides is not sustainable and can in the long term not be the foundation of future integrated pest managements.

6 Summary

Stable flies (*Stomoxys calcitrans*) are a common problem in animal husbandry worldwide. Nuisance, especially by biting flies, can have a considerable negative impact on animal well-being, health and productivity. Furthermore, stable flies are known vectors of a range of pathogens. Insecticides constitute the mainstay for their control. However, if insecticides are applied in a non-strategic manner, there are risks of developing insecticide resistance within a few generations due to the high reproductive rate of stable flies. The objective of this study was to assess the occurrence of insecticide resistance in *Stomoxys calcitrans* on dairy farms in Brandenburg, Germany. Moreover, it aspired to propose best-bet strategies for on-farm pest control aiming to minimize the use of insecticides.

Based on a telephone questionnaire survey performed in June 2015 with 52 dairy farms in Brandenburg, 40 farms were selected for an on-farm cross-sectional survey. In this survey the FlyBox®-method was used in order to evaluate the susceptibility of stable flies to a deltamethrin impregnated polyester fabric. For confirmatory evaluation, *Stomoxys* populations from 10 farms were caught and colonies established in the laboratory. The susceptibility of the emerging F1 to F3 generations was tested with current methods under controlled conditions. In a first step, the LD_{95} of deltamethrin and azamethiphos were defined with established sensitive laboratory strains of *S. calcitrans*. Then the toxicity of both was assessed by topical application of the LD_{95} and multiples of it. The larvicidal effects of two insect growth regulators, cyromazine and pyriproxyfen, were evaluated at different concentrations based on the manufacturers' recommendations.

The questionnaire survey revealed that pyrethroids are the most frequently used insecticides (78.9 %) with deltamethrin being the dominant active ingredient (41 %). Furthermore, the on-farm survey using the FlyBox®-method indicated deltamethrin resistance in all of the 40 tested strains (100 %). In the laboratory tests, 24 hours following topical application of deltamethrin and azamethiphos to all of the 10 strains using the LD_{95} of 2.3 and 4.9 ng/fly, respectively, mortalities below 90 % were encountered. This led to the conclusion that these populations were resistant to the tested insecticides. Nine out of the 10 populations (90 %) even showed a mortality below 40 % at the LD_{95} of both deltamethrin and azamethiphos. Forty to 80 % of the mortality rates of multiples of the LD_{95} of azamethiphos and deltamethrin, respectively, were below 90 %. The insect growth regulators cyromazine and pyriproxyfen tested at the recommended concentrations resulted in an inhibition rate of 100 % of all 10 populations.

The study revealed that there is an urgent need to conduct further field surveys in different livestock production systems in order to draw general conclusions concerning the resistance status in Germany and the EU. Those studies would facilitate the elaboration of better integrated pest management strategies in order to warrant the use of insecticides as a last

resort only within a given cascade of possible control measures. This would sensitise farmers as well as veterinarians on resistance development and its impact on the environment. The design of genetic markers for the detection of insecticide resistance should be promoted in order to simplify further testing. In conclusion, the legal regulation of some insecticides should be evaluated regularly protecting human health and preventing negative impacts on honey bees, hymenoptera and other non-target insects.

7 Zusammenfassung

Vorkommen und Verbreitung von Insektizidresistenzen bei Wadenstechern (*Stomoxys calcitrans*) in Milchviehbetrieben im Bundesland Brandenburg, Deutschland

Wadenstecher (*Stomoxys calcitrans*) stellen weltweit ein häufiges Problem in der Nutzviehhaltung dar. Die Belästigung, die diese Fliegen vor allem durch ihre schmerzhaften Stiche bewirken, können erhebliche negative Auswirkungen auf das Wohlergehen, die Gesundheit und die Produktivität der Nutztiere haben. Darüber hinaus sind Wadenstecher bekannte Vektoren von einer Reihe von Pathogenen. Insektizide bilden die Hauptbekämpfungsmaßnahme für ihre Kontrolle. Wenn diese allerdings nicht strategisch angewendet werden, besteht das Risiko, dass sich aufgrund der hohen Reproduktionsrate der Wadenstecher innerhalb weniger Generationen Insektizidresistenzen entwickeln.

Ziel dieser Studie war es, das Vorkommen von Insektizidresistenzen bei *Stomoxys calcitrans* auf Milchviehbetrieben in Brandenburg zu beurteilen. Darüber wurde angestrebt, Strategien für die landwirtschaftliche Schädlingsbekämpfung zu entwickeln, um den Einsatz von Insektiziden in Zukunft zu minimieren.

Auf Grundlage einer telefonischen Fragebogenumfrage, die im Juni 2015 mit 52 Milchviehbetrieben in Brandenburg durchgeführt wurde, wurden 40 Betriebe für eine Feldstudie ausgewählt. In diesem Teil der Studie wurde die FlyBox®-Methode verwendet, um die Empfindlichkeit von Wadenstechern gegen ein mit Deltamethrin imprägniertes Polyestergewebe zu bewerten. Für die Überprüfung der Ergebnisse dieses Studienteils wurden Stomoxys-Populationen auf 10 Betrieben gefangen, um diese im Labor als Populationen zu etablieren. Die Empfindlichkeit der gezüchteten F1- bis F3-Generationen wurde mit aktuellen Methoden unter kontrollierten Bedingungen getestet. In einem ersten Schritt wurde dafür die LD_{95} von Deltamethrin und Azamethiphos mit etablierten sensitiven *S.calcitrans*-Laborstämmen bestimmt. Im Anschluss daran wurde die Toxizität beider mittels der topikalen Applikation der LD_{95} und Vielfacher davon beurteilt. Die larvizide Wirkung von zwei Insektenwachstumsregulatoren, Cyromazin und Pyriproxyfen, wurde in verschiedenen Konzentrationen um die Herstellerempfehlung herum bewertet.

Die Fragebogenumfrage ergab, dass Pyrethroide die am häufigsten verwendeten Insektizide (78,9 %) sind, wobei Deltamethrin der dominierende Wirkstoff (41 %) ist. Darüber hinaus zeigte die Felduntersuchung mit der FlyBox®-Methode, dass alle 40 getesteten Stämmen (100 %) resistent gegenüber Deltamethrin sind. In den Labortests wurden 24 Stunden nach der topikalen Applikation der LD_{95} von 2,3 bzw. 4,9 ng/Fliege von Deltamethrin und Azamethiphos bei allen 10 Stämmen Paralyseraten erreicht, die unter 90% lagen. Dies führte zu der Schlussfolgerung, dass diese Populationen gegen die getesteten Insektizide resistent sind.

Neun dieser 10 Populationen (90%) zeigten sogar eine Mortalität unter 40% bei der LD_{95} von Deltamethrin und Azamethiphos. Vierzig bis 80% der Paralyseraten von Vielfachen der LD_{95} von Azamethiphos bzw. Deltamethrin lagen unter 90%. Die empfohlene Herstellerdosis der getesteten Insektenwachstumsregulatoren Cyromazin und Pyriproxyfen führte bei allen 10 Populationen zu Inhibitionsraten von 100%.

Die Studie hat gezeigt, dass eine dringende Notwendigkeit besteht, weitere Untersuchungen in anderen Nutztierhaltungssystemen durchzuführen, um allgemeine Schlussfolgerungen über den Resistenzstatus in Deutschland und der EU ziehen zu können. Diese Studien würden die Ausarbeitung eines verbesserten integrierten Schädlingsmanagements unterstützen, um so den Einsatz von Insektiziden als letztes Mittel der Wahl in einer Kaskade möglicher Bekämpfungsmethoden zu gewährleisten. Dies würde sowohl Landwirte als auch Tierärzte für die Resistenzentwicklung und ihre Bedeutung für die Umwelt sensibilisieren. Die Entwicklung von genetischen Markern sollte gefördert werden, um zukünftige Untersuchungen zu vereinfachen. Zudem sollte die gesetzliche Regulierung mancher Insektizide regelmäßig evaluiert werden, um die menschliche Gesundheit zu schützen und negativen Auswirkungen auf Honigbienen, Hymenopteren und andere Nicht-Ziel-Insekten vorzubeugen.

8 Glossary

WHO (2016):

Ace-1	A target-site resistance gene for carbamate and organophosphate insecticides conferring insensitive acetylcholinesterase. The resistance is caused by a single mutation, G119S, of the *Ace-1* gene.
Cross resistance	Resistance towards substances of different chemical groups, even where the insect population or strain has not been selected by exposure to the latter.
Discriminating dose	A fixed dose of an insecticide ingredient dissolved in a solvent that is topically applied on the insect body; used to discriminate the proportions of susceptible and resistant phenotypes in a sample of a population.
F1 progeny	Generally means “first generation offspring”, but in this context refers to the use of adults raised from the eggs of wild-caught female flies to obtain an age-standardized sample of the wild population for use in bioassay tests for resistance.
Kdr (knockdown mutation)	Knockdown resistance is caused by a series of genes involving a mutation in the sodium ion channel, the target site of pyrethroids and organochlorine compounds and conferring resistance to these insecticides.
Larvicide	A chemical substance applied to larval habitats to kill larvae.
LD_{95}	Lethal dosis at which 95 % of a sensitive stable fly population die.
Multi resistance	Resistance to more than one class of insecticide.
pen	Mutation of the pen resistance factor gene, which is localised on chromosome III is

	characterized by reducing the penetration rate of insecticide through the cuticle.
Resistance	Ability of an insect strain to tolerate doses of toxic substances that would cause the death of the majority of sensitive individuals of the same species.
Side resistance	Resistance to the same chemical class with the same mode of action.
Susceptible population	A population that has not been subjected to insecticidal pressure and in which resistant individuals are either absent or rare.
Synergist	A substance that does not itself have insecticidal properties, but which, when mixed and applied with insecticides of a particular class, considerably enhances their potency by inhibiting an enzyme that normally acts to detoxify the insecticide in the insect system.

9 Annex

Questionnaire analysis - *Stomoxys calcitrans*

Enterprise	
Address	
Telefone number	
N°	
Dairy farm?	○ Yes ○ No
Organic or conventional husbandry?	○ Organic ○ Conventional
Size of herd?	○ < 50 ○ 50 – 200 ○ > 200 ○ How many: _____
Husbandry system Flooring conditions Dung removal technique Dung management	*Tethered housing:* ○ Yes ○ No *Freestall type:* ○ Deep litter stable ○ Open cubicle stable ○ With litter ○ Without litter ○ Concrete floor ○ Rubber mat ○ Slatted floor ○ Partly slatted floor *With litter – solid manure:* Continuous dung removal: ○ manual ○ Complete litter exchanging procedure ○ Several layer system ○ Partly automated/ mobile ○ automated ○ with slider/ chain conveyer/ push rod on a concrete floor

	○ with slider/ chain conveyer/ push rod on a perforated/ slatted floor Discontinous dung removal: ○ Deep litter procedure *Without litter - slurry:* ○ Continuous passage through perforated floors to slurry pit ○ Discontinuous procedure: opening of gate valves at intervals ○ Slurry removal through pipes ○ Manual cleaning with water hose • *Storage in stable*: ○ Solid manure in deep litter ○ Slurry in deep slurry pits • *Storage outside of the stable:* ○ Solid manure on concrete slab ○ Slurry in containers ○ aboveground ○ underground ○ Further processing in attached biogas plant
With run? Possibility to graze?	○ Yes ○ No ○ Yes ○ No
Problems with flies? Quantitative appraisal of fly abundance?	○ Yes ○ No ○ low ○ medium ○ high
During which season is the fly abundance the highest?	
Problems with blood-sucking flies? Defensive movement in the milking parlor? Differentiation to stable fly possible?	○ Yes ○ No
Quantitative appraisal of haematophagous fly abundance?	○ I don't know ○ low ○ medium ○ high

During which season is the haematophagous fly abundance the highest?	
What kind of control measures do you apply against flies?	○ None ○ Chemical control measures (insecticides) ○ Biological control measures (for example pteromalid wasps) ○ Physical control measures (sticky traps, uv-light-traps) ○ Other:_____
Have you changed the product in use within the last 3 years?	○ Yes ○ No
Insecticides in use in the past Mode of application	○ Pour on ○ Wall spray ○ Net ○ Ear tags ○ Fumigant ○ As a liquid pour-on for slurry or manure ○ Other:_____
Brand	○ Bayofly ○ Latroxin Delta ○ Butox ○ Neporex ○ Baycidal ○ Agita ○ Quickbayt ○ SPY ○ LarvEx ○ Other:_____
Current insecticides in use Mode of application	○ Pour on ○ Wall spray ○ Net ○ Ear tags ○ Fumigant ○ As a liquid pour-on for slurry or manure Other:_____
Brand	○ Bayofly ○ Latroxin Delta ○ Butox ○ Neporex ○ Baycidal

	○ Agita ○ Quickbayt ○ SPY ○ LarvEx ○ Other:_____
Period of application	
Frequency of application	
How satisfied are you with your kind of control measurement?	○ Not satisfied ○ Fairly satisfied ○ Very satisfied
Abnormalities? Comparison before and now?	
Have you noticed a loss in effectivity?	○ Yes ○ No

10 Bibliography

98/8/EC, 1998. 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market. Off. J. Eur. Communities: Legis, 1-63.

528/2012, E., 2012. EU-Regulation no. 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products. Off. J. of European Union L 167, 1-123.

2001/82/EC, 2001. Directive 2001/82/Ec Of The European Parliament And Of The Council of 6 November 2001 on the Community code relating to veterinary medicinal products.

2377/90, E.N. 1990. Council Regulation (EEC) No 2377/90 of 26 June 1990 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin, 1-8.

Abasa, R.O., 1983. Effects of temperature, relative humidity, lipid and water content on post-oviposition development of eggs of *Stomoxys calcitrans*. Entomologia experimentalis et applicata 33, 259-262.

