

Culicoides trapping with Rothamsted suction traps before and during the bluetongue epidemic of 2006 in Belgium

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Abstract

The collection of biting midges was taking place some months before the first bluetongue outbreak in Belgium in August 2006. The Walloon Agricultural Research Centre had been monitoring aphid populations at two sites annually in Belgium (Gembloux and Libramont), using two stationary '12-m' Rothamsted suction traps. For the Gembloux trap, collections of insects captured daily from 11 May 2006 onwards were already available at the time of the outbreak. An examination of these samples revealed the presence of *Culicoides*, some species of which are considered as potential vectors of the bluetongue virus (BTV). The trapping was therefore extended beyond the normal aphid activity period and the *Culicoides* captured were identified to species level. From 11 May to 31 December 2006, the Gembloux trap caught 664 *Culicoides* specimens belonging to 19 species comprising known BTV-vectors. The second trap, at Libramont, was reactivated from 12 September to 13 October and caught 97 specimens belonging to nine species, all of which had been found at the Gembloux site. Among the 19 species identified, four were new to Belgian fauna: *Culicoides achrayi*, *C. deltus*, *C. lupicaris* and *C. newsteadi*. This paper examines the overall phenology and the physiological status of *Culicoides* in 2006 before and during the bluetongue epidemic. It discusses the potential of the Rothamsted suction trap to monitor *Culicoides*.

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Keywords: *Culicoides*; Rothamsted suction trap; Bluetongue; BTV; Vector

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1. Introduction

The Rothamsted ‘12-m’ suction trap (RST), the design and construction of which were described by Macaulay et al. (1988), is currently being used to monitor aerial population dynamics of small insects, especially aphids. Developed in 1964 at Rothamsted, UK, a trapping network has since been established throughout the UK and western and eastern Europe in order to forecast outbreaks of aphids of economic importance (Barbagallo, 1982) and to provide data for studies on the dynamics of insect populations (Macaulay et al., 1988). In Belgium, there are only two such traps (Fig. 1), one at Gembloux and the other at Libramont; established in 1980, they are used for a fixed period annually to monitor the occurrence of aphids daily at a height of 12 m (Bernard, 1982).

The development of a bluetongue epidemic in 2006 in Belgium meant that updated entomological data were needed on the occurrence (presence and abundance) in this country of biting midges in the genus *Culicoides* (Diptera: Ceratopogonidae), which includes some species considered to be potential bluetongue vectors. An entomological monitoring programme was set up with great urgency by the Belgian Federal Agency for the Security of the Food Chain (FASFC) and included among its partners the Walloon Agricultural Research Centre (CRA-W). CRA-W possessed biting midges collected using the RST before and during the bluetongue epidemic outbreak. CRA-W’s objectives were to determine the diversity and abundance of *Culicoides* species in the aerial plankton at a height of 12 m and to examine the physiological status of females in order to gain a better insight into the prevalence of a specific status among wind-borne biting midges caught at this altitude. Belgian *Culicoides* diversity was studied by Goetghebuer (1952) and the last updated list had 36 species (Gosseries, 1991). The identification of species would determine if potential BTV-vectors could be caught using the RST and would provide new distribution data for Belgium. This paper analyses and discusses the overall catches of *Culicoides* using the RST, the physiological status of females, the phenology in relation to local weather and the potential of the RST to monitor *Culicoides*.

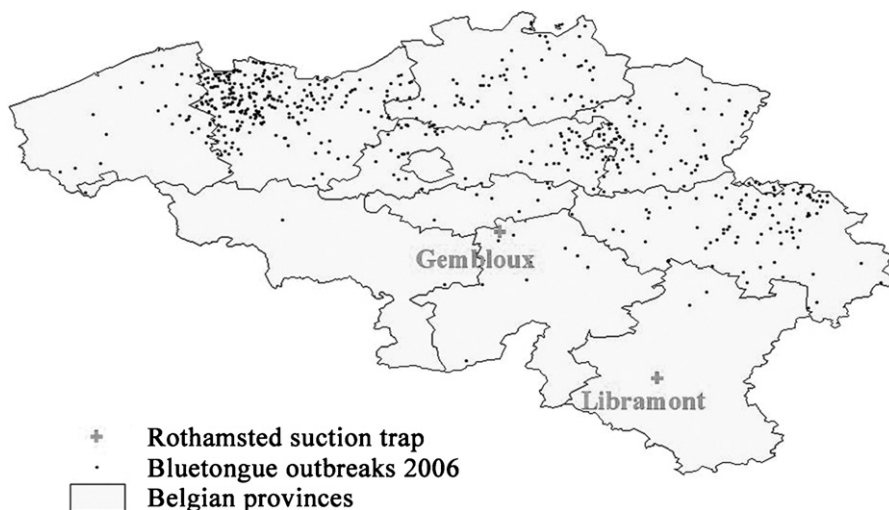


Fig. 1. Permanent location of the two Rothamsted suction traps (RSTs) in Belgium in the context of the BTV outbreaks in 2006.

2. Materials and methods

2.1. Insect samples studied

The RST at Gembloux was operated in 2006 as part of a potato aphid monitoring programme from 11 May. After separating the aphids from other insects, the latter were kept for further study. When the bluetongue epidemic occurred in Belgium, an examination of these other insects revealed the presence of *Culicoides* specimens among them. To monitor the *Culicoides* activity, the use of the RST was therefore extended through the winter. The insects were thus sampled at Gembloux from 11 May to 31 December 2006 and at Libramont from 12 September to 13 October 2006.

The RST, which provides a continuous standard sample, consists of a 3 m-high box and a vertical 9 m-long pipe on the top, with a trap inlet 12 m above ground. The closed box contains an electric fan, an expansion chamber to reduce airspeed, a collecting net and a collecting bottle filled with water and a few detergent drops for insect samples. The trap samples air at a rate of 45 m³/min (Macaulay et al., 1988). When functioning, the suction traps operate around the clock capturing day- and night-flying arthropods. The collecting bottle is changed at 8 h 30 min daily except at weekends and the trap catch is conserved in a 70% alcohol solution for further examination.

2.2. Trapping sites

The two RSTs in Belgium belonging to CRA-W are located permanently at Gembloux (50°33'42"N–4°42'38"E) in Namur province and at Libramont (49°55'37"N–5°21'36"E) in Luxembourg province, a distance of 90 km from the Gembloux site. With regard to the officially confirmed bluetongue outbreaks in Belgium (Thiry et al., 2006), Gembloux is near the centre of Belgium not far from the sites of some BTV outbreaks in 2006, but Libramont is in the southern part of the country in a region not affected by the disease (Fig. 1).

The trap belonging to the Department of Biological Control and Plant Genetic