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Short communication

Chemical composition of silage residues sustaining the larval development of the *Culicoides obsoletus*/*Culicoides scoticus* species (Diptera: Ceratopogonidae)

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ABSTRACT

Culicoides (Diptera: Ceratopogonidae) are biological vectors of bluetongue virus (BTV). Bluetongue is a viral disease that affects domestic and wild ruminants. Since its recent emergence in northern Europe, this disease has caused considerable economic losses to the sheep and cattle industry. The biotopes, and more particularly the chemical characteristics which are suitable for larval development of the main vector species, are still relatively unknown. This study shows that the larvae of biting midges belonging to the species *Culicoides obsoletus* and *Culicoides scoticus* are able to breed in different types of silage residue (maize, grass, sugar beet pulp and their combinations). The chemical composition of substrates strongly influences the presence of the immature stages of these biting midges. Higher lignin and insoluble fibre contents seem to favour their presence and could play the role of a physical support for semi-aquatic larvae. In contrast, higher concentrations of magnesium and calcium are negatively correlated with the presence of these two species. These data will help to locate and monitor the breeding sites of these species and could contribute to the control of these insects on farms.

1. Introduction

Bluetongue (BT) is an emerging viral disease in northern Europe (Thiry et al., 2006). This disease, which affects domestic and wild ruminants, was reported for the first time in northern Europe in August 2006. During the second half of 2006, over 2000 cases of BT were recorded in Europe; 399 and 296 sheep and cattle farms in Belgium, respectively, were infected (http://www.afsca.be). In 2007, a greater clinical severity was recorded, associated with increased rates of mortality and morbidity (Szmaragd et al., 2007; Saegerman et al., 2008b). A total of 6870 farms were affected in Belgium (http://www.afsca.be).

From late 2006, the epizoonosis extended to other neighbouring countries, for example France where 27,413 outbreaks of BTV-8 were reported in 2008. Direct and indirect economic losses related to serotype 8 of bluetongue were considerable, although difficult to assess (Saegerman et al., 2008b). The biting midges responsible for the transmission of BTV belong to the genus *Culicoides*, but in a particular area, only a few species may act as biological vectors of the virus (Saegerman et al., 2008a).

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Very few data exist on *Culicoides* breeding sites and their physicochemical characteristics, in particular for the main northern European species of bluetongue virus vectors (Kettle & Lawson, 1952; Rieb, 1982; Chaker, 1983; Zimmer et al., 2008). *Culicoides obsoletus* (Meigen) and *Culicoides scoticus* Downes and Kettle form the *Obsoletus* complex. The possibility of transmission of BTV serotype 8 has been shown in northern Europe for this complex (Carpenter et al., 2008). Hoffmann et al. (2009) consider *C. obsoletus* as a relevant vector of bluetongue disease. This represents the most abundant and widely distributed species (Zimmer et al., 2009). The breeding sites of these species are poorly documented. Leaf compost and tree holes have been reported for *C. obsoletus* (Murray, 1957; Chaker, 1983). Buxton (1960) reared *C. scoticus* on large fungi. Zimmer et al. (2010) have recently identified for the first time a suitable breeding site for these two species directly located inside a cowshed, i.e. in dried dung left on the walls of the buildings. This last breeding site has been confirmed for *C. obsoletus* by Ninio et al. (2011), who reported numerous emergences from used litter samples collected inside a dairy cow building. Maize silage residues, usually located close to livestock buildings, are also known to be suitable for both species (Zimmer et al., 2008). However, other types of silage (grass, sugar beet pulp and various mixtures) are regularly used to feed livestock.

The objective of this study was to evaluate the suitability of different types of silage residues as breeding sites for the *Obsoletus* complex and to chemically characterise those substrates.

2. Materials and methods

2.1. Study sites

Twenty Belgian farms selected from the list of BTV sentinel farms (AFSCA – Agence Fédérale pour la Sécurité de la Chaîne Alimentaire) were initially chosen for the study. They were located in the provinces of Namur and Walloon Brabant, both in the south of Belgium. The presence of silage residues near the silos of these farms was observed between the 25th and 28th of February 2008, and those with no silage residues were excluded from the study. Fourteen farms in which we found silage residues of one or several types (maize, grass, maize + grass or maize + sugar beet pulp) were selected. Sampling took place over a period of three weeks between the 5th and 25th of March 2008. The sampling was organised to collect each type of silage material at least once during each sampling week.

2.2. Sampling of silage residues

Silos encountered were covered with a protective canvas topped tires. For each silo, all heaps of residues with different localisation at ground level (for example located in corners or between tires) were sampled independently. A total of 77 independent samples of silage residues were collected from the 14 study sites. Each sample comprised a 1.5 l volume, collected using a small garden shovel. These samples were not sealed or placed in direct sunlight. Back at the laboratory, 1 l was used for experiments and the remaining material was equally divided into two plastic bags. These last samples, each containing 250 ml of substrate, were deaerated and stored at −18 °C for further physicochemical analysis. Each 1 l sample was divided into two:

- 0.5 l for the extraction of immature stages (larvae and pupae) using a direct flotation technique;
- 0.5 l for the collection of adult *Culicoides* following the incubation of samples in an emergence device.

2.3. Extraction of immature Culicoides spp. stages

For the extraction of larvae and pupae, a direct flotation technique was used, developed and adapted from Linley and Kettle (1964), Glukhova (1967) and Linley and Adams (1972). The first 0.5 l sample was transferred into a wide-mouth container and a saturated solution of magnesium sulphate ($MgSO_4$, density 1.20 kg/m^3) was added. The immature stages of the insects were collected at the surface with forceps while the debris was sedimenting. The larvae and pupae of *Culicoides* were sorted based on the general key of Glukhova (1977), counted under a binocular microscope (10–40× magnification) and stored in 80% ethanol.

2.4. Collection of adult Culicoides

This was performed by inducing the emergence of insects from the substrate kept at a temperature of 24 °C (±2 °C) using a thermostat. The second 500 ml sample was deposited in an emergence device as previously described by Kremer (1965).

Every two days during a five week period, adults were harvested by means of a mouth-operated aspirator and preserved in 80% ethanol. *Culicoides* were sorted by gender using a stereomicroscope (10–40×) and identified to the species level using the identification key of Delécolle (1985), with the exception of female *C. obsoletus* and *C. scoticus* individuals which could not be morphologically separated and were identified to the complex level.

2.5. Laboratory analyses

Fresh silage samples were extracted with distilled water (100 g silage/900 g water) for 24 h and the pH was measured (Microprocessor pH meter, pH 320/SET, WTW, Weilheim, Germany). Silage samples were placed in forced-air ovens at 60 °C, dried to a constant weight and then ground to pass through a 1-mm screen (Cyclotec 1.093, Foss Tecator AB, Höganäs, Sweden). Dry and organic matters were determined according to standardised methodology described by the Association of Analytical Communities (AOAC, 1990).

Crude protein (Kjeldahl N × 6.25), crude cellulose (according to the Weende method), neutral detergent fibre (NDF) (Van Soest et al., 1991), acid detergent fibre (ADF) (Van Soest et al., 1991) and acid detergent lignin (ADL) (Van Soest et al., 1991) were estimated by near infrared spectroscopy (NIRS). The mineral content (phosphorus, calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+), sodium (Na^+)