Susceptibility of Two European strains of Stomoxys calcitrans (L.) to Cypermethrin, Deltamethrin, Fenvalerate, λ-cyhalothrin, Permethrin and Phoxim

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ABSTRACT

Susceptibility of two French strains of stable flies *Stomoxys calcitrans* (L.) to six insecticides was assayed, using an exposure technique (1-hour contact) with treated filter papers. Three replicates per insecticide, per concentration (10 concentrations per insecticide), per fly strain (ENVT, Cabanac) and fly category (blood-engorged – non-blood-engorged) were performed using a total of 14,400 adult flies in this trial. The LD_{50} and LD_{90} are higher for the blood-engorged flies than the non-blood-engorged flies for both strains of S. calcitrans. The LD_{90} (mg/m²) of the engorged flies for both strains were respectively: cypermethrin (637.9, 54.9), deltamethrin (264.3, 28.1), fenvalerate (2392.5, 125.1), λ-cyhalothrin (118.2, 41.3), permethrin (353.7, 88.1), and phoxim (194, 226.8). Phoxim, which has not been used in the ENVT for several years, showed the same susceptibility for both strains. The LD90 values obtained for the Cabanac strain (organic farm) were 1 to 4 times lower than the recommended doses of all five pyrethroids. For the ENVT strain (blood-engorged flies), the LD_{90} was 7.1 and 22.6 times over the recommended doses of both deltamethrin and fenvalerate respectively, which are commonly used insecticides on this site.

INTRODUCTION

The market for insecticides used to control flies on cattle is growing. Cattle breeders are

seeking ways to minimize production losses and disease transmission while respecting animal welfare. Among Diptera, stable flies (*Stomoxys calcitrans* Linnaeus, 1758) are of great medical and economic importance due to their world-wide distribution and the high population densities commonly found in field[1]. Somme[2] reported that an average of 75% of flies from 12 Norwegian farms were stable flies.

Dougherty *et al*.[3] observed cows infested with 0, 50, and 100 stable flies. These flies induced linear increases of head and ear movements, skin twitches, and tail swishes. Similar results were observed by Vitela *et al*.[4] Schwinghammer *et al*.[5] discovered that 100 horn flies (*Haematobia irritans*) and 25 stable flies per animal were sufficient to increase specific physiological responses such as heart rates, respiration rates, and rectal temperatures, due to their painful bites. Since cattle lose energy when trying to fight off flies, pain and blood losses may lead to the reduction of weight and milk production. In grazing as well as in feedlot yearling steers, differences in weight gains ranged from 0.02 to 0.2 kg per animal, per day, during the 3 or 4 observation months.[6-9] Bruce and Decker[10] reported that stable fly infestations caused a reduction in milk yield. Moreover, the milk fat production was higher in the treated herd than in the control herd.[11,12] The annual losses of the U.S. cattle industry reached a total of $2,211 million[13].

Male and female *Stomoxys calcitrans* are biting flies. Numerous agents are transmitted mechanically by stable flies: *Bacillus anthracis, Brucella* spp., bovine diarrhea virus, *Trypanosoma evansi* and probably *Besnoitia besnoiti*. In addition to the pain they inflict, stable flies cause skin lesions after biting specific parts of the animal's body[1, 14-18].

Many field trial studies are conducted on the control of cattle flies using insecticides. They usually deal with *Haematobia irritans*, horse flies, *Musca domestica*, *Musca autumnalis* and *Stomoxys calcitrans*.[19-24] Only a few publications report drug effectiveness against *S. calcitrans* alone. Hogsette *et al*.[25] removed a stable fly population from dairy cattle within 24 hours with permethrin tapes applied to their tails. The control of stable flies with insecticides applied to cattle is limited, due to low drug concentrations in the lower legs where stable flies normally feed, and especially due to the short time of contact between the flies and their hosts when taking a blood meal[25].