Abbas, N., Khan, H.A.A., Shad, S.A., 2014. Resistance of the house fly *Musca domestica* (Diptera: Muscidae) to lambda-cyhalothrin: mode of inheritance, realized heritability, and cross-resistance to other insecticides. Ecotoxicology 23, 791-801.

Abbott, W., 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18, 265-267.

Acevedo, G.R., Zapater, M., Toloza, A.C., 2009. Insecticide resistance of house fly, Musca domestica (L.) from Argentina. Parasitology research 105, 489-493.

Agee, H., Patterson, R., 1983. Spectral sensitivity of stable, face, and horn flies and behavioral responses of stable flies to visual traps (Diptera: Muscidae). Environmental Entomology 12, 1823-1828.

Agency, E.P. 2017. Prohibited Products (Ministry of Environment and Food of Denmark).

Akca, I., Tuncer, C., Guler, A., Saruhan, I., 2009. Residual toxicity of 8 different insecticides on honey bee (*Apis mellifera* Hymenoptera: Apidae). J. Anim. Vet. Adv 8, 436-440.

Akiner, M.M., Çaglar, S.S., 2012. Monitoring of five different insecticide resistance status in Turkish house fly *Musca domestica* L.(Diptera: Muscidae) populations and the relationship between resistance and insecticide usage profile. Türkiye Parazitolojii Dergisi 36, 87.

Albuquerque, T.A., Zurek, L., 2014. Temporal changes in the bacterial community of animal feces and their correlation with stable fly oviposition, larval development, and adult fitness. Frontiers in microbiology 5.

Amir, O., Peveling, R., 2004. Effect of triflumuron on brood development and colony survival of free-flying honeybee, *Apis mellifera* L. Journal of Applied Entomology 128, 242-249.

Anderson, J.R., 1978. Mating behavior of *Stomoxys calcitrans*: Effects of a blood meal on the mating drive of males and its necessity as a prerequisite for proper insemination of females. Journal of Economic Entomology 71, 379-386.

Anderson, J.R., Tempelis, C., 1970. Precipitin test identification of blood meals of Stomoxys calcitrans (L.) caught on California poultry ranches, and observations of digestion rates of bovine and citrated human blood. Journal of Medical Entomology 7, 223-229.

Arends, M., 2016. Die Wirksamkeit von Insektizid-behandelten Netzen zur Bekämpfung von Vektoren und Lästlingsinsekten mit veterinärmedizinischer und medizinischer Bedeutung in Burkina Faso, West-Afrika. Freie Universität Berlin, Thesis, Journal No.: 3832.

Ashrafi, S.H., 1964. The cultivation and nutritional requirements of *Stomoxys calcitrans*. Bulletin of the World Health Organization 31, 519.

Bailey, D., TL, W., Smittle, B., 1973. Flight and dispersal of stable fly - Diptera - Muscidae. Journal of Economic Entomology 66, 410-411.

Bailey, D.L., Whitfield, T., LaBrecque, G., 1975. Laboratory biology and techniques for mass producing the stable fly, *Stomoxys calcitrans* (L.)(Diptera: Muscidae). Journal of Medical Entomology 12, 189-193.

Baker, A., 1918. The Effect of Stable and Horn Fly Attacks on Milk Production. Book: The Effect of Stable and Horn Fly Attacks on Milk Production., 91-93.

Baldacchino, F., Muenworn, V., Desquesnes, M., Desoli, F., Charoenviriyaphap, T., Duvallet, G., 2013. Transmission of pathogens by *Stomoxys* flies (Diptera, Muscidae): a review. Parasite 2013, 20-26.

Barker, R.J., Taber, S., 1977. Effects of diflubenzuron fed to caged honey bees. Environmental Entomology 6, 167-168.

Barré, N., 1981. Les stomoxes ou «mouches bœuf» à la réunion. Pouvoir pathogène, écologie, moyen de lutte. GERDAT-IEMVT, Maisons-Alfort, France, 90.

Bates, P., 2012. External Parasites of Small Ruminants. A practical guide to their prevention and control.169 256 p.

Bauer, B., Gitau, D., Oloo, F., Karanja, S., 2006. Evaluation of a preliminary test to protect zero-grazed dairy cattle with insecticide-treated mosquito netting in western Kenya. Tropical animal health and production 38, 29-34.

Beckmann, M., Haack, K.J., 2003. Insektizide für die Landwirtschaft: Chemische Schädlingsbekämpfung. Chemie in unserer Zeit 37, 88-97.

Bell, H.A., Robinson, K.A., Weaver, R.J., 2010. First report of cyromazine resistance in a population of UK house fly (*Musca domestica*) associated with intensive livestock production. Pest Management Science 66, 693-695.

Berberian, D., 1938. Successful Transmission of Cutaneous Leishmaniasis by the Bites of *Stomoxys calcitrans*. Proceedings of the Society for Experimental Biology and Medicine 38, 254-256.

Beresford, D., Sutcliffe, J., 2006. Studies on the effectiveness of Coroplast sticky traps for sampling stable flies (Diptera: Muscidae), including a comparison to Alsynite. Journal of Economic Entomology 99, 1025-1035.

Beresford, D., Sutcliffe, J., 2009. The effect of *Macrocheles muscaedomesticae* and *M. subbadius* (Acarina: Macrochelidae) phoresy on the dispersal of *Stomoxys calcitrans* (Diptera: Muscidae). Systematic and Applied Acarology Special Publications 23, 1-30.

Bergé, J., Feyereisen, R., Amichot, M., 1998. Cytochrome P450 monooxygenases and insecticide resistance in insects. Philosophical Transactions of the Royal Society of London B: Biological Sciences 353, 1701-1705.

Berkebile, D.R., 1995. Developmental sites of immature stages of the stable fly and house fly (Diptera: Muscidae) found outside of cattle feedlot pens and the overwintering of the stable fly. ETD collection for University of Nebraska - Lincoln. *AA*I9604401.

Berkebile, D.R., Thomas, G.D., Campbell, J.B., 1994. Overwintering of the stable fly (Diptera: Muscidae) in southeastern Nebraska. Journal of Economic Entomology 87, 1555-1563.

Berry, I., Foerster, K., Ilcken, E., 1976. Prediction model for development time of stable flies. Transactions of the ASAE 19, 123-0127.

Berry, I., Kunz, S., Foerster, K., 1977. A dynamic model of the physiological development of immature stable flies. Annals of the Entomological Society of America 70, 173-176.

Berry, I., Stage, D., Campbell, J., 1983. Populations and economic impacts of stable flies on cattle. Transactions of the ASAE 26, 873-0877.

Bigalke, R.D., 1968. New concepts on the epidemiological features of bovine besnoitiosis as determined by laboratory and field investigations. Onderstepoort Journal of Veterinary Research 35, 3-137.

Birkemoe, T., Soleng, A., Aak, A., 2009. Biological control of *Musca domestica* and *Stomoxys calcitrans* by mass releases of the parasitoid *Spalangia cameroni* on two Norwegian pig farms. BioControl 54, 425-436.

Bishopp, F., 1913. The Stable Fly (*Stomoxys calcitrans*, L.), an important live-stock pest. Journal of Economic Entomology 6, 112-126.

Bishopp, F., Dove, W., Parman, D., 1915. Notes on certain points of economic importance in the biology of the house fly. Journal of Economic Entomology 8, 54-71.

Bishopp, F.C., 1920. The stable fly: how to prevent its annoyance and its losses to live stock. US Department of Agriculture. Farmer's Bulletin 1097, 13-21.

Bisset, J., Rodriguez, M., Soca, A., Pasteur, N., Raymond, M., 1997. Cross-resistance to pyrethroid and organophosphorus insecticides in the southern house mosquito (Diptera: Culicidae) from Cuba. Journal of Medical Entomology 34, 244-246.

Bitman, J., Cecil, H.C., Harris, S.J., Fries, G.F., 1968. Estrogenic activity of o, p'-DDT in the mammalian uterus and avian oviduct. Science 162, 371-372.

Blank, J., 2008. Die Wirkung eines Insektizid-behandelten Netzzaunes zum Schutz von Pferden gegen Weidefliegen.Thesis, Journal No.: 3073, Freie Universität Berlin.

Boire, S., Bay, D., Olson, J., 1988. An evaluation of various types of manure and vegetative material as larval breeding media for the stable fly. Southwestern Entomologist 13, 247-249.

Bouet, G., Roubaud, E., 1912. Expériences de transmission des trypanosomiases animales d'Afrique occidentale française par les stomoxes. Bulletin de la Société de Pathologie Exotique 5, 544-550.

Brain, C.K., 1912. *Stomoxys calcitrans* Linn. Annals of the Entomological Society of America 5, 421-430.

Brain, C.K., 1913. *Stomoxys calcitrans* Linn, Part II. Annals of the Entomological Society of America 6, 197-201.

Brengues, C., Hawkes, N.J., Chandre, F., McCarroll, L., Duchon, S., Guillet, P., Manguin, S., Morgan, J., Hemingway, J., 2003. Pyrethroid and DDT cross-resistance in Aedes aegypti is correlated with novel mutations in the voltage-gated sodium channel gene. Medical and Veterinary Entomology 17, 87-94.

Broce, A.B., 1988. An improved Alsynite trap for stable flies, *Stomoxys calcitrans* (Diptera: Muscidae). Journal of Medical Entomology 25, 406-409.

Broce, A.B., Hogsette, J., Paisley, S., 2005. Winter feeding sites of hay in round bales as major developmental sites of *Stomoxys calcitrans* (Diptera: Muscidae) in pastures in spring and summer. Journal of Economic Entomology 98, 2307-2312.

Brown, A., 1961. The challenge of insecticide resistance. Bulletin of the Entomological Society of America 7, 6-19.

Brown, T.M., Brogdon, W.G., 1987. Improved detection of insecticide resistance through conventional and molecular techniques. Annual review of entomology 32, 145-162.

Bruce, W., Decker, G.C., 1958. The relationship of stable fly abundance to milk production in dairy cattle. Journal of Economic Entomology 51, 269-274.

Brues, C.T., 1913. The Geographical Distribution of the stable fly, *Stomoxys calcitrans*. Journal of Economic Entomology 6, 459-477.

Bull, D., Meola, R., 1993. Effect and fate of the insect growth regulator pyriproxyfen after application to the horn fly (Diptera: Muscidae). Journal of Economic Entomology 86, 1754-1760.

Burg, J., Axtell, R., 1984. Monitoring house fly, *Musca domestica* (Diptera: Muscidae), populations in caged-layer poultry houses using a baited jug-trap. Environmental Entomology 13, 1083-1090.

Burg, J., Neely, D., Williams, N., Knapp, F.W., 1994. Retention and attempted mechanical transmission of *Ehrlichia risticii* by *Stomoxys calcitrans*. Medical and Veterinary Entomology 8, 43-46.

Burg, J., Roberts, A., Williams, N., Powell, D., Knapp, F.W., 1990. Attempted transmission of *Ehrlichia risticii* (Rickettsiaceae) with *Stomoxys calcitrans* (Diptera: Muscidae). Journal of Medical Entomology 27, 874-877.

Buschman, L.L., Patterson, R.S., 1981. Assembly, mating, and thermoregulating behavior of stable flies under field conditions. Environmental Entomology 10, 16-21.

Busvine, J., 1951. Mechanism of resistance to insecticide in house flies. Nature, 193-195.

Busvine, J.R., 1957. Insecticide-resistant strains of insects of public health importance. Transactions of the Royal Society of Tropical Medicine and Hygiene 51, 11-31.

Buxton, B., Hinkle, N., Schultz, R., 1985. Role of insects in the transmission of bovine leukosis virus: potential for transmission by stable flies, horn flies, and tabanids. American journal of veterinary research 46, 123-126.

Çakir, Ş., Sarikaya, R., 2005. Genotoxicity testing of some organophosphate insecticides in the *Drosophila* wing spot test. Food and Chemical Toxicology 43, 443-450.

Campbell, J., Berry, I., Boxler, D., Davis, R., Clanton, D., Deutscher, G., 1987. Effects of stable flies (Diptera: Muscidae) on weight gain and feed efficiency of feedlot cattle. Journal of Economic Entomology 80, 117-119.

Campbell, J., Skoda, S., Berkebile, D., Boxler, D., Thomas, G., Adams, D., Davis, R., 2001. Effects of stable flies (Diptera: Muscidae) on weight gains of grazing yearling cattle. Journal of Economic Entomology 94, 780-783.

Campbell, J., White, R., Wright, J., Crookshank, R., Clanton, D., 1977. Effects of stable flies on weight gains and feed efficiency of calves on growing or finishing rations. Journal of Economic Entomology 70, 592-594.

Carlson, D., Mayer, M., Silhacek, D., James, J., Beroza, M., Bierl, B., 1971. Sex attractant pheromone of the house fly: isolation, identification and synthesis,76-78.

Castro, B.G.d., Souza, M., Bittencourt, A.J., 2007. Aerobic bacterial microbiota in *Stomoxys calcitrans*: preliminary studies in Brazil. Revista Brasileira de Parasitologia Veterinária 16, 193-197.

Catangui, M.A., Campbell, J.B., Thomas, G.D., Boxler, D.J., 1993. Average daily gains of Brahman-Crossbred and English×Exotic feeder heifers exposed to low, medium, and high levels of stable flies (Diptera: Muscidae). Journal of Economic Entomology 86, 1144-1150.

Catangui, M.A., Campbell, J.B., Thomas, G.D., Boxler, D.J., 1997. Calculating economic injury levels for stable flies (Diptera: Muscidae) on feeder heifers. Journal of Economic Entomology 90, 6-10.

CDC, 2010. Guideline for evaluating insecticide resistance in vectors using the CDC bottle bioassay. Atlanta: The Centers Centers for Disease Control Prevention, 5-28.

