Sensitivity of *Culicoides obsoletus* (Meigen) (Diptera: Ceratopogonidae) to deltamethrin determined by an adapted WHO standard susceptibility test

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SUMMARY

Bluetongue is a disease of major economic concern in Europe. Its causative agent, bluetongue virus (BTV), is transmitted by several *Culicoides* species (mainly *Culicoides imicola* and *Culicoides obsoletus* in Europe). The application of insecticides on animals may reduce transmission of BTV, however, no formulation is currently licensed specifically against *Culicoides* midges. The present study assesses the susceptibility of *C. obsoletus* to deltamethrin using an adapted World Health Organization (WHO) susceptibility test. Midges were exposed to different dosages of deltamethrin for 1 h, and mortality after 1 h and 24 h was recorded. Results indicated that deltamethrin is highly toxic to *C. obsoletus* since a dose of $1{\cdot}33 \times 10^{-4}$% was enough to kill 50% of the population (LD_{50}) in 24 h. The deltamethrin concentration needed to kill 90% of the population (LD_{90}) was $5{\cdot}55 \times 10^{-4}$%. The results obtained in the present work could help to create a system that can be used to assess insecticide resistance and susceptibility of *Culicoides* biting midges.

Key words: *Culicoides obsoletus*, bluetongue, insecticide bioassay, LD_{50}, LD_{90}, vector control.

INTRODUCTION

Bluetongue (BT) is a widespread viral disease of ruminants, inflicting mortality rates of up to 13% and 41% in cattle and sheep, respectively (Conraths *et al*. 2009). The pathogen agent is an *Orbivirus* that is transmitted by competent vector species of *Culicoides* Latreille biting midges. A number of species can transmit the bluetongue virus (BTV) but *Culicoides imicola* Kieffer is considered the main vector of BTV in Africa and southern Europe (Boorman *et al*. 1985; Mellor *et al*. 1985) while *Culicoides pulicaris* Linnaeus, *Culicoides dewulfi* Goetghebuer, *Culicoides obsoletus* (Meigen) and *Culicoides chiopterus* (Meigen) are considered vectors in northern Europe (Purse *et al*. 2004; Conte *et al*. 2007; Balenghien, 2008; Meiswinkel *et al*. 2008; Stephan *et al*. 2009). Of these species, *C. obsoletus* is the most abundant in Europe (EFSA, 2008) and is considered as the main vector species in Europe (Jennings and Mellor, 1988; Carpenter *et al*. 2006; Hoffmann *et al*. 2009). This species, as well as *C. chiopterus* and *Culicoides scoticus* Downes & Kettle, has also been implicated in the transmission of Schmallenberg virus, which emerged recently in northern Europe (Elbers *et al*. 2013).

The insecticide susceptibility of European species of *Culicoides* to deltamethrin is poorly documented. The responses of several species of *Culicoides* to a range of organochlorines, organophosphates, carbamates and pyrethroids has been documented (Hill and Roberts, 1947; Kline and Roberts, 1981; Floore, 1985; Holbrook, 1994; Braverman *et al*. 2004; Schmahl *et al*. 2008, 2009; Venail *et al*. 2011) and the results obtained with insecticides based on pyrethroids – such as deltamethrin – show promise for the control of *Culicoides* midges (Mehlhorn *et al*. 2008*a*, *b*; Schmahl *et al*. 2008, 2009; Venail *et al*. 2011).

In an attempt to standardize the technique of assessing the response of insects to chemical treatments, the World Health Organization (WHO, 1981) developed an *in vitro* method that proved successful for mosquitoes and was subsequently adapted to work on biting midges (Venail *et al*. 2011). The present work could aid to standardize a protocol to assess the sensitivity of *Culicoides* to insecticides and the results obtained could be used to develop commercial products with the correct doses of deltamethrin, thus avoiding the misuse of active components in the field that may be toxic to non-target insects or

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that might lead to the rapid development of resistance in *Culicoides* populations.

This study evaluates the sensitivity to deltamethrin of a field population of *C. obsoletus* using the standardized World Health Organization (WHO) test.

MATERIALS AND METHODS

Insect collection

Adult *Culicoides* midges were collected at a dairy farm named *Ca's Boter* (39°30′N; 3°7′S,) located in Felanitx on the Balearic Island of Majorca, Spain. Insects were collected using either Onderstepoort (Agricultural Research Council-OVI, South Africa) or Mini-CDC (John Hock Company, USA) light traps, which were operated from dusk to dawn (19:30 to 7:00) between April and June 2010. A piece of roughly folded paper towel was placed inside the collection container of the trap to shelter trapped insects from the air flow of the fan. Early in the morning captured midges were taken to the insectary inside a thermally insulated container. At the insectary, midges were transferred to WHO chambers (WHO, 1981) in batches of ≈100 individuals and maintained in a dim light environment at 25 ± 2 °C and 75–85% relative humidity (RH) with 5% sucrose solution for 24 h. After this time, any dead *Culicoides* were discarded and live ones were used to assess the insecticide.