Historically, stable fly control programs have focused on adult populations with the application of insecticides to cattle and stable walls. However, no information is available on the susceptibility of European stable fly populations. The aim of this study was to test the insecticide susceptibility of two *S. calcitrans* populations isolated in the South-West of France: the first one was isolated at the National Veterinary School of Toulouse where insecticides (mainly pyrethroids) are commonly applied to cattle, and the second, from an organic farm in the vicinity of Toulouse city, where there are no records of insecticide use for more than 10 years.

Six insecticides were tested: one organophosphate and five pyrethroids. The first family of chemicals inhibits the enzyme acetylcholinesterase, whereas the second family (pyrethroids) acts on sodium channels of nerve cells. The test was performed by exposing stable flies to insecticide-impregnated papers for 1 hour. This method was adapted from those suggested during the 8th International Symposium on Ectoparasites of Pets to test sand fly and mosquito susceptibility to insecticides.

MATERIALS AND METHODS

Insecticides

Six neurotoxic insecticides were used in this study: five pyrethroids (cypermethrin, deltamethrin, fenvalerate, λ-cyhalothrin, permethrin) and one organophosphate (phoxim).

All compounds were produced by PESTANAL® and purchased from Sigma Aldrich (Saint-Quentin Fallavier, France). All the doses were expressed in mg of active ingredient per m². Pure acetone was used to

dilute these active ingredients until required concentrations were reached and also to obtain a negative test control.

To establish the 50% lethal dose (LD50) and the 90% lethal dose (LD90), insects were exposed to ten gradually increasing doses: cypermethrin [0 to 2,000] mg/m,^{2} deltamethrin [0 to 370] mg/m,^{2} fenvalerate [0 to 3392] mg/m,^{2} λ–cyhalothrin [0 to 400] mg/m,^{2} permethrin [0 to 2000] phoxim [0 to 750] mg/m.^{2}.The recommended dosage by the manufacturer for cattle was within the range of tested doses and calculated according to the body surface area.[27]

$$S(m2) = 0.1 \times [BodyWeight]_{(kg)}.0.685$$
For example, the recommended dose of deltamethrin was 30 ml for cattle with a body weight of 400 kg (6 m^2), that is to say 37.5 mg / m^2 (7.5 mg /ml for Butox 7.5 Pour on® Intervet, Beaucouzé, France).

Stable Fly Colonies Used in the Experiments

Two strains of *S. calcitrans* were established in the Laboratory of Parasitology at the National Veterinary School of Toulouse (ENVT).

- ENVT strain. At the veterinary school, cattle and stable walls were frequently treated with deltamethrin, while horses and wall boxes were treated with fenvalerate. We were unable to obtain accurate historical records of insecticide applications, but the insecticide pressure was high due to the epizootic of bluetongue in 2008 and 2009. Adult stable flies were manually removed from cattle or caught by using six Vavoua traps for 12 hours in May 2009 at the campus of ENVT. This trap was initially developed in West Africa for the control of tsetse flies ,[28] but is now widely used for the capture of *S. calcitrans*. [29,30] *Stomoxys calcitrans* were able to be identified according to Zumpt.[1] Each stable fly was examined individually under a magnifying glass to discard individuals with phoretically-attached macrochelid mites. Then, flies were reared under laboratory conditions
- Cabanac strain. This second colony was

collected in 2010 on an organic cattle farm where there has been no insecticide spraying in the past 10n years. This farm is located 70 km southwest of Toulouse.

Rearing Methods

Both adult fly colonies were maintained in cages (30 x 30 x 30 cm) and were given each a specific type of diet. The first group was fed in Petri dishes containing a piece of cotton soaked in honey and water (non-blood-engorged flies), whereas the second group was fed with honey, water, and blood using a double-chambered glass feeder made by a local glassblower (blood-engorged flies). This system allows the blood to be kept at a stable temperature of 38.4°C, by having warm water circulating through the outer chamber using an electric heat pump (ED-5 Heating Circulator with Open Bath, Julabo, Seelbach, Germany).