Cerf, D.C., Georghiou, G.P., 1974. Cross resistance to juvenile hormone analogues in insecticide-resistant strains of *Musca domestica* L. Pest Management Science 5, 759-767.

Cetin, H., Erler, F., Yanikoglu, A., 2009. Survey of insect growth regulator (IGR) resistance in house flies (*Musca domestica* L.) from southwestern turkey. Journal of Vector Ecology 34, 329-337.

Chandre, F., Darriet, F., Manguin, S., Brengues, C., Carnevale, P., Guillet, P., 1999. Pyrethroid cross resistance spectrum among populations of *Anopheles gambiae* ss from Cote d'Ivoire. Journal of the American Mosquito Control Association 15, 53-59.

Cheng, T.-H., Kesler, E.M., 1961. A three-year study on the effect of fly control on milk production by selected and randomized dairy herds. Journal of Economic Entomology 54, 751-757.

Chia, L.S., Baxter, J.A., Morrison, P.E., 1982. Quantitative relationship between ingested blood and follicular growth in the stable fly, *Stomoxys calcitrans*. Canadian Journal of Zoology 60, 1917-1921.

Chihota, C., Rennie, L., Kitching, R., Mellor, P., 2003. Attempted mechanical transmission of lumpy skin disease virus by biting insects. Medical and Veterinary Entomology 17, 294-300.

Cilek, J., 2003. Attraction of colored plasticized corrugated boards to adult stable flies, *Stomoxys calcitrans* (Diptera: Muscidae). Florida Entomologist 86, 420-423.

Cilek, J., Greene, G., 1994. Stable fly (Diptera: Muscidae) insecticide resistance in Kansas cattle feedlots. Journal of Economic Entomology 87, 275-279.

Clark, A., Shamaan, N., 1984. Evidence that DDT-dehydrochlorinase from the house fly is a glutathione S-transferase. Pesticide Biochemistry and Physiology 22, 249-261.

Cohn, B.A., Wolff, M.S., Cirillo, P.M., Sholtz, R.I., 2007. DDT and breast cancer in young women: new data on the significance of age at exposure. Environmental Health Perspectives, 1406-1414.

Coler, R., Boucias, D., Frank, J., Maruniak, J., GARCIA-CANEDO, A., Pendland, J., 1993. Characterization and description of a virus causing salivary gland hyperplasia in the housefly, *Musca domestica*. Medical and Veterinary Entomology 7, 275-282.

Coppage, D.L., Matthews, E., 1974. Short-term effects of organophosphate pesticides on cholinesterases of estuarine fishes and pink shrimp. Bulletin of Environmental Contamination and Toxicology 11, 483-488.

Corbel, V., N'guessan, R., Brengues, C., Chandre, F., Djogbenou, L., Martin, T., Akogbéto, M., Hougard, J.-M., Rowland, M., 2007. Multiple insecticide resistance mechanisms in *Anopheles gambiae* and *Culex quinquefasciatus* from Benin, West Africa. Acta tropica 101, 207-216.

Coronado, A., Butler, J., Becnel, J., Hogsette, J., 2004. Artificial feeding in the stable fly *Stomoxys calcitrans* and their relationship with the blood meal destination. In: Proceedings of the 1st International Symposium and 2nd National Symposium on Hemoparasites and Their Vectors, pp. 50-51.

Cortinas, R., Jones, C.J., 2006. Ectoparasites of cattle and small ruminants. Veterinary Clinics of North America: Food Animal Practice 22, 673-693.

Crespo, D.C., Lecuona, R.E., Hogsette, J.A., 1998. Biological Control: An Important Component in Integrated Management of *Musca domestica* (Diptera: Muscidae) in Caged-Layer Poultry Houses in Buenos Aires, Argentina. Biological Control 13, 16-24.

Cruz-Vázquez, C., García-Vázquez, Z., Fernández-Ruvalcaba, M., George, J.E., 2005. Susceptibilidad de *Stomoxys calcitrans* (L.) a la permetrina en establos lecheros de Aguascalientes, México Susceptibility of *Stomoxys calcitrans* (L.) to permethrin in dairy farms of Aguascalientes, Mexico. Vet. Méx 36, 4.

Davies, T., Field, L., Usherwood, P., Williamson, M., 2007. DDT, pyrethrins, pyrethroids and insect sodium channels. IUBMB life 59, 151-162.

Deplazes, P., Eckert, J., von Samson-Himmelstjerna, G., Zahner, H., 2012. Lehrbuch der Parasitologie für die Tiermedizin. Stuttgart: Georg Thieme Verlag.

Desquesnes, M., Dia, M.L., 2003a. Mechanical transmission of Trypanosoma congolense in cattle by the African tabanid *Atylotus agrestis*. Experimental Parasitology 105, 226-231.

Desquesnes, M., Dia, M.L., 2003b. *Trypanosoma vivax*: mechanical transmission in cattle by one of the most common African tabanids, *Atylotus agrestis*. Experimental Parasitology 103, 35-43.

Desquesnes, M., Dia, M.L., 2004. Mechanical transmission of *Trypanosoma vivax* in cattle by the African tabanid *Atylotus fuscipes*. Veterinary Parasitology 119, 9-19.

Devonshire, A.L., Moores, G.D., 1982. A carboxylesterase with broad substrate specificity causes organophosphorus, carbamate and pyrethroid resistance in peach-potato aphids (*Myzus persicae*). Pesticide Biochemistry and Physiology 18, 235-246.

DeVries, D., Georghiou, G., 1980. A wide spectrum of resistance to pyrethroid insecticides in *Musca domestica*. Cellular and Molecular Life Sciences 36, 226-227.

Dougherty, C., Knapp, F., Burrus, P., Willis, D., Burg, J., Cornelius, P., Bradley, N., 1993. Stable flies (*Stomoxys calcitrans* L.) and the behavior of grazing beef cattle. Applied Animal Behaviour Science 35, 215-233.

Dougherty, C., Knapp, F., Burrus, P., Willis, D., Cornelius, P., 1995. Behavior of grazing cattle exposed to small populations of stable flies (*Stomoxys calcitrans* L.). Applied Animal Behaviour Science 42, 231-248.

Douris, V., Steinbach, D., Panteleri, R., Livadaras, I., Pickett, J.A., Van Leeuwen, T., Nauen, R., Vontas, J., 2016. Resistance mutation conserved between insects and mites unravels the benzoylurea insecticide mode of action on chitin biosynthesis. Proceedings of the National Academy of Sciences, 201618258.

Doyle, M.S., Swope, B.N., Hogsette, J.A., Burkhalter, K.L., Savage, H.M., Nasci, R.S., 2011. Vector competence of the stable fly (Diptera: Muscidae) for West Nile virus. Journal of Medical Entomology 48, 656-668.

EC-Regulation, 1907/2006. Verordnung (Eg) Nr. 1907/2006 Des Europäischen Parlaments Und Des Rates.

EC-Regulation, 2005. 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. EEC.

El-Din, H., El-Samni, M., Attia, M., 1990. Latent effects of some insect growth regulators on the body weight of workers of the honey bee *Apis mellifera carnica*. Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz 63, 67-68.

El-Wakeil, N.E., 2013. Botanical pesticides and their mode of action. Gesunde Pflanzen 65, 125-149.

Elissa, N., Mouchet, J., Rivière, F., Meunier, J.-Y., Yao, K., 1993. Resistance of Anopheles gambiae ss to pyrethroids in Côte d'Ivoire. In: Annales-Societe Belge De Medecine Tropicale, pp. 291-291.

Enan, E.E., 2005. Molecular and pharmacological analysis of an octopamine receptor from American cockroach and fruit fly in response to plant essential oils. Archives of insect biochemistry and physiology 59, 161-171.

Estienne, M., Knapp, F., Boling, J., Burg, J., 1991. Physiological and nutritional responses of beef steers exposed to stable flies (Diptera: Muscidae). Journal of Economic Entomology 84, 1262-1265.

Eto, M., 1990. Biochemical mechanisms of insecticidal activities, In: Controlled Release, Biochemical Effects of Pesticides, Inhibition of Plant Pathogenic Fungi. Springer, pp. 65-107.

Fankhauser, B., Irwin, J.P., Stone, M.L., Chester, S.T., Soll, M.D., 2015. Repellent and insecticidal efficacy of a new combination of fipronil and permethrin against stable flies (*Stomoxys calcitrans*). Parasites & Vectors 8, 61.

FAO, 1967. Report of the first session of the Food and Agriculture Organization Panel of Experts on Integrated Pest Control. Rome (Italy) , Sept. 18-22 19 pp.

FAO, 2004. Resistance Management and Integrated Parasite Control in Ruminants – Guidelines, Module 1 – Ticks: Acaricide Resistance: Diagnosis, Management and Prevention. Food and Agriculture Organization, Animal Production and Health Division Rome, pp. 53.

Farnham, A.W., 1973. Genetics of resistance of pyrethroid-selected houseflies, *Musca domestica* L. Pest Management Science 4, 513-520.

Farnham, A.W., Murray, A.W., Sawicki, R.M., Denholm, I., White, J.C., 1987. Characterization of the structure-activity relationship of kdr and two variants of super-kdr to pyrethroids in the housefly (*Musca domestica* L.). Pest Management Science 19, 209-220.

Fay, R., 1939. A Control for the Larvae of Houseflies in Manure Piles. Journal of Economic Entomology 32, 851-854.

Ferris, D., Hanson, R., Dicke, R., Roberts, R., 1955. Experimental transmission of vesicular stomatitis virus by Diptera. Journal of Infectious Diseases 96, 184-192.

Feyereisen, R., 1995. Molecular biology of insecticide resistance. Toxicology letters 82, 83-90.

Ffrench-Constant, R.H., Anthony, N., Aronstein, K., Rocheleau, T., Stilwell, G., 2000. Cyclodiene insecticide resistance: from molecular to population genetics. Annual review of entomology 45, 449-466.

Fleming, R., Retnakaran, A., 1985. Evaluating Single Treatment Data Using Abbott's Formula With Reference to Insecticides. Journal of Economic Entomology, 78, 1179-1181.

Foil, L., Hogsette, J., 1994. Biology and control of tabanids, stable flies and horn flies. Revue scientifique et technique (International Office of Epizootics) 13, 1125-1158.

Foil, L., Meek, C., Adams, W., Issel, C., 1983. Mechanical transmission of equine infectious anemia virus by deer flies (*Chrysops flavidus*) and stable flies (*Stomoxys calcitrans*). American journal of veterinary research, 44, 155-156.

Foil, L.D., Younger, C.D., 2006. Development of treated targets for controlling stable flies (Diptera: Muscidae). Veterinary Parasitology 137, 311-315.

Freeborn, S.B., Regan, W., 1932. Fly Sprays for Dairy Cows–A Progress Report. Journal of Economic Entomology 25, 167-174.

Freeborn, S.B., Regan, W.M., Folger, A.H., 1925. The relation of flies and fly sprays to milk production. Journal of Economic Entomology 18, 779-790.

Friesen, K., Chen, H., Zhu, J., Taylor, D.B., 2015. External morphology of stable fly (Diptera: Muscidae) larvae. Journal of Medical Entomology, 52, 626-637.

Friesen, K.M., Johnson, G.D., 2012. Reproductive potential of stable flies (Diptera: Muscidae) fed cattle, chicken, or horse blood. Journal of Medical Entomology 49, 461-466.

Gangishetti, U., Breitenbach, S., Zander, M., Saheb, S.K., Müller, U., Schwarz, H., Moussian, B., 2009. Effects of benzoylphenylurea on chitin synthesis and orientation in the cuticle of the *Drosophila* larva. European journal of cell biology 88, 167-180.

Garabrant, D.H., Held, J., Langholz, B., Peters, J.M., Mack, T.M., 1992. DDT and related compounds and risk of pancreatic cancer. Journal of the National Cancer Institute 84, 764-771.

Geden, C., Garcia-Maruniak, A., Lietze, V., Maruniak, J., Boucias, D., 2011. Impact of house fly salivary gland hypertrophy virus (MdSGHV) on a heterologous host, *Stomoxys calcitrans*. Journal of Medical Entomology 48, 1128-1135.

Geden, C., Hogsette, J. 2001. Research and Extension Needs for Integrated Management Programs for Livestock and Poultry, 269 pp.

Geden, C.J., 2012. Status of biopesticides for control of house flies. Journal of Biopesticides 5, 1-11.

Geden, C.J., Moon, R.D., 2009. Host ranges of gregarious muscoid fly parasitoids: *Muscidifurax raptorellus* (Hymenoptera: Pteromalidae), *Tachinaephagus zealandicus* (Hymenoptera: Encyrtidae), and *Trichopria nigra* (Hymenoptera: Diapriidae). Environmental Entomology 38, 700-707.

Georghiou, G.P., 1990. Overview of insecticide resistance, In: ACS Publications. Managing Resistance to Agrochemicals, Chapter 2, 18–4.

Georghiou, G.P., 1994. Principles of insecticide resistance management. Phytoprotection 75, 51-59.

Georghiou, G.P., Taylor, C.E., 1977a. Genetic and biological influences in the evolution of insecticide resistance. Journal of Economic Entomology 70, 319-323.

Georghiou, G.P., Taylor, C.E., 1977b. Operational influences in the evolution of insecticide resistance. Journal of Economic Entomology 70, 653-658.

Gfeller, E.W.M.F.J. 1999. *Stomoxys calcitrans*: topical application (micro droplet) bioassays with fifteen standard chemicals. Novartis Animal Health Inc. Technical Report. Author-Report No.: 628, 1-7.

Gfeller, F.J., 2014. Zuchtmethode für *Stomoxys calcitrans*. Novartis Animal Health Inc., Bioprotection Laboratory, 1-7.