Insecticide susceptibility test

The standard WHO test procedure (WHO, 1981) was modified to assess the lethal effects of differing doses of deltamethrin on *C. obsoletus*. Filter papers (Whatman # 1, 90 g m^{-2}, 12 × 25 cm) impregnated with different doses of deltamethrin (0·0001, 0·0005, 0·001, 0·005 and 0·01%) in acetone-silicone solution – 2 mL per paper; 67% acetone, 33% silicone – were supplied by a WHO collaborative centre (Vector Control Research Unit, Universiti Sains Malaysia). Nets supplied along with the WHO test kit were replaced by fine gauze to avoid escape of midges and their transfer from the maintenance to the test chambers (and vice versa) was easily conducted after cold anaesthetizing the insects for 3 min at −4 °C.

Papers without insecticide and impregnated only with 2 mL of acetone-silicone mixture were supplied by the same institution and used as a control.

WHO treated chambers with batches of ≈100 unsorted *Culicoides* were held horizontally for 1 h at 25 ± 2 °C, 75–85% RH. At least three replicates per concentration were conducted although more replicates were performed when some of the insects survived the treatment (Table 1). After exposure, insects killed by the immediate action of the insecticide were counted and identified, while those still alive were transferred to clean chambers and maintained in the insectary with 5% sucrose solution. After 24 h, alive and dead midges were again counted and identified. If the mortality of control groups was between 5 and 20%, the mortality rate of the treatment groups was corrected by applying the Abbot formula (Abbott, 1925). If mortality of control groups was >20%, the results were discarded. Data of the tested samples were pooled if mortality <5% was noted for the control. All midges were identified using the key of Rawlings (1996) although a subsample of 793 specimens belonging to the Obsoletus complex were sent to CIRAD (Centre International de Recherche de l'Agriculture et du Dévéloppement) for molecular analysis as females of this complex are difficult to identify morphologically.

Data analysis

Data obtained from the different assays were pooled and subjected to Probit analysis (Finney, 1971) with the program XLStat 2011 (Addinsoft) and the Lethal Dose 50 (LD_{50}) and LD_{90} (Raymond, 1985) were obtained. Only the numbers collected of *C. obsoletus* were high enough to analyse statistically as individual species. Statistical differences were considered significant at $P < 0{\cdot}05$. Cumulative per cent mortality was transformed into probit units and plotted against the logarithm of dose of toxicant with the analytic program Statplus 2009. The log-dose probit (Ld-P) mortality line obtained was used to measure the variability of the strain.

RESULTS

A total of 2737 *Culicoides* midges belonging to 11 different species were assayed, namely: Obsoletus complex (78·4%), *C. circumscriptus* Kieffer (15·1%), *C. newsteadi* Austen (3·6%), *C. maritimus* Kieffer (0·9%), *C. univittatus* Vimmer (0·8%), *C. cataneii* Clastrier (0·6%), *C. longipennis* Khalaf (0·3%), *C. pictipennis* Staeger (0·1%), *C. puncticollis* Becker (0·1%) and *C. imicola* (0·1%). 2145 specimens belonged to the Obsoletus complex. Molecular analyses on specimens belonging to the Obsoletus complex revealed that only five (0·63%) of the 793 tested belonged to *C. scoticus* Downes & Kettle, while the remaining (788 individuals; 99·37%) belonged to *C. obsoletus*.

The mean mortality observed for the Obsoletus complex was >50% at a deltamethrin concentration of 0·0005% and >90% at a deltamethrin concentration of 0·001%. Doses ⩾0·005% led invariably to 100% mortality. At 24 h post-exposure the mortality observed ranged from 1·8 (delt. conc. = 0·0001%) to 1·01 (delt. conc. = 0·001%) times higher than the mortality observed at 1 h post-exposure (Table 1).

After 1 h exposure, and following correction using the Abbot formula, the deltamethrin LD_{50} observed for the Obsoletus complex was $LD_{50} = 1{\cdot}89 \times 10^{-4}$%

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Table 1. Effect of deltamethrin on the mortality of *Culicoides obsoletus* under laboratory conditions

Deltamethrin concentration (%)	∑ *Culicoides* assayed (range)	*N*	$\overline{X}$ % mort ± S.D. (1 h)	$\overline{X}$% mort ± S.D. (24 h)
Control	171 (41–78)	3	2 ± 3·0a	9 ± 9·1a
0·0001	389 (38–165)	6	23 ± 17·7a	46 ± 22·0a
0·0005	621 (67–185)	6	81 ± 22·6a	83 ± 16·7a
0·001	409 (54–103)	5	98 ± 16·3a	99 ± 1·4a
0·005	187 (27–106)	3	100 ± 0·0a	100 ± 0·0a
0·01	179 (28–115)	3	100 ± 0·0a	100 ± 0·0a

Means in rows with the same letter are not significantly different (P <0·05).