Bovine blood was poured in the inner chamber, which was sealed with a thin synthetic membrane (Parafilm 3M, Pechiney Plastic Packaging, Chicago, IL). The glass feeder was placed in contact with the upper side of the cage, thus enabling the stable flies to pierce the thin membrane in order to draw blood. The blood was collected weekly from a blood donor calf. This 14-month old calf was not treated with topical or systemic parasiticides within 3 months before the start of the study.

Blood was collected in 4ml Venosafe tubes, containing 60 USP U Lithium Heparin (Terumo Europe N.V., Leuven, Belgium) to prevent coagulation. The blood was stored in a refrigerator at 3-4 °C. The heparinized blood and the membrane were changed daily. Stable flies were bred at 25°C (±2°C), 50% (±10% RH) humidity, and under a 12 Light:12 Dark photoperiod. Immediately after emergence, adult populations from group 1 and 2 had free access to water and honey or to water, honey, and blood, respectively. More details regarding rearing methods are provided by Salem *et al*. [31] A batch of 20 blood-engorged and non-blood-engorged flies, aged between 5 and 10 days (approximately 10 males and 10 females per vial),

was released with ten different insecticide concentrations. Three replicates per insecticide, per concentration, per fly strain (ENVT and Cabanac), and meal category (blood-engorged – non-blood-engorged) were performed using a total of 14,400 adult flies in this trial.

Exposure to Insecticides

Insecticide sensitivity of *Stomoxys calcitrans* was tested in this study by exposing them to impregnated filter papers. Half filter paper discs (Whatman, 185 mm diameter) were cut, forming a cone when properly folded. They were impregnated with 2 ml acetone (control) or with an insecticide-acetone solution. Once dry, they were coiled into a cone shape (Figure 1). Whatman filter paper sealing discs of 90 mm diameter (Healthcare UL Limited, Little Chalfont, UK) were treated in the same way with 940 µl of solution. The cones were placed in a cylindrical plastic container (5 cm diameter X 10 cm height) covered with glass featuring a 1-cm-diameter hole.

Twenty adult *S. calcitrans* were then removed from a colony with an aspirator and blown into the cone through the hole. The cone was then carefully sealed with a filter paper disc. The glass was removed and replaced by the container cap (Figure 1). The flies were not anesthetized during the manipulation. Following 1-hour exposure, the cone was opened in a glass cage and the flies were counted. The mortality criteria were based on the WHO criteria: flies were considered "dead" when they were dead, moribund, or showing uncoordinated movements.

Data Analysis

The results were expressed in percentages of dead flies. The results analysis was performed with the Win Non Lin version 4.01 software (Pharsight, Mountain View, CA.). The dose-effect relationships were analyzed using a sigmoid E_{max} model:

$$\text{Effect} = \frac{E_{max} \times D^{\delta}}{LD_{50}^{\delta} + D^{\delta}}$$

D stands for the dose of insecticide,

Emax is the maximum lethal effect, LD_{50} is the dose producing 50% maximum effect, and γ is the slope of the dose-response curve. For each drug, the LD_{90} was calculated using the estimated values of E_{max}, LD_{50} and γ

Results

All 1,440 stable flies (72 control groups of 20 flies each) were alive after 1ne-hour contact with pure acetone-impregnated papers. Each drug reached a maximum effect (Emax) of 100% of mortality. The LD_{50} and the LD_{90} were calculated for the different drugs, both strains (ENVT strain and Cabanac strain) and for both categories of flies (blood-engorged and non-blood-engorged flies, Table 1). The dose-response curves are presented for three drugs: deltamethrin and fenvalerate (frequently used at the ENVT only, Figures 2 and 3 respectively) and phoxim (not used at the ENVT for more than 20 years and in the organic farm for more than 13 years, Figure 4).