Gfeller, F.J., Breuer, M. 2012. Study IDL 1053 Larvicide activity of Pyri-Shield against a Musca domestica field strain from the USA (Code: Green Valley and Green Valley II), Final Report. Novartis Animal Health Inc. Bioprotection Laboratory,1-12.

Giannakakis, A., Fletcher, B., 1985. Morphology and distribution of antennal sensilla of *Dacus tryoni* (Froggatt) (Diptera: Tephritidae). Australian Journal of Entomology 24, 31-35.

Gibbs, E., Johnson, R., Gatehouse, A., 1973. laboratory technique for studying the mechanical transmission of bovine herpes mammillitis virus by the stable fly (*Stomoxys calcitrans* L.). Research in Veterinary Science, 145-147.

Gill, S.S., Cowles, E.A., Pietrantonio, P.V., 1992. The mode of action of *Bacillus thuringiensis* endotoxins. Annual review of entomology 37, 615-634.

Gilles, J., David, J.-F., Duvallet, G., 2005. Temperature effects on development and survival of two stable flies, *Stomoxys calcitrans* and *Stomoxys niger niger* (Diptera: Muscidae), in La Reunion island. Journal of Medical Entomology 42, 260-265.

Gilles, J., David, J.F., Duvallet, G., De La Rocque, S., Tillard, E., 2007. Efficiency of traps for *Stomoxys calcitrans* and *Stomoxys niger niger* on Reunion Island. Medical and Veterinary Entomology 21, 65-69.

Gingrich, R., 1965. *Bacillus thuringiensis* as a feed additive to control dipterous pests of cattle. Journal of Economic Entomology 58, 363-364.

Glaser, R., 1924. Rearing flies for experimental purposes with biological notes. Journal of Economic Entomology 17, 486-496.

Goss, P.J., McKenzie, J.A., 1996. Selection, refugia, and migration: simulation of evolution of dieldrin resistance in *Lucilia cuprina* (Diptera: Calliphoridae). Journal of Economic Entomology 89, 288-301.

Green, B., Foil, L., Hagius, S., Issel, C., 1996. Stability of equine infectious anemia virus in *Aedes aegypti* (Diptera: Culicidae), *Stomoxys calcitrans* (Diptera: Muscidae), and *Tabanus fuscicostatus* (Diptera: Tabanidae) stored at -70 degrees C. Journal of the American Mosquito Control Association 12, 334-336.

Greene, G.L., Hogsette, J., Patterson, R., 1989. Parasites That Attack Stable Fly and House Fly (Diptera: Muscidae) Pupa ria During the Winter on Dairies in Northwestern Florida. Journal of Economic Entomology 82, 412-415.

Grundy, D.L., Still, C.C., 1985. Inhibition of acetylcholinesterases by pulegone-1, 2-epoxide. Pesticide Biochemistry and Physiology 23, 383-388.

Guengerich, F., 1991. Reactions and significance of cytochrome P-450 enzymes. Journal of Biological Chemistry 266, 10019-10022.

Guglielmone, A., 1995. Epidemiology of babesiosis and anaplasmosis in South and Central America. Veterinary Parasitology 57, 109-119.

Gunjima, K., 1992. Oral toxicities of some pyrethroids to the housefly, *Musca domestica* L. (Diptera: Muscidae). Applied Entomology and Zoology 27, 319-324.

Hafez, M., Gamal-Eddin, F., 1959. Ecological studies on *Stomoxys calcitrans* L. and sitiens Rond, in Egypt, with suggestions on their control (Diptera: Muscidae). Bulletine de la Societe Entomologique d'Egypte 43, 245-283.

Hale, K.M., 2011. Proximate causation of stable fly (*Stomoxys calcitrans* (L.)) host use: the influence of phenology and host blood suitability.

Hall, R., Thomas, G., Berry, I., Fischer, F., Foehse, M., 1983. Relative abundance of stable flies, *Stomoxys calcitrans* (L.)(Diptera: Muscidae), at dairies, feedlots, and pastures in Missouri. Journal of the Kansas Entomological Society, 223-228.

Hammock, B.D., Soderlund, D.M., 1986. Chemical strategies for resistance management. Pesticide Resistance: strategies and tactics for management, 111-129.

Hansens, E.J., 1951. The stable fly and its effect on seashore recreational areas in New Jersey. Journal of Economic Entomology 44, 482-487.

Harris, E., Grose, J., Saliba, L., Lucia, M.Z., 1976. Control of Farm Flies in Malta—I. Toxicities of insecticides to laboratory and Maltese field strains of *Musca domestica* and *Stomoxys calcitrans*. PANS 22, 201-206.

Harris, R.L., 1964. Laboratory tests to determine susceptibility of adult horn fly and stable fly to insecticides. Journal of Economic Entomology 57, 492-494.

Harris, R.L., Frazar, E., Grossman, P., Graham, O., 1966. Mating habits of the stable fly. Journal of Economic Entomology 59, 634-636.

Hatch Jr, E., 1911. The house fly as a carrier of disease. The Annals of the American Academy of Political and Social Science 37, 168-179.

Hawkins, J.A., Adams, W., Cook, L., Wilson, B., Roth, E., 1973. Role of horse fly (*Tabanus fuscicostatus* Hine) and stable fly (*Stomoxys calcitrans* L.) in transmission of equine infectious anemia to ponies in Louisiana. American journal of veterinary research 34, 1583.

Heath, A., Levot, G., 2015. Parasiticide resistance in flies, lice and ticks in New Zealand and Australia: mechanisms, prevalence and prevention. New Zealand veterinary journal 63, 199-210.

Heimbucher, J., 1982. Insektizide gegen Ektoparasiten und Lästlinge bei Haustieren - eine kritische Bestandsaufnahme in Österreich erhältlicher Präparate. Wiener Tierärztliche Monatsschrift.

Hewitt, C.G., 1909. Memoirs: The Structure, Development, and Bionomics of the house fly, *Musca domestica*, Linn. Journal of Cell Science 2, 347-414.

Hienton, T.E. 1974. Summary of investigations of electric insect traps. United States Department of Agriculture, Economic Research Service. Technical Bulletin No. 1498. Agricultural Research Service. Washington D.C. 20402.

Hieu, T.T., Kim, S.-I., Lee, S.-G., Ahn, Y.-J., 2010. Repellency to *Stomoxys calcitrans* (Diptera: Muscidae) of plant essential oils alone or in combination with *Calophyllum inophyllum* nut oil. Journal of Medical Entomology 47, 575-580.

Hildebrand, J., 2017. Vorkommen und Verbreitung von Insektizidresistenzen bei Fliegen (*Musca domestica*) in Schweinehaltungen im Bundesland Schleswig-Holstein, Deutschland. Thesis. Journal No.: 3899. Freie Universität Berlin.

Hoch, A.L., Gargan 2nd, T., Bailey, C.L., 1985. Mechanical transmission of Rift Valley fever virus by hematophagous Diptera. The American journal of tropical medicine and hygiene 34, 188-193.

Hoffmann, G., 1987a. Resistenzentwicklung bei Hygieneschädlingen einschließlich Ektoparasiten–Definition und gegenwärtige Situation. Zbl. Bakt. Hyg., Orig. B 185, 207-213.

Hoffmann, G., 1987b. Successful fly control-preventing resistance development. Landtechnik (Germany, FR). Bundesgesundheitsamt Berlin (Germany, F.R.). Institut für Wasser-, Boden- und Lufthygiene.

Hogsette, J., Farkas, R., 2000. Secretophagous and haematophagous higher Diptera. Contributions to a Manual of Palearctic Dipteran. Science Herald, Budapest, 769-792.

Hogsette, J.A., Nalli, A., Foil, L.D., 2008. Evaluation of different insecticides and fabric types for development of treated targets for stable fly (Diptera: Muscidae) control. Journal of Economic Entomology 101, 1034-1038.

Hogsette, J.A., Ruff, J.P., 1985. Stable fly (Diptera: Muscidae) migration in northwest Florida. Environmental Entomology 14, 170-175.

Hogsette, J.A., Ruff, J.P., 1986. Evaluation of flucythrinate- and fenvalerate- impregnated ear tags and permethrin ear tapes for fly (Diptera: Muscidae) control on beef and dairy cattle in northwest Florida. Journal of Economic Entomology 79, 152-157.

Hogsette, J.A., Ruff, J.P., 1990. Comparative attraction of four different fiberglass traps to various age and sex classes of stable fly (Diptera: Muscidae) adults. Journal of Economic Entomology 83, 883-886.

Hogsette, J.A., Ruff, J.P., 1996. Permethrin-impregnated yarn: longevity of efficacy and potential use on cylindrical fiberglass stable fly (Diptera: Muscidae) traps. Journal of Economic Entomology 89, 1521-1525.

Hogsette, J.A., Ruff, J.P., Jones, C.J., 1987. Stable fly biology and control in northwest Florida. Journal of Agricultural Entomology 4, 1-11.

Holzgrefe, B., 2013. Wirksamkeit Insektizid-behandelter Netze zum Schutz von Schweinen vor Tsetsefliegen und Trypanosomeninfektionen in Suhum, Eastern Region, Ghana. Thesis. Journal No.: 3570. Freie Universität Berlin.

Houlbert, C.C., 1920. Les Insectes: anatomie et physiologie générales, introduction a l'etude de l'entomologie biologique. Encyclopédie scientifique. Bibliotheque de zoologie, 343-352.

Howard, J., Wall, R., 1995. The use of triflumuron on sugar-baited targets for autosterilization of the housefly, Musca domestica. Entomologia experimentalis et applicata 77, 159-165.

Huang, H.-S., Hu, N.-T., Yao, Y.-E., Wu, C.-Y., Chiang, S.-W., Sun, C.-N., 1998. Molecular cloning and heterologous expression of a glutathione S-transferase involved in insecticide resistance from the diamondback moth, Plutella xylostella. Insect biochemistry and molecular biology 28, 651-658.

Huang, J., Kristensen, M., Qiao, C.-l., Jespersen, J.B., 2004. Frequency of kdr gene in house fly field populations: correlation of pyrethroid resistance and kdr frequency. Journal of economic entomology 97, 1036-1041.

Huckett, H.C., 1965. The Muscidae of Northern Canada, Alaska, and Greenland (Diptera). Memoirs of the Entomological Society of Canada 97, 5-369.

Hugh-Jones, M., Blackburn, J., 2009. The ecology of *Bacillus anthracis*. Molecular aspects of medicine 30, 356-367.

Hummadi, M., Maki, B., 1970. Studies on the breeding of *Stomoxys calcitrans* stable fly. Baghdad Univ Iraq Natur Hist Mus Bull.

Imai, C., 1987. Control of insecticide resistance in a field population of houseflies, Musca domestica, by releasing susceptible flies. Researches on population ecology 29, 129-146.

Ingles, P.J., Adams, P.M., Knipple, D.C., Soderlund, D.M., 1996. Characterization of voltage-sensitive sodium channel gene coding sequences from insecticide-susceptible and knockdown-resistant house fly strains. Insect biochemistry and molecular biology 26, 319-326.

IRAC 2017. The Insecticide Resistance Action Committee. 24.03.2018. http://www.irac-online.org/.

Ishaaya, I., Horowitz, A.R., 1995. Pyriproxyfen, a novel insect growth regulator for controlling whiteflies: mechanisms and resistance management. Pest Management Science 43, 227-232.

Isman, M.B., 2000. Plant essential oils for pest and disease management. Crop protection 19, 603-608.

Jabeen, F., Chaudhry, A.S., Manzoor, S., Shaheen, T., 2015. Examining pyrethroids, carbamates and neonicotenoids in fish, water and sediments from the Indus River for potential health risks. Environmental monitoring and assessment 187, 29.

Jandowsky, A., 2009. Vorkommen und Verbreitung von Insektizidresistenzen bei Fliegen (*Musca domestica*) in Milchviehbetrieben im Bundesland Brandenburg, Deutschland. Thesis. Journal No.: 3340. Freie Universität Berlin.

Jandowsky, A., Clausen, P.-H., Schein, E., Bauer, B., 2010. Vorkommen und Verbreitung von Insektizidresistenzen bei Fliegen (*Musca domestica*) in Milchviehbetrieben im Bundesland Brandenburg, Deutschland. Der Praktische Tierarzt 91, 590-598.

Jeanbourquin, P., Guerin, P.M., 2007. Sensory and behavioural responses of the stable fly Stomoxys calcitrans to rumen volatiles. Medical and Veterinary Entomology 21, 217-224.

Joffe, T., Gunning, R.V., Allen, G.R., Kristensen, M., Alptekin, S., Field, L.M., Moores, G.D., 2012. Investigating the potential of selected natural compounds to increase the potency of pyrethrum against house flies *Musca domestica* (Diptera: Muscidae). Pest Management Science 68, 178-184.

Johnson, G., Panella, N., Hale, K., Komar, N., 2010. Detection of West Nile virus in stable flies (Diptera: Muscidae) parasitizing juvenile American white pelicans. Journal of Medical Entomology 47, 1205-1211.

Jones, C.J., Hogsette, J., Patterson, R., Milne, D., Propp, G., Milio, J., Rickard, L., Ruff, J., 1991. Origin of stable flies (Diptera: Muscidae) on west Florida beaches: electrophoretic analysis of dispersal. Journal of Medical Entomology 28, 787-795.

Jones, C.J., Weinzierl, R.A., 1997. Geographical and temporal variation in pteromalid (Hymenoptera: Pteromalidae) parasitism of stable fly and house fly (Diptera: Muscidae) pupae collected from Illinois cattle feedlots. Environmental Entomology 26, 421-432.

Jones, C.M., Smith, C., 1966. Stable flies. Insect colonization and mass production. New York: Academic Press Inc.,169-172.

Kaufman, P., Rutz, D., Frisch, S., 2001a. Sticky traps for large scale house fly (Diptera: Muscidae) trapping in New York poultry facilities. Journal of Agricultural and Urban Entomology 18, 43-49.