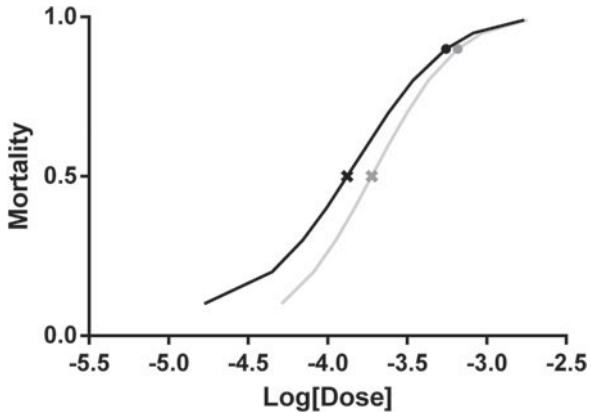

Fig. 1. Sensitivity of *C. obsoletus* when exposed to various concentrations of deltamethrin for 1 h (grey line) and after 24 h post exposure (black line) indicating LD_{50} (crosses) and LD_{90} (dots).

Fig. 2. Regression model showing the variability of response to deltamethrin of the *Culicoides* population assayed. Grey line represents the experimental points while black line represents the regression line (Ld-P).

and a $LD_{90} = 6{\cdot}57 \times 10^{-4}$% (Fig. 1). LD_{50} and LD_{90} were slightly lower at 24 h post-exposure ($LD_{50} = 1{\cdot}33 \times 10^{-4}$% and $LD_{90} = 5{\cdot}55 \times 10^{-4}$%) than after 1 h exposure (Fig. 1).

The natural mortality observed was close to 2% at 1 h post-exposure and lower than 10% 24 h post-exposure (Table 1).

Slope values of the Log. Dose-Probit Line (Ldp) showed homogeneity in population response to the insecticide treatment (slope 2·11; $R^2 = 0{\cdot}96$). The straight line observed for the Ldp indicated a unimodal response of the population to the treatments (Fig. 2).

DISCUSSION

The population of *C. obsoletus* studied showed a high sensitivity to deltamethrin. The lowest concentration tested increased the mortality rate of the population by approximately 40% and concentrations $\geqslant 0{\cdot}005$% killed all midges assayed in less than 1 h, thus indicating the effectiveness of this insecticide in laboratory conditions. Deltamethrin affected the insects mainly during the first hour of contact and any residual effect was only evident when the lowest concentrations were applied.

The results obtained in the present work are consistent with other bioassays that demonstrate the highly potent effect of deltamethrin on *Culicoides* populations (Bishop *et al*. 2001; Doherty *et al*. 2001; Melville *et al*. 2001; Mehlhorn *et al*. 2008*a*; Schmahl *et al*. 2008). Furthermore, the protocol used in the present bioassay offers a reproducible and standardized method to test *Culicoides* midges to insecticides. In a similar study, Venail *et al*. (2011) obtained a $LD_{90} = 2{\cdot}03 \times 10^{-3}$% 24 h post-treatment, thus indicating that the population of *C. obsoletus* assayed in the present trial was 3·5 times more susceptible to deltamethrin than the population studied by Venail *et al*. (2011). This difference in tolerance observed between populations of the same species suggests that previous exposure to deltamethrin due to farm practices could have had an effect on the susceptibility of the *Culicoides* populations to this insecticide.

Standardized WHO tests are used to assess the susceptibility and possible resistances of certain insects – especially mosquitoes – to insecticides in laboratory conditions. However, field studies are essential to determine the real effect of the insecticide over the target population and associated fauna. The environmental temperature should also be considered since pyrethroids can exhibit lower toxicity at higher temperatures (Hodjati and Curtis, 1999). Knowledge of the temperature effects on deltamethrin toxicity could avoid the continuous use of sub-lethal doses of pyrethroids during the summer season in southern Europe that may lead to resistance development.

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In the present work we have assessed the sensitivity of one of the main vector species – *C. obsoletus* – of BTV in Europe. However, assessment of the other important species in Europe – *C. imicola, C. pulicaris* or *C. chiopterus* – should be conducted in future works to contrast the results with previous studies (Venail *et al*. 2011) and establish its susceptibility to the insecticide. Furthermore, more field trials with discriminating doses of insecticide applied on animals should be conducted since the results obtained so far with permethrins have shown limited efficacy (Mullens *et al*. 2001; Venail *et al*. 2011). The repellent and insecticide effect of deltamethrin could, however, decrease the biting rate of the midges or reduce the size of the vector population thus affecting the infection rate and preventing the transmission of BTV.

The results obtained in the present trial showed that the population of *C. obsoletus* assayed is highly susceptible to deltamethrin treatments. Field trials with this insecticide would reveal its potential for the control of the *Culicoides* populations and determine if the reduction of the biting rate would be high enough to generate a significant impact on the transmission of BTV.

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