Regarding the effect of blood meal on insecticide efficiency, the ratios of LD_{50} or LD_{90} for blood-engorged flies over LD_{50} or LD_{90} for non-blood-engorged flies were calculated for each strain. The LD_{50} and the LD_{90} were higher for the blood-engorged flies than for the non-blood-engorged flies. This difference was observed in both strains of *S. calcitrans*. The ratios of LD_{50} for blood-engorged over non-blood-engorged flies were, respectively for the ENVT strain and Cabanac strain : 1.7 and 2.1 for phoxim; 3.5 and 2.5 for deltamethrin; 2.1 and 1.6 for cypermethrin;, 2.3 and 2 for fenvalerate, 4.2 and 1.7 for λ-cyhalothrin and 1.3 and 1.7 for permethrin.

Differences in stable fly susceptibility to insecticides between the organic farm and the ENVT campus were able to be identified (Table 1). Indeed, the ratios between the LD50 and LD90 of both strains (ENVT/Cabanac) ranged from 5 to 10 for three pyrethroids (cypermethrin, deltamethrin and fenvalerate) and from 3 to 3.8 for the λ-cyhalothrin and the permethrin, respectively, when calculated based on the blood-

Table 1. Susceptibility of the two strains of S. calcitrans to insecticide residues on filter papers.

Population	LD50 (mg/m²) (± S.D.)	LD90 (mg/m²)	Ratio 50[a] LD50	Ratio 90[b] LD90	SR50[c]	SR90[d]	Ratio T[e]
Cypermethrin (recommended dose: 125 mg/m²)							
Cabanac non-blood-engorged	11.5 (0.4)	30.3	-	-	-	-	0.24
Cabanac blood-engorged	18.9 (0.9)	54.9	1.6	1.8	-	-	0.43
ENVT non-blood-engorged	50.4 (2.1)	212.6	-	-	4.4	7	1.7
ENVT blood-engorged	104.3 (7.4)	637.9	2.1	3	5.5	11.6	5.1
Deltamethrin (recommended dose: 37.5 mg/m²)							
Cabanac non-blood-engorged	2.9 (0.1)	9.7	-	-	-	-	0.26
Cabanac blood-engorged	7.2 (0.6)	28.1	2.5	2.9	-	-	0.7
ENVT non-blood-engorged	12.4 (1.3)	104.1	-	-	4.3	10.7	2.8
ENVT blood-engorged	43.9 (7)	264.3	3.5	2.5	6.1	9.4	7.1
Fenvalerate (recommended dose: 106 mg/m²)							
Cabanac non-blood-engorged	21.4 (2.1)	92.1	-	-	-	-	0.9
Cabanac blood-engorged	42.6 (1.6)	125.1	2	1.4	-	-	1.2
ENVT non-blood-engorged	181.9 (9.8)	500.9	-	-	8.5	5.4	4.7
ENVT blood-engorged	417.7 (34.2)	2392.5	2.3	4.8	9.8	19.1	22.6
λ-cyhalothrin (recommended dose: 50 mg/m²)							
Cabanac non-blood-engorged	6.5 (0.3)	17.5	-	-	-	-	0.4
Cabanac blood-engorged	11.4 (0.8)	41.3	1.7	2.4	-	-	0.8
ENVT non-blood-engorged	8.2 (0.3)	22.6	-	-	1.3	1.3	0.5
ENVT blood-engorged	34.1 (0.7)	118.2	4.2	5.2	3	2.9	2.4
Permethrin (recommended dose: 125 mg/m²)							
Cabanac non-blood-engorged	20.2 (0.6)	46.01	-	-	-	-	0.4
Cabanac blood-engorged	35.4 (1.1)	88.1	1.7	1.9	-	-	0.7
ENVT non-blood-engorged	105.6 (3.3)	232.6	-	-	5.2	5.1	1.9
ENVT blood-engorged	135.4 (5.7)	353.7	1.3	1.5	3.8	4	2.8
Phoxim (recommended dose: 750 mg/m²)-							
Cabanac non-blood-engorged	52.9 (2.7)	102.4	-	-	-	-	0.1
Cabanac blood-engorged	112.7 (6.3)	226.8	2.1	2.2	-	-	0.3
ENVT non-blood-engorged	59.4 (1.5)	99.7	-	-	1.1	1	0.1
ENVT blood-engorged	100.6 (5.7)	194	1.7	1.9	0.9	0.9	0.3