Kaufman, P., Rutz, D., Frisch, S., 2005. Large sticky traps for capturing house flies and stable flies in dairy calf greenhouse facilities. Journal of Dairy Science 88, 176-181.

Kaufman, P.E., Scott, J.G., Rutz, D.A., 2001b. Monitoring insecticide resistance in house flies (Diptera: Muscidae) from New York dairies. Pest Management Science 57, 514-521.

Kavitha, P., Rao, J.V., 2008. Toxic effects of chlorpyrifos on antioxidant enzymes and target enzyme acetylcholinesterase interaction in mosquito fish, Gambusia affinis. Environmental Toxicology and Pharmacology 26, 192-198.

Keiding, J., 1986. House fly; biology and control, In: House fly; biology and control. OMS.

Keiding, J., 1999. Review of the global status and recent development of insecticide resistance in field populations of the housefly, *Musca domestica* (Diptera: Muscidae). Bulletin of Entomological Research.

Khan, H.A.A., Akram, W., Haider, M.S., 2015. Genetics and mechanism of resistance to deltamethrin in the house fly, *Musca domestica* L., from Pakistan. Ecotoxicology 24, 1213-1220.

Khater, H.F., Ramadan, M.Y., El-Madawy, R.S., 2009. Lousicidal, ovicidal and repellent efficacy of some essential oils against lice and flies infesting water buffaloes in Egypt. Veterinary Parasitology 164, 257-266.

Kinn, D., 1966. Predation by the mite, *Macrocheles muscaedomesticae* (Acarina: Macrochelidae), on three species of flies. Journal of Medical Entomology 3, 155-158.

Kirchner, T., Liebrich, S. 2017. EU-Kommission will für Bienen schädliche Pflanzenschutzmittel verbieten.24.03.2017. Süddeutsche Zeitung. http://www.sueddeutsche.de/wirtschaft/neonicotinoide-eu-kommission-will-bienen-schaedliche-pflanzenschutzmittel-verbieten-1.3435072.

Kocan, K.M., De la Fuente, J., Guglielmone, A.A., Meléndez, R.D., 2003. Antigens and alternatives for control of *Anaplasma marginale* infection in cattle. Clinical Microbiology Reviews 16, 698-712.

Kočišová, A., Novák, P., Toporčák, J., Petrovský, M., 2002. Development of resistance in field housefly (*Musca domestica*): Comparison of effect of classic spray regimes versus integrated control methods. Acta Veterinaria Brno 71, 401-405.

Koehler, P., Patterson, R., 1982. Stable fly control with fiberglass panels. IFAS, University of Florida, Cooperative Extension Service Livestock Protection Pointer 14.

Kramer, J., Steinkraus, D., 1981. Culture of Entomophthora muscae in vivo and its infectivity for six species of muscoid flies. Mycopathologia 76, 139-143.

Krinsky, W.L., 1976. Review Article: Animal Disease Agents Transmitted by Horse Flies and Deer Flies (Diptera: Tabanidae). Journal of Medical Entomology 13, 225-275.

Kristensen, M., Jespersen, J.B., 2003. Larvicide resistance in *Musca domestica* (Diptera: Muscidae) populations in Denmark and establishment of resistant laboratory strains. Journal of economic entomology 96, 1300-1306.

Kristensen, M., Jespersen, J.B., 2008. Susceptibility to thiomethoxam in *Musca domestica* from Danish livestock farms. Pest Management Science 64, 126-132.

Kristensen, M., Spencer, A.G., Jespersen, J.B., 2001. The status and development of insecticide resistance in Danish populations of the housefly Musca domestica L. Pest management science 57, 82-89.

Kühlhorn, F., 1961. Über die Bedeutung des Fliegenzufluges in Viehställe und seine Behinderung. Gesundheitswes. u. Desinfekt 53.

Kühlhorn, F., 1965. Untersuchungen über die Beziehungen zwischen den Luftbewegungen und dem Verteilungsverhalten von Dipteren in Stallräumen. Gesundheitswes. Desinfekt 57, 81-85.

Kühlhorn, F., 1983. Dipterenfeinde in Stallungen. Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz 56, 109-113.

Kulkarni, A.P., Hodgson, E., 1980. Metabolism of insecticides by mixed function oxidase systems. Pharmacology & therapeutics 8, 379-475.

Künast, C., Güntzrodt, C., 1981. Vergleichende Laboruntersuchungen über Lockstoff und Köder bei der Stubenfliege (*Musca domestica* L.). Anzeiger für Schädlingskunde 54, 131-135.

Kunz, S., Berry, I., Foerster, K., 1977. The development of the immature forms of *Stomoxys calcitrans*. Annals of the Entomological Society of America 70, 169-172.

Kunz, S., Murrell, K., Lambert, G., James, L., Terrill, C., 1991. Estimated losses of livestock to pests. CRC handbook of pest management in agriculture 1, 69-98.

Kunz, T.H., 1976. Observations on the winter ecology of the bat fly *Trichobius corynorhini* Cockerell (Diptera: Streblidae). Journal of Medical Entomology 12, 631-636.

Labib, I., Rady, M., 2001. Application of *Bacillus thuringiensis* in poultry houses as a biological control agent against the housefly, *Musca domestica sorbens*. Journal of the Egyptian Society of Parasitology 31, 531-544.

Larrain, S., Salas, F., 2008. House Fly (*Musca domestica* L.) (Diptera Muscidae) Development in Different Types of Manure. Chilean journal of agricultural research 68, 192-197.

Larsen, E.B., Thomsen, M., 1940. The influence of temperature on the development of some species of diptera. Vidensk. Medd. fra Dansk naturh. Foren. 104.

Laveissière, C., Grebaut, P., 1990. The trapping of tsetse flies (Diptera: Glossinidae). Improvement of a model: the Vavoua trap. Tropical Medicine and Parasitology: official organ of Deutsche Tropenmedizinische Gesellschaft and of Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ) 41, 185-192.

Lee, R.M., Davies, D., 1979. Feeding in the stable fly, *Stomoxys calcitrans* (Diptera: Muscidae) I. Destination of blood, sucrose solution and water in the alimentary canal, the effects of age on feeding, and blood digestion. Journal of Medical Entomology 15, 541-554.

Legner, E., Bay, E., White, E., 1967. Activity of parasites from Diptera: *Musca domestica*, *Stomoxys calcitrans*, *Fannia canicularis*, and *F. femoralis*, at sites in the Western Hemisphere. Annals of the Entomological Society of America 60, 462-468.

Legner, E., Brydon, H., 1966. Suppression of dung-inhabiting fly populations by pupal parasites. Annals of the Entomological Society of America 59, 638-651.

Legner, E., McKeen, W., Warkentin, R., 1990. Inoculation of three pteromalid wasp species (Hymenoptera: Pteromalidae) increases parasitism and mortality of *Musca domestica* L. pupae in poultry manure. Bulletin of the Society for Vector Ecology 15, 149-155.

Legner, E., Olton, G., 1971. Distribution and relative abundance of dipterous pupae and their parasitoids in accumulations of domestic animal manure in the southwestern United States. Hilgardia 40, 505-535.

Leon;, L.L.L.H.P.D., 2012. Effects of pyriproxyfen and buprofezin on immature development and reproduction in the stable fly. Medical and Veterinary Entomology 26, 379-385.

Lewis, C., 1971. Superficial sense organs of the antennae of the fly, *Stomoxys calcitrans*. Journal of Insect Physiology 17, 449-461.

Lewis, J., Sawicki, R., 1971. Characterization of the resistance mechanisms to diazinon, parathion and diazoxon in the organophosphorus-resistant SKA strain of house flies (*Musca domestica* L.). Pesticide Biochemistry and Physiology 1, 275-285.

Liénard, E., Salem, A., Grisez, C., Prévot, F., Bergeaud, J., Franc, M., Gottstein, B., Alzieu, J., Lagalisse, Y., Jacquiet, P., 2011. A longitudinal study of *Besnoitia besnoiti* infections and seasonal abundance of Stomoxys calcitrans in a dairy cattle farm of southwest France. Veterinary Parasitology 177, 20-27.

Liénard, E., Salem, A., Jacquiet, P., Grisez, C., Prévot, F., Blanchard, B., Bouhsira, E., Franc, M., 2013. Development of a protocol testing the ability of *Stomoxys calcitrans* (Linnaeus, 1758) (Diptera: Muscidae) to transmit *Besnoitia besnoiti* (Henry, 1913)(Apicomplexa: Sarcocystidae). Parasitology Research 112, 479-486.

Lietze, V.-U., Geden, C.J., Blackburn, P., Boucias, D.G., 2007. Effects of salivary gland hypertrophy virus on the reproductive behavior of the housefly, *Musca domestica*. Applied and Environmental Microbiology 73, 6811-6818.

Lietze, V.-U., Keesling, J.E., Lee, J.A., Vallejo, C.R., Geden, C.J., Boucias, D.G., 2013. Muscavirus (MdSGHV) disease dynamics in house fly populations–How is this virus transmitted and has it potential as a biological control agent? Journal of Invertebrate Pathology 112, S40-S43.

Liu, N., Yue, X., 2000. Insecticide resistance and cross-resistance in the house fly (Diptera: Muscidae). Journal of Economic Entomology 93, 1269-1275.

Liu, S., Li, A.Y., Lohmeyer, K.H., Perez De Leon, A., 2012. Effects of pyriproxyfen and buprofezin on immature development and reproduction in the stable fly. Medical and Veterinary Entomology 26, 379-385.

Lodha, K., 1961. Studies on the life cycle of the stable fly *Stomoxys calcitrans* (L.). Indian Vet. J 38, 541-547.

Löscher, W., Ungemach, F.R., Kroker, R., 2006. Pharmakotherapie bei Haus-und Nutztieren. Berlin: Parey.

Lotmar, R., 1949. Beobachtungen über Nahrungsaufnahme und Verdauung bei *Stomoxys calcitrans*. Mitteilungen. Schweizerische Entomologische Gesellschaft. 22, 97-115.

Low, W.Y., Ng, H.L., Morton, C.J., Parker, M.W., Batterham, P., Robin, C., 2007. Molecular evolution of glutathione S-transferases in the genus *Drosophila*. Genetics 177, 1363-1375.

Lundholm, E., 1987. Thinning of eggshells in birds by DDE: mode of action on the eggshell gland. Comparative Biochemistry and Physiology Part C: Comparative Pharmacology 88, 1-22.

Lysyk, T., 1998. Relationships between temperature and life-history parameters of *Stomoxys calcitrans* (Diptera: Muscidae). Journal of Medical Entomology 35, 107-119.

Lysyk, T., Kalischuk-Tymensen, L., Rochon, K., Selinger, L., 2010. Activity of *Bacillus thuringiensis* isolates against immature horn fly and stable fly (Diptera: Muscidae). Journal of Economic Entomology 103, 1019-1029.

Lysyk, T., Krafsur, E., 1993. Relationship between pterin accumulation and ovarian development in the stable fly *Stomoxys calcitrans* (L.)(Diptera: Muscidae). The Canadian Entomologist 125, 869-879.

Macadam, I., 1977. Control of *Dermatophilus congolensis* infection. The Veterinary record 100, 411-411.

Machtinger, E.T., 2011. Studies on the Field Ecology, Breeding Biology and Parasitism of house flies, musca domestica and stable flies, *Stomoxys Calcitrans*, (Diptera: Muscidae) to Improve Integrated Pest Management for North Florida Small Equestrian Farms. Thesis: Master of Science. University of Florida.

Magoc, L., Yen, J.L., Hill-Williams, A., McKenzie, J.A., Batterham, P., Daborn, P.J., 2005. Cross-resistance to dicyclanil in cyromazine-resistant mutants of *Drosophila melanogaster* and *Lucilia cuprina*. Pesticide biochemistry and physiology 81, 129-135.

Maia, M.F., 2009. Impact of insecticide-treated nets protecting cattle in zero-grazing units on nuisance and biting insects in the forest region of Kumasi, Ghana. Thesis, Journal No.: 3298. Freie Universität Berlin.

Marcon, P.C., Thomas, G.D., Siegfried, B.D., Campbell, J.B., 1997. Susceptibility of stable flies (Diptera: Muscidae) from southeastern Nebraska beef cattle feedlots to selected insecticides and comparison of 3 bioassay techniques. Journal of Economic Entomology 90, 293-298.

Mascari, T., Mitchell, M., Rowton, E., Foil, L., 2007. Laboratory evaluation of diflubenzuron as a feed-through for control of immature sand flies (Diptera: Psychodidae). Journal of Medical Entomology 44, 171-174.

Masmeatathip, R., Ketavan, C., Duvallet, G., 2006. Morphological studies of *Stomoxys* spp.(Diptera: Muscidae) in central Thailand. Kasetsart J.(Nat. Sci.) 40, 872-881.

Matsumura, F., 2010. Studies on the action mechanism of benzoylurea insecticides to inhibit the process of chitin synthesis in insects: A review on the status of research activities in the past, the present and the future prospects. Pesticide Biochemistry and Physiology 97, 133-139.

Mellor, J., 1919. Observations on the habits of certain flies, especially of those breeding in manure. Annals of Applied Biology 6, 53-88.

Mellor, P., Kitching, R., Wilkinson, P., 1987. Mechanical transmission of capripox virus and African swine fever virus by *Stomoxys calcitrans*. Research in Veterinary Science 43, 109-112.

Melvin, R., 1931. Notes on the biology of the stable fly, *Stomoxys calcitrans* Linn. Annals of the Entomological Society of America 24, 436-438.

Melvin, R., 1932. Physiological studies on the effect of flies and fly sprays on cattle. Journal of Economic Entomology 25, 1151-1164.

Meola, R.W., Harris, R., Meola, S., Oehler, D., 1977. Dietary-Induced Secretion of Sex Pheromone and Development of Sexual Behavior in the Stable Fly. Environmental Entomology 6, 895-897.

Meyer, J., Petersen, J., 1982. Sampling stable fly and house fly pupal parasites on beef feedlots and dairies in eastern Nebraska (*Musca domestica*, *Stomoxys calcitrans*). Southwest Entomology.

Meyer, J., Petersen, J., 1983. Characterization and seasonal distribution of breeding sites of stable flies and house flies (Diptera: Muscidae) on eastern Nebraska feedlots and dairies. Journal of Economic Entomology 76, 103-108.

Mihok, S., Maramba, O., Munyoki, E., Kagoiya, J., 1995. Mechanical transmission of *Trypanosoma* spp. by african stomoxyinae (Diptera: Muscidae). Tropical Medicine and Parasitology: official organ of Deutsche Tropenmedizinische Gesellschaft and of Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ) 46, 103-105.

Miller, R., Pickens, L., Morgan, N., Thimlian, R., Wilson, R., 1973. Effect of stable flies on feed intake and milk production of dairy cows. Journal of Economic Entomology 66, 711-714.

Miller, R., Pickens, L., Potts, W., 1993a. Comparison of traps and an integrated program to manage house flies and stable flies on dairy farms. Journal of Agricultural Entomology (USA).

Miller, R., Rutz, D., Pickens, L., Geden, C., 1993b. Evaluation of traps and the parasitoid Muscidifurax raptor Girault and Sanders to manage house flies and stable flies on dairy farms. Journal of Agricultural Entomology (USA).

Miller, R., Schmidtmann, E., Wauchope, R., Clegg, C., Herner, A., Weber, H., 1996. Urine delivery of cyromazine for suppressing house and stable flies (Diptera: Muscidae) in outdoor dairy calf hutches. Journal of Economic Entomology 89, 689-694.

Miller, T., 1988. Mechanisms of resistance to pyrethroid insecticides. Parasitology Today 4, 8-12.

Mitzmain, M., 1913. The Bionomics of *Stomoxys calcitrans*, Linnaeus; a preliminary account. Philippine Journal of Science 8, 29-48.

Miura, T., Takahashi, R., 1974. Insect developmental inhibitors. Effects of candidate mosquito control agents on nontarget aquatic organisms. Environmental Entomology 3, 631-636.

Miyamoto, J., Hirano, M., Takimoto, Y., Hatakoshi, M., 1993. Insect growth regulators for pest control, with emphasis on juvenile hormone analogs, ACS Publications.

Mnzava, A.P., Knox, T.B., Temu, E.A., Trett, A., Fornadel, C., Hemingway, J., Renshaw, M., 2015. Implementation of the global plan for insecticide resistance management in malaria vectors: progress, challenges and the way forward. Malaria journal 14, 173.

Moon, R.D., 2002. Muscid flies (Muscidae). Medical and Veterinary Entomology, 279-301.

Moorhouse, D., 1972. Cutaneous lesions on cattle caused by stable fly. Australian veterinary journal 48, 643-644.

Moraes, A.P.R., Angelo, I.d.C., Fernandes, E.K., Bittencourt, V.R., Bittencourt, A.J., 2008. Virulence of *Metarhizium anisopliae* to eggs and immature stages of *Stomoxys calcitrans*. Annals of the New York Academy of Sciences 1149, 384-387.

Morgan, D., Bailie, H., 1980. A field trial to determine the effect of fly control using permethrin on milk yields in dairy cattle in the UK. The Veterinary record 106, 121-123.

Morgan, P.B., Patterson, R., LaBrecque, G., Weidhaas, D., Benton, A., Whitfield, T., 1975. Rearing and release of the house fly pupal parasite *Spalangia endius* Walker. Environmental Entomology 4, 609-611.

Morrison, P., Venkatesh, K., Thompson, B., 1982. The role of male accessory-gland substance on female reproduction with some observations of spermatogenesis in the stable fly. Journal of Insect Physiology 28, 607611-609614.

Mount, G.A., Lofgren, C., Bowman, M., Acree, F., 1966. Fate of dieldrin applied topically to stable flies susceptible and resistant to dieldrin. Journal of Economic Entomology 59, 1352-1353.

Moussa, A.Y., 1978. A new virus disease in the housefly, *Musca domestica* (Diptera). Journal of Invertebrate Pathology 31, 204-216.

Moyses, E.W., Gfeller, F.J., 2001. Topical application as a method for comparing the effectiveness of insecticides against cat flea (Siphonaptera: Pulicidae). Journal of Medical Entomology 38, 193-196.

Mramba, F., Broce, A., Zurek, L., 2007. Vector competence of stable flies, *Stomoxys calcitrans* L.(Diptera: Muscidae), for *Enterobacter sakazakii*. Journal of Vector Ecology 32, 134-139.

Muhammed, S., Butler, J., Carlson, D., 1975. Stable fly sex attractant and mating pheromones found in female body hydrocarbons. Journal of Chemical Ecology 1, 387-398.

Mullen, G.R., Durden, L.A., 2009. Medical and veterinary entomology. Burlington: Academic press.

Mullens, B., Lii, K.S., Mao, Y., Meyer, J., Peterson, N., Szijj, C., 2006. Behavioural responses of dairy cattle to the stable fly, *Stomoxys calcitrans*, in an open field environment. Medical and Veterinary Entomology 20, 122-137.

Muma, M.H., Hixson, E., 1949. Effects of weather, sanitation and chlorinated chemical residues on house and stable fly populations on Nebraska farms.

Muraleedharan, K., 2005. Pyrethroid insecticides, sumicidin and butox for the effective control of *Stomoxys calcitrans*. JPD 29, 164-165.

Mutero, A., Pralavorio, M., Bride, J.-M., Fournier, D., 1994. Resistance-associated point mutations in insecticide-insensitive acetylcholinesterase. Proceedings of the national academy of sciences 91, 5922-5926.

Nasonkin, I., Alikasifoglu, A., Ambrose, C., Cahill, P., Cheng, M., Sarniak, A., Egan, M., Thomas, P.M., 1999. A novel sulfonylurea receptor family member expressed in the embryonic *Drosophila* dorsal vessel and tracheal system. Journal of Biological Chemistry 274, 29420-29425.

Nelder, M.P., Lloyd, J.E., Loftis, A.D., Reeves, W.K., 2008. Coxiella. Emerging Infectious Diseases, 1002-1004.

Newstead, R., 1906. On the life-history of *Stomoxys calcitrans*, Linn. Journal of Economic Biology 1, 157-166.

Olafson, P.U., Pitzer, J.B., Kaufman, P.E., 2011. Identification of a mutation associated with permethrin resistance in the para-type sodium channel of the stable fly (Diptera: Muscidae). Journal of Economic Entomology 104, 250-257.

Olson, D., Christensen, G., 1980. Effects of water pollutants and other chemicals on fish acetylcholinesterase (*in vitro*). Environmental Research 21, 327-335.

Oppenoorth, F., 1965. Biochemical genetics of insecticide resistance. Annual Review of Entomology 10, 185-206.

Oppenoorth, F., Van Asperen, K., 1960. Allelic genes in the housefly producing modified enzymes that cause organophosphate resistance. Science 132, 298-299.

Oppenoorth, F., Van der Pas, L., Houx, N., 1979. Glutathione S-transferase and hydrolytic activity in a tetrachlorvinphos-resistant strain of house fly and their influence on resistance. Pesticide Biochemistry and Physiology 11, 176-188.

Palacios, S.M., Bertoni, A., Rossi, Y., Santander, R., Urzúa, A., 2009. Insecticidal activity of essential oils from native medicinal plants of Central Argentina against the house fly, *Musca domestica* (L.). Parasitology Research 106, 207-212.

Pap, L., Farkas, R., 1994. Monitoring of resistance of insecticides in house fly (*Musca domestica*) populations in Hungary. Pest Management Science 40, 245-258.

Patterson, R., LaBrecque, G., Williams, D., Weidhaas, D., 1981. Control of the Stable Fly, *Stomoxys calcitrans* (Diptera: Muscidae), on St. Croix, US Virgin Islands, Using Integrated Pest Management Measures: III. Field techniques and population control. Journal of Medical Entomology 18, 203-210.

Pawson, B., Petersen, J., 1988. Dispersal of *Muscidifurax zaraptor* (Hymenoptera: Pteromalidae), a filth fly parasitoid, at dairies in eastern Nebraska. Environmental Entomology 17, 398-402.

Peakall, D.B., Lincer, J.L., Risebrough, R.W., Pritchard, J.B., Kinter, W.B., 1973. DDE-induced egg-shell thinning: structural and physiological effects in three species. Comparative and general Pharmacology 4, 305-313.

Petersen, J., Meyer, J., 1983. Host preference and seasonal distribution of pteromalid parasites (Hymenoptera: Pteromalidae) of stable flies and house flies (Diptera: Muscidae) associated with confined livestock in eastern Nebraska. Environmental Entomology 12, 567-571.

Peterson, A., 1916. The head-capsule and mouth-parts of Diptera, Vol 52. University of Illinois: Illinois Biological Monographs.

Pickens, L., 1991. Battery-powered, electrocuting trap for stable flies (Diptera: Muscidae). Journal of Medical Entomology 28, 822-830.
Pickens, L., 1995. Baited fly traps--1900 to 1995. IPM practitioner: the newsletter of integrated pest management.
Pickens, L., Miller, R., 1987. Techniques for trapping flies on dairy farms. Journal of Agricultural Entomology 4, 305-313.
Pinto, M.C., Prado, A.P.d., 2001. Resistance of *Musca domestica* L. populations to cyromazine (insect growth regulator) in Brazil. Memórias do Instituto Oswaldo Cruz 96, 729-732.
Pitzer, J.B., Kaufman, P.E., Tenbroeck, S.H., 2010. Assessing permethrin resistance in the stable fly (Diptera: Muscidae) in florida by using laboratory selections and field evaluations. Journal of Economic Entomology 103, 2258-2263.
Pospischil, R., Londershausen, M., Szomm, K., Turberg, A., 1996a. Resistance in German house fly populations (*Musca domestica* L., Diptera) - summary of recent studies. In: Proceedings of the 2nd international Conference in Insect Pests in the Urban Environment, pp. 255-262.
Pospischil, R., Szomm, K., Londershausen, M., Schröder, I., Turberg, A., Fuchs, R., 1996b. Multiple resistance in the larger house fly *Musca domestica* in Germany. Pest Management Science 48, 333-341.
Quarles, W., 2006. Biological control of nuisance flies with pupal parasitoids. The IPM practitioner: the newsletter of integrated pest management.
Ranade, D., 1970. The development of the eyes in *Stomoxys calcitrans* Linn.(Diptera—cyclorrhapha—muscidae—stomoxydinae). In: Proceedings of the Indian Academy of Sciences-Section B, pp. 145-149.
Ranson, H., Rossiter, L., Ortelli, F., Jensen, B., Xuelan, W., Collins, F.H., Hemingway, J., 2001. Identification of a novel class of insect glutathione S-transferases involved in resistance to DDT in the malaria vector Anopheles gambiae. Biochemical Journal 359, 295-304.
Rasmussen, R., Campbell, J., 1981. Investigation of environmental factors and their relationship to populations of the stable fly, *Stomoxys calcitrans* (L.). Environmental Entomology 10, 798-800.
Ratcliffe, D., 1970. Changes attributable to pesticides in egg breakage frequency and eggshell thickness in some British birds. Journal of Applied Ecology, 67-115.
Retnakaran, A., Percy, J., 1985. Fertilization and special modes of reproduction. Comprehensive Insect Physiology, Biochemistry and Pharmacology, Oxford: Pergamon Press, 231-294.
Ribbeck, R., Betke, P., Muller, P., Schumann, H., Hiepe, T., 1987. Stallfliegen - Schadwirkung und Bekämpfung in der Intensiventierproduktion. Monatshefte fur Veterinärmedizin, 42, 517-521.
Richard, J., Pier, A., 1966. Transmission of *Dermatophilus congolensis* by *Stomoxys calcitrans* and *Musca domestica*. American journal of Veterinary Research 27, 419-423.
Richter, A., Steuber, S., 2010. Antiparasitica, Vol 3rd ed. H.-H. Frey, W. Loescher, Stuttgart: Enke Verlag, Germany.
Riddiford, L., 1976. Juvenile hormone control of epidermal commitment *in vivo* and *in vitro*, The juvenile hormones. Springer, pp. 198-219.
Rinkevich, F.D., Hedtke, S.M., Leichter, C.A., Harris, S.A., Su, C., Brady, S.G., Taskin, V., Qiu, X., Scott, J.G., 2012. Multiple origins of kdr-type resistance in the house fly, *Musca domestica*. PLoS One 7, e52761.
Robert, V., Carnevale, P., 1991. Influence of deltamethrin treatment of bed nets on malaria transmission in the Kou valley, Burkina Faso. Bulletin of the World Health Organization 69, 735.
Robertson, J.L., Savin, N., Russell, R.M., Preisler, H.K., 2007. Bioassays with arthropods. CRC press.
Robinson, G.E., 1985. Effects of a juvenile hormone analogue on honey bee foraging behaviour and alarm pheromone production. Journal of Insect Physiology 31, 277-282.

Rohrmann, K.M.A., 2010. Die Wirksamkeit insektizidbehandelter Netze zum Schutz von Rindern vor Gnitzen und Lästlingsinsekten in Milchviehstallungen. Thesis, Journal No.: 3344, Freie Universität Berlin.

Romero, A., Broce, A., Zurek, L., 2006. Role of bacteria in the oviposition behaviour and larval development of stable flies. Medical and Veterinary Entomology 20, 115-121.