S.D. : Standard Deviation
[a] LD50 blood-engorged flies / LD50 non-blood-engorged flies
[b] LD90 blood-engorged flies / LD90 non-blood-engorged flies
[c] Susceptibility ratio 50: lethal dose 50 value of ENVT strain divided by lethal dose 50 value of Cabanac strain
[d] Susceptibility ratio 90: lethal dose 90 value of ENVT strain divided by lethal dose 90 value of Cabanac strain
[e] LD90 / Recommended dose

Figure 1. *Schema of the filter cone system*
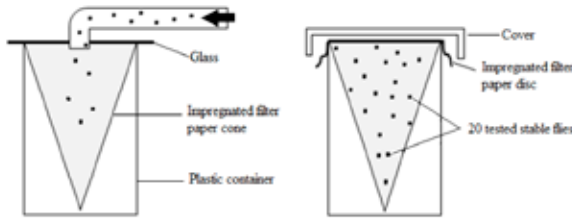
engorged stable flies. The susceptibility to phoxim, not used in the ENVT for more than 20 years in the organic farm, was the same for both strains.

DISCUSSION

The Filter Paper Cone Method.

The implementation of this method, used to assess insecticide efficacy, is very simple. This bioassay technique performed in the laboratory was considered safe for the 1,440 flies of the control groups.

Flies are in contact with the insecticide or acetone-impregnated filter paper for almost an hour, except when they fly inside the cone. The flies were in permanent contact with the paper by placing them between two treated fabrics, as also described by Hogsette e al. [32] It was not the case with Marçon et al. [33] using impregnated filter papers placed in plastic Petri dishes. In this study, the flies are not anesthetized in order to avoid the mortality risk. We are able to test simultaneously different products and different concentrations on 20 flies per group. The method suggested by Ehrhardt [34] is more time-consuming. He observed only two flies simultaneously for 5 minutes in a syringe with a filter paper. The time of contact during the test (1 hour) was chosen so as to prevent possible variations in individual exposure in correlation with the required time to introduce the 20 insects into the device. It is longer than the natural contact duration with the coat of cattle in the field. Indeed, stable flies can take one 35,36 to two or even three blood meals a day, 37,38 each lasting 4 to 30 minutes. 37,39

Susceptibility to Insecticides of Both Strains Isolated in the South-West of France

The LD_{50} and LD_{90} of the Cabanac strain were similar to those published by Marçon et al. [33] for permethrin with the Kerrville susceptible strain. This strain was used by Marçon as a standard reference for insecticide susceptibility because it has been isolated from insecticide exposure since 1952. The LD_{50} and LD_{90} were respectively 14.5 and 24.1 mg/m² after 4-hour exposure. In this study, the LD50 was 20 - 35.4 mg/m2 and the LD90 was 46 - 88.1 mg/m2 for non-blood-engorged and blood-engorged flies, respectively, after 1-hour exposure. Ehrhardt [34] exposed flies to deltamethrin-impregnated papers for 5 minutes and obtained an LD_{50} of 3 mg/m2 for the susceptible strain from Mauritius. The engorgement state is not reported. In the present study, the deltamethrin LD_{50} was 2.9 (non-blood-engorged) and 7.2 (blood-engorged) mg/m².

For all drugs, the insecticide doses producing 90% mortality (LD_{90}) were

Figure 2. *The mortality rates for the two strains of blood-engorged S. calcitrans after one hour contact with deltamethrin residues on filter papers.*
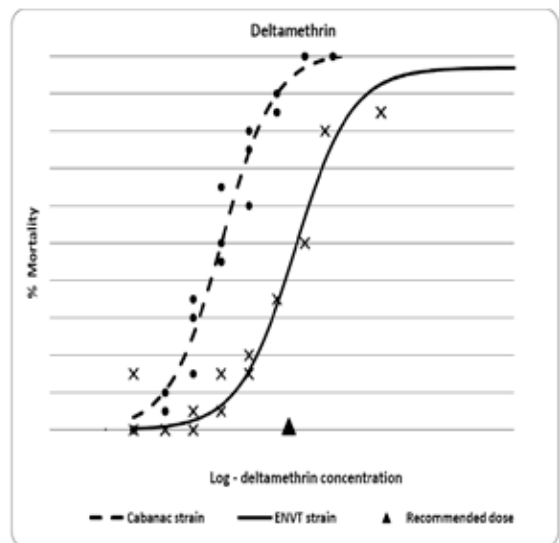
compared to the recommended doses. When considering the five pyrethroids, LD_{90} values for the Cabanac strain were within the recommended dose range of fenvalerate (Table 1 and Figure 3) but were four times lower than the recommended dose of cypermethrin and deltamethrin (Table 1 and Figure 2), meaning that maximum effectiveness should be anticipated for all drugs against this strain. For the ENVT strain (blood-engorged flies), the LD_{90} recommended dose ratio ranged from 2.4 to 22.6, meaning that recommended doses of the five pyrethroids should not be able to produce sufficient effectiveness against this strain. Interestingly, greater decreases in potency were obtained with deltamethrin and fenvalerate (ratio of 7.1 and 22.6, respectively), two commonly used insecticides on the site.

When considering the effects of the recommended doses against the ENVT strain, our results indicated that the recommended dose of deltamethrin (37.5 mg/m^2) was close to the LD50 (43.9 mg/m^2, Table 1) and observations of Figure 2 confirm that it would produce about 40-50% mortality. For fenvalerate, the recommended dose (106 mg/m^2) was much lower than the LD_{50} (417.7 mg/m^2, Table 1) and observations of Figure 3 confirm that it would produce no more than 10-15% mortality.

In this study, the LD_{90} ratio between the less susceptible strain (ENVT blood-engorged flies) and the more susceptible strain (Cabanac blood-engorged flies) ranged from 9 to 19 for cypermethrin, fenvalerate, and deltamethin. These ratios were 4 for permethrin and 2.9 for cyhalothrin. These ratios were higher than those published by Marçon et a,[33] who compared three field strains with the Kerrville susceptible strain in the U.S.

CONCLUSION

If we consider the Cabanac strain as a susceptible strain (no exposure to insecticides in this organic farm since 13 years), the ENVT strain was resistant to the five tested pyrethroids. Today, the treatment of cattle

Figure 3. The mortality rates for the two strains of blood-engorged S. calcitrans after one hour contact with fenvalerate residues on filter papers.
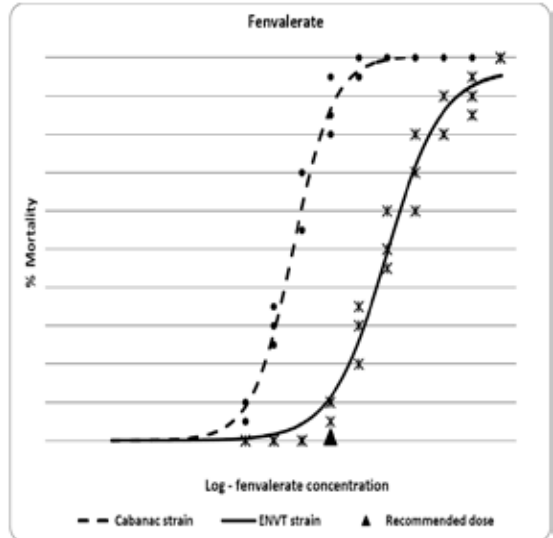
with pyrethroids at the recommended dose on the site of the ENVT obviously cannot control stable fly populations. The use of other insecticide families such as avermectines (eprinomectin), or organophosphates (phoxim) on animals as well as insect growth regulators (cyromazin, triflumuron) in the environment could be recommended in this area where resistance is found.

ACKNOWLEDGEMENT

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