Roth, M., Richards, R.H., Dobson, D.P., Rae, G.H., 1996. Field trials on the efficacy of the organophosphorus compound azamethiphos for the control of sea lice (Copepoda: Caligidae) infestations of farmed Atlantic salmon (*Salmo salar*). Aquaculture 140, 217-239.

Roush, R.T., 1993. Occurrence, genetics and management of insecticide resistance. Parasitology Today 9, 174-179.

Rutz, D.A., Axtell, R.C., 1979. Sustained releases of *Muscidifurax raptor* (Hymenoptera: Pteromalidae) for house fly (*Musca domestica*) control in two types of caged-layer poultry houses. Environmental Entomology 8, 1105-1110.

Salem, A., Bouhsira, E., Emmanuel Liénard, D., 2012a. Susceptibility of two European strains of *Stomoxys calcitrans* (L.) to cypermethrin, deltamethrin, fenvalerate, λ-cyhalothrin, permethrin and phoxim. Ali Salem, 5103.

Salem, A., Franc, M., Jacquiet, P., Bouhsira, E., Liénard, E., 2012b. Feeding and breeding aspects of *Stomoxys calcitrans* (Diptera: Muscidae) under laboratory conditions. Parasite 19, 309-317.

Schaub, L., Sardy, S., Capkun, G., 2002. Natural variation in baseline data: when do we call a new sample 'resistant'? Pest Management Science 58, 959-963.

Schmidtmann, E., 1991. Suppressing immature house and stable flies in outdoor calf hutches with sand, gravel, and sawdust bedding. Journal of Dairy Science 74, 3956-3960.

Schmidtmann, E., Miller, R., Muller, R., 1989. Effect of experimental bedding treatments on the density of immature *Musca domestica* and *Stomoxys calcitrans* (Diptera: Muscidae) in outdoor calf hutches. Journal of Economic Entomology 82, 1134-1139.

Schnieder, T., 2006. Veterinärmedizinische Parasitologie,Josef Boch and Rudolf Supperer, 6, Berlin: Parey.

Schofield, S., Torr, S., 2002. A comparison of the feeding behaviour of tsetse and stable flies. Medical and Veterinary Entomology 16, 177-185.

Scholl, P., Petersen, J., Stage, D., Meyer, J., 1981. Open silage as an overwintering site for immature stable flies in eastern Nebraska (*Stomoxys calcitrans*, *Musca domestica*). Southwest Entomology.

Schoof, H., 1964. Laboratory culture of *Musca, Fannia*, and *Stomoxys*. Bulletin of the World Health Organization 31, 539.

Schowalter, T., Klowden, M., 1979. Blood meal size of the stable fly, *Stomoxys calcitrans*, measured by the HiCN (hemoglobin-cyanide) method (Pest of livestock). Mosquito News (USA).

Schreck, C., Posey, K., Smith, D., 1978. Durability of permethrin as a potential clothing treatment to protect against blood-feeding arthropods. Journal of Economic Entomology 71, 397-400.

Schricker, B., Stephen, W., 1970. The effect of sublethal doses of parathion on honeybee behaviour. I. Oral administration and the communication dance. Journal of Apicultural Research 9, 141-153.

Schuberg, A., Böing, W., 1914. On the transmission of diseases by indigenous biting insects. Arbeiten aus dem Kaiserlichen Gesundheitsamte (Berlin), 47, 491-512.

Schuberg, A., Kuhn, P., 1912. Über die Übertragung von Krankheiten durch einheimische stechende Insekten. Arbeiten aus dem Kaiserlichen Gesundheitsamte (Berlin), 40, 209-234.

Schwinghammer, K., Knapp, F., Boling, J., Schillo, K., 1986. Physiological and nutritional response of beef steers to infestations of the stable fly (Diptera: Muscidae). Journal of Economic Entomology 79, 1294-1298.

Scoles, G.A., Broce, A.B., Lysyk, T.J., Palmer, G.H., 2005. Relative efficiency of biological transmission of *Anaplasma marginale* (Rickettsiales: Anaplasmataceae) by

Dermacentor andersoni (Acari: Ixodidae) compared with mechanical transmission by *Stomoxys calcitrans* (Diptera: Muscidae). Journal of Medical Entomology 42, 668-675.
Scott, J.G., Alefantis, T.G., Kaufman, P.E., Rutz, D.A., 2000. Insecticide resistance in house flies from caged-layer poultry facilities. Pest Management Science 56, 147-153.
Scott, J.G., Roush, R., Tabashnik, B., 1990. Pesticide resistance in arthropods. Pesticide resistance in arthropods. Boston: Springer.
Scott, J.W., 1922. Insect transmission of swamp fever or infectious anemia of horses. University of Wyoming, Agricultural Experiment Station.
Seraydar, K., Kaufman, P., 2015. Does behaviour play a role in house fly resistance to imidacloprid-containing baits? Medical and Veterinary Entomology 29, 60-67.
Sfara, V., Zerba, E.N., Alzogaray, R.A., 2006. Toxicity of pyrethroids and repellency of diethyltoluamide in two deltamethrin-resistant colonies of *Triatoma infestans* Klug, 1834 (Hemiptera: Reduviidae). Memórias do Instituto Oswaldo Cruz 101, 89-94.
Shah, R.M., Abbas, N., Shad, S.A., Varloud, M., 2015a. Inheritance mode, cross-resistance and realized heritability of pyriproxyfen resistance in a field strain of *Musca domestica* L.(Diptera: Muscidae). Acta tropica 142, 149-155.
Shah, R.M., Shad, S.A., Abbas, N., 2015b. Mechanism, stability and fitness cost of resistance to pyriproxyfen in the house fly, *Musca domestica* L.(Diptera: Muscidae). Pesticide biochemistry and physiology 119, 67-73.
Shen, J., Plapp, F., 1990. Cyromazine resistance in the house fly (Diptera: Muscidae): genetics and cross-resistance to diflubenzuron. Journal of Economic Entomology 83, 1689-1697.
Simmons, S., 1944. Observations on the biology of the stable fly in Florida. Journal of Economic Entomology 37, 680-686.
Simmons, S., Dove, W., 1941. Breeding places of the stable fly or "dog fly" *Stomoxys calcitrans* (L.) in northwestern Florida. Journal of Economic Entomology 34, 457-462.
Skidmore, P., 1985. The biology of the Muscidae of the world, Vol 29. Springer Science & Business Media. Dr W Junk Publishers Group.
Skoda, S.R., Thomas, G.D., Campbell, J.B., 1991. Developmental sites and relative abundance of immature stages of the stable fly (Diptera: Muscidae) in beef cattle feedlot pens in eastern Nebraska. Journal of Economic Entomology 84, 191-197.
Skovgård, H., 2004. Sustained releases of the pupal parasitoid *Spalangia cameroni* (Hymenoptera: Pteromalidae) for control of house flies, *Musca domestica* and stable flies *Stomoxys calcitrans* (Diptera: Muscidae) on dairy farms in Denmark. Biological Control 30, 288-297.
Skovgård, H., Jespersen, J., 1999. Activity and relative abundance of hymenopterous parasitoids that attack puparia of *Musca domestica* and *Stomoxys calcitrans* (Diptera: Muscidae) on confined pig and cattle farms in Denmark. Bulletin of Entomological Research 89, 263-269.
Smith, C., 1995. Searching for safe methods of flea control. Journal of the American Veterinary Medical Association 206, 1137-1143.
Smith, J.P., Hall, R.D., Thomas, G.D., 1985. Field studies on mortality of the immature stages of the stable fly (Diptera: Muscidae). Environmental Entomology 14, 881-890.
Smith, J.P., Hall, R.D., Thomas, G.D., 1987. Field parasitism of the stable fly (Diptera: Muscidae). Annals of the Entomological Society of America 80, 391-397.
Smith, P.F., Howerth, E.W., Carter, D., Gray, E.W., Noblet, R., Mead, D.G., 2009. Mechanical transmission of vesicular stomatitis New Jersey virus by Simulium vittatum (Diptera: Simuliidae) to domestic swine (*Sus scrofa*). Journal of Medical Entomology 46, 1537-1540.
Soderlund, D.M., Bloomquist, J.R., 1990. Molecular mechanisms of insecticide resistance, Pesticide resistance in arthropods. Springer, pp. 58-96.
Sømme, L., 1958. The number of stable flies in Norwegian barns, and their resistance to DDT. Journal of Economic Entomology 51, 599-601.
Sømme, L., 1961. On the overwintering of house flies (Musco domestica L.) and stable flies (*Stomoxys calcitrans* (L.)) in Norway. Norsk Entomologisk Tidsskrift 11, 191-223.

Sømme, L., 1962. Notes on resistance to chlorinated hydrocarbon insecticides in the stable fly (*Stomoxys calcitrans* (L.))(Diptera). Norsk Entomologisk Tidsskrift 12.

Stenersen, J., 1966. Cross resistance to Dilan and DDT plus WARF Antiresistant in DDT resistant stable flies *Stomoxys calcitrans* (L). Norsk Entomologisk Tidsskrift 13.

Stenersen, J., Sømme, L., 1963. Notes on cross-resistance and genetics of resistance to the DDT-group insecticides in the stable fly (*Stomoxys calcitrans* (L.))(Diptera). Norsk Entomologisk Tidsskrift 12, 113-117.

Straif, S., Maier, W., Seitz, H., 1990. Regurgitation as a potential mechanism of pathogen transmission in the biting fly *Stomoxys calcitrans*. Zeitschrift für Angewandte Zoologie 77, 357-366.

Stuyck, C., 2014. Phenological relationships of nesting barn swallows in a swallow-fly-cattle system and their potential role in suppression of pest flies in a warming climate. Clemson University, ProQuest Dissertations Publishing, UMI No.: 1558050.

Sukontason, K., Sukontason, K.L., Piangjai, S., Boonchu, N., Chaiwong, T., Ngern-klun, R., Sripakdee, D., Vogtsberger, R.C., Olson, J.K., 2004. Antennal sensilla of some forensically important flies in families Calliphoridae, Sarcophagidae and Muscidae. Micron 35, 671-679.

Sullivan, J.J., Goh, K.S., 2008. Environmental fate and properties of pyriproxyfen. Journal of Pesticide Science 33, 339-350.

Sutcliffe, J.F., d'Cambre, C., Downe, A., 1993. Effects of two blood-feeding regimes on mortality and female reproduction in a laboratory colony of stable flies, *Stomoxys calcitrans*. Medical and Veterinary Entomology 7, 111-116.

Sutherland, B., 1978. Nutritional values of different blood diets expressed as reproductive potentials in adult *Stomoxys calcitrans* L. (Diptera: Muscidae). Onderstepoort J Vet Res 45, 209-212.

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

Szalanski, A.L., Taylor, D.B., Peterson, R.D., 1996. Population genetics and gene variation of stable fly populations (Diptera: Muscidae) in Nebraska. Journal of Medical Entomology 33, 413-420.

Talley, J., Broce, A., Zurek, L., 2009. Characterization of stable fly (Diptera: Muscidae) larval developmental habitat at round hay bale feeding sites. Journal of Medical Entomology 46, 1310-1319.

Tang, A.H., Tu, C., 1994. Biochemical characterization of Drosophila glutathione S-transferases D1 and D21. Journal of Biological Chemistry 269, 27876-27884.

Tarry, D., Bernal, L., Edwards, S., 1991. Transmission of bovine virus diarrhoea virus by blood feeding flies. The Veterinary Record 128, 82-84.

Taylor, D.B., Berkebile, D., 2006. Comparative efficiency of six stable fly (Diptera: Muscidae) traps. Journal of Economic Entomology 99, 1415-1419.

Taylor, D.B., Friesen, K., Zhu, J.J., Sievert, K., 2012a. Efficacy of cyromazine to control immature stable flies (Diptera: Muscidae) developing in winter hay feeding sites. Journal of Economic Entomology 105, 726-731.

Taylor, D.B., Moon, R.D., Campbell, J.B., Berkebile, D.R., Scholl, P.J., Broce, A.B., Hogsette, J.A., 2010. Dispersal of stable flies (Diptera: Muscidae) from larval development sites in a Nebraska landscape. Environmental Entomology 39, 1101-1110.

Taylor, D.B., Moon, R.D., Mark, D.R., 2012b. Economic impact of stable flies (Diptera: Muscidae) on dairy and beef cattle production. Journal of Medical Entomology 49, 198-209.

Taylor, D.B., Moon, R.D., Mark, D.R., 2012c. Economic impact of stable flies (Diptera: Muscidae) on dairy and beef cattle production. Journal of Medical Entomology 49, 198-209.

Thacker, J.R., 2002. An introduction to arthropod pest control. Cambridge: University Press.

Tharwat, M., Hassan, A., Moustafa, A., Mahran, A., 1994. Effects of industrial formulations of *Bacillus thuringiensis* on the biology of *Musca domestica* L.(Diptera: Muscidae). The Journal of the Egyptian Public Health Association 70, 151-163.

Thimijan, R., Pickens, L., Morgan, N., 1970. A trap for house flies. Journal of Economic Entomology 63, 1030-1031.

Thimijan, R., Pickens, L., Morgan, N., Miller, R., 1972. House fly capture as a function of number of traps in a dairy barn. Journal of Economic Entomology 65, 876-877.

Thomas, G.D., Skoda, S.R., Berkebile, D.R., Campbell, J.B., 1996. Scheduled sanitation to reduce stable fly (Diptera: Muscidae) populations in beef cattle feedlots. Journal of Economic Entomology 89, 411-414.

Thomsen, E., Thomsen, M., 1937. Über das Thermopraeferendum der Larven einiger Fliegenarten. Zeitschrift für vergleichende Physiologie 24, 343-380.

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.

Traversa, D., Otranto, D., Iorio, R., Carluccio, A., Contri, A., Paoletti, B., Bartolini, R., Giangaspero, A., 2008. Identification of the intermediate hosts of *Habronema microstoma* and *Habronema muscae* under field conditions. Medical and Veterinary Entomology 22, 283-287.

Tsukamoto, M., Narahashi, T., Yamasaki, T., 1965. Genetic control of low nerve sensitivity to DDT in insecticide-resistant house flies. Botyu-Kagaku - Scientific Insect Control 30.

Tunaz, H., Uygun, N., 2004. Insect growth regulators for insect pest control. Turkish Journal of Agriculture and Forestry 28, 377-387.

Turell, M.J., Knudson, G.B., 1987. Mechanical transmission of *Bacillus anthracis* by stable flies (*Stomoxys calcitrans*) and mosquitoes (*Aedes aegypti* and *Aedes taeniorhynchus*). Infection and immunity 55, 1859-1861.

Uebel, E., Sonnet, P., Bierl, B., Miller, R., 1975. Sex pheromone of the stable flyl: Isolation and preliminary identification of compounds that induce mating strike behavior. Journal of Chemical Ecology 1, 377-385.

Unterstenhöfer, G., 1970. Zur Beeinflussung der Resistenzentwicklung, Chemie der Pflanzenschutz-und Schädlingsbekämpfungsmittel. Springer, pp. 77-86.

Urdaz-Rodriguez, J., Fosgate, G., Alleman, A., Rae, D., Donovan, G., Melendez, P., 2009. Seroprevalence estimation and management factors associated with high herd seropositivity for *Anaplasma marginale* in commercial dairy farms of Puerto Rico. Tropical animal health and production 41, 1439-1448.

Vais, H., Williamson, M.S., Devonshire, A.L., Usherwood, P.N.R., 2001. The molecular interactions of pyrethroid insecticides with insect and mammalian sodium channels. Pest management science 57, 877-888.

Valentine, G., Marquez, L., Pammi, M., 2016. Zika virus-associated microcephaly and eye lesions in the newborn. Journal of the Pediatric Infectious Diseases Society 5, 323-328.

van den Berg, H., 2009. Global status of DDT and its alternatives for use in vector control to prevent disease. Environmental health perspectives 117, 1656.

Vazirianzadeh, B., Jervis, M., Kidd, N.A., 2007. The effects of oral application of cyromazine and triflumuron on house fly larvae. Journal of Arthropod-Borne Diseases 1, 7-13.

Venkatesh, K., Morrison, P., 1980. Crop filling and crop emptying by the stable fly *Stomoxys calcitrans* L. Canadian Journal of Zoology 58, 57-63.

Villatte, F., Ziliani, P., Marcel, V., Menozzi, P., Fournier, D., 2000. A high number of mutations in insect acetylcholinesterase may provide insecticide resistance. Pesticide Biochemistry and Physiology 67, 95-102.

Voegtline, A., Ozburn, G.W., Gill, G.D., 1965. The relation of weather to biting activity of *Stomoxys calcitrans* (L.) along Lake Superior. Papers of the Michigan Acadamy of Science, Arts, and Letters 50, 107-114.

Vogel, H., 1968. Züchtung von *Empusa muscae* und deren Verwendung zur Fliegenbekämpfung. Anzeiger für Schädlingskunde 41, 76-77.

Vontas, J.G., Graham, J., Hemingway, J., 2001. Glutathione S-transferases as antioxidant defence agents confer pyrethroid resistance in Nilaparvata lugens. Biochemical Journal 357, 65-72.

Voss, G., 1988. Insecticide/acaricide resistance: industry's efforts and plans to cope. Pest Management Science 23, 149-156.

Walsh, S.B., Dolden, T.A., Moores, G.D., Kristensen, M., Lewis, T., Alan, L., Williamson, M.S., 2001. Identification and characterization of mutations in house fly (*Musca domestica*) acetylcholinesterase involved in insecticide resistance. Biochemical Journal 359, 175-181.

Wang, Y., 1970. E. and Gill GD: 1970, Effect of temperature and relative humidity on mortality of adult stable flies. Journal of Economic Entomology 63, 1666-1668.

Ware, G. 1983. Pesticides, theory and application. San Francisco: Freeman, 308 pp.

Watson, D., Geden, C., Long, S., Rutz, D., 1995. Efficacy of *Beauveria bassiana* for controlling the house fly and stable fly (Diptera: Muscidae). Biological control 5, 405-411.

Watson, D., Rutz, D., Keshavarz, K., Waldron, J.K., 1998. House fly (*Musca domestica* L.) survival after mechanical incorporation of poultry manure into field soil. The Journal of Applied Poultry Research 7, 302-308.

Weidhaas, D., Haile, D., 1978. A theoretical model to determine the degree of trapping required for insect population control. Bulletin of the Entomological Society of America 24, 18-20.

Weiher, W., Bauer, B., Mehlitz, D., Nijhof, A.M., Clausen, P.-H., 2014. Field trials assessing deltamethrin (Butox®) treatments of sheep against Culicoides species. Parasitology research 113, 2641-2645.

WHO, 1957. Expert Committee on Insecticides (1957) Seventh report. World Health Organization techn. Rep. Ser 125.

WHO, 1976. Resistance of vectors and reservoirs of disease to pesticides. Twenty-second report of the WHO Expert Committee on Insecticides. Technical Report Series. No.: 585.

WHO, 1991. Vector Control Series. The house fly training and information guide-intermediate level. Geneva: World Health Organization.

WHO, 2013. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. Geneva: World Health Organization.

WHO, 2016. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. Geneva: World Health Organization.

Wieman, G., Campbell, J., Deshazer, J., Berry, I., 1992. Effects of stable flies (Diptera: Muscidae) and heat stress on weight gain and feed efficiency of feeder cattle. Journal of Economic Entomology 85, 1835-1842.

Williams, D., Rogers, A., Hester, P., Ruff, J., Levy, R., 1980. Preferred breeding media of the stable fly, *Stomoxys calcitrans*, in northwestern Florida. Mosquito News 40, 276-279.

Williams, D.F., 1973. Sticky traps for sampling populations of *Stomoxys calcitrans*. Journal of Economic Entomology 66, 1279-1280.

Williamson, M.S., Denholm, I., Bell, C.A., Devonshire, A.L., 1993. Knockdown resistance (kdr) to DDT and pyrethroid insecticides maps to a sodium channel gene locus in the house fly (*Musca domestica*). Molecular and General Genetics MGG 240, 17-22.

Williamson, M.S., Martinez-Torres, D., Hick, C.A., Devonshire, A.L., 1996. Identification of mutations in the houseflypara-type sodium channel gene associated with knockdown resistance (kdr) to pyrethroid insecticides. Molecular and General Genetics MGG 252, 51-60.

Wright, J.E., Oehler, D.D., Johnson, J.H., Cannon, J., 1975. Control of house fly and stable fly breeding in rhinoceros dung with an insect growth regulator used as a feed additive. Journal of wildlife diseases 11, 522-524.

Yarmut, Y., Brommer, H., Weisler, S., Shelah, M., Komarovsky, O., Steinman, A., 2008. Ophthalmic and cutaneous habronemiasis in a horse: case report and review of the literature. Israel Journal of Veterinary Medicine 63, 87.

Zaspel, D., 2009. Insektizidhaltige Netze zum Schutz von Pferden gegen Bremsen und Lästlingsinsekten auf Weiden in Brandenburg. Thesis, Journal No.: 3239. Freie Universität Berlin.

Zhang, L., Kasai, S., Shono, T., 1998. In vitro metabolism of pyriproxyfen by microsomes from susceptible and resistant housefly larvae. Archives of insect biochemistry and physiology 37, 215-224.

Zhu, J., Wienhold, B., Wehrle, J., Davis, D., Chen, H., Taylor, D., Friesen, K., Zurek, L., 2014. Efficacy and longevity of newly developed catnip oil microcapsules against stable fly oviposition and larval growth. Medical and Veterinary Entomology 28, 222-227.

Zhu, J.J., Li, A.Y., Pritchard, S., Tangtrakulwanich, K., Baxendale, F.P., Brewer, G., 2011. Contact and fumigant toxicity of a botanical-based feeding deterrent of the stable fly, *Stomoxys calcitrans* (Diptera: Muscidae). Journal of Agricultural and Food Chemistry 59, 10394-10400.

Zumpt, F., 1973. The Stomoxyine biting flies of the world. Diptera: Muscidae. Taxonomy, biology, economic importance and control measures. Stuttgart: Gustav Fischer Verlag.

11 List of publications

Reissert, S., Bauer, B., Steuber,S., Sievert, K., Clausen, P.-H., 2016. **Occurrence and distribution of insecticide resistance in stable flies (*Stomoxys calcitrans*) in the federal state of Brandenburg (Germany).** In: Tagung der Deutschen Veterinärmedizinischen Gesellschaft, Fachgruppe Parasitologie und parasitäre Krankheiten, May 2-4, Berlin, Germany, p. 109

Reissert, S., Jandowsky, A., Bauer, B., Steuber,S., Sievert, K., Clausen, P.-H., 2016. **Occurrence of insecticide resistance in nuisance insects on dairy farms in the federal state of Brandenburg, Germany - a global problem of intensive livestock husbandry systems?** In: Tropical Animal Diseases and Veterinary Public Health: Joining forces to meet future global challenges, First Joint AITVM – STVM Conference, September 4-8, Berlin, Germany, p. 97.

Reissert, S., Steuber,S., Bauer, B., Clausen, P.-H., 2017. **Insecticide resistance in stable flies (*Stomoxys calcitrans*) on dairy farms in Brandenburg, Germany.** In: WAAVP – 26th International Conference of the World Association for the Advancement of Veterinary Parasitology - in Conjunction with the 53rd MSPTM Annual Conference. Combating zoonoses: strength in east-west partnerships, September 4-8, Kuala Lumpur, Malaysia, p. 141. No.: 4624.

Reissert, S., Bauer, B., Steuber, S., Sievert, K., Clausen, P.-H., 2018. **Occurrence of insecticide resistance in stable flies (*Stomoxys calcitrans*) on dairy farms in the federal state of Brandenburg, Germany.** In: 28th Annual Meeting of the German Society for Parasitology, March 21-24, Berlin, Germany, p. 102. No.: DRE-O-05.

12 Acknowledgements

Throughout the time of working on this thesis, I could always rely on the support and cooperation of a number of people whom I would like to thank here.

My sincerest and cordial gratitude is dedicated to my professors and mentors: Prof. Dr Peter-Henning Clausen, Dr Burkhard Bauer and Dr Stephan Steuber for entrusting me with this project and for providing constructive scientific and moral support at all times. I would like to express my deepest thankfulness for receiving guidance in my practical work and for the kind and patient correction of this thesis.

I would also like to thank the Federal Office of Cosumer Protection and Food Safety (BVL) for making this study possible by funding it and forming a cooperation with the Institute of Parasitology and Tropical Veterinary Medicine of the Freie Universität Berlin.

Furthermore, I particularly want to thank Dr Kai Sievert for offering advice at all times and for providing me with a pyriproxyfen sample at such short notice.

I am grateful for the dedicated guidance I received from Dr Anabell Jandowsky and Dr Jana Hildebrand via E-mail and on the telephone before and during my study.

My sincerest thanks also goes to PD Dr Jürgen Krücken for his excellent help when coping with statistical and laboratory problems, and to Peggy Hoffmann-Köhler for her enthusiastic help in the laboratory when the larvae did not want to grow.

I like to thank Dr Erik Schmolz, Dr Carola Kuhn, Dr Birgit Habedank and especially Marion Lindner and Birgit Snelinski from the Federal Environment Office (UBA) for introducing me to the breeding system and the topical application of *Stomoxys calcitrans* and for providing me with stable fly pupae.

My sincere thanks go also to Dr Heike Williams, Melanie Gros and Bernd Jürgens from MSD Animal Health Innovation GmbH for showing me their stable fly breeding system, for always providing quick advice and for sending me *Stomoxys calcitrans* pupae at relatively short notice.

Many thanks go to those who always found professional answers to my questions in the laboratory and who offered friendly help at the institute at all times: Dr Erich Zweygarth, Ard Nijhof, PhD, Louise Le Bel, Antje Hoppenheit, PhD, Sabrina Ramünke and Sarah Knorr. I additionally want to thank Dr Roswitha Merle for her advice on the statistical evaluation of this study.

I want to express my gratitude to Prof. Dr Uwe Rösler of the Institute for Animal Hygiene and Environmental Health for letting me use their laboratory when testing my stable flies.

Finally yet importantly, I like to thank Dr Hans Peter Heckert, Dr Lutz Urzynicok and Dr Hagen Mallow for providing me with addresses of dairy farms or for helping me distribute my questionnaire analysis. The same applies to Marlene Neumann as well as to all the dairy farmers who took the time and interest to participate in this study.

In the end, I want to express my gratitude to all the ParaDocs and postdocs who through their team spirit and humour made my time at the institute marvellous and memorable.

My greatest thanks is devoted to my friends and family. I want to thank especially my parents for their kind and consistent support. I specifically want to express my gratitude to my father for his encouraging way of proofreading my thesis. I also want to thank Elena Sixtus particularly for (the good times and) the constructive conversations about statistical problems. And Patricia van de Wetering for making (not only) sports enjoyable. My special gratefulness is also dedicated to poor Norbert, as well as to Louis and Hartmut. And above all, heartfelt thanks to Alexander Oppermann: thank you for your caring and constant support. You are the best ☺.

13 Declaration of authorship

I hereby certify that this thesis has been composed by me and is based on my own work, unless stated otherwise. No other person's work has been used without due acknowledgement in this thesis. All references and verbatim extracts have been quoted, and all sources of information, including graphs and data sets, have been specifically acknowledged.

Berlin, 08.11.2018 Sophia Reissert

mbvberlin mensch und buch verlag

49,90 Euro | ISBN: 978-3-86387-940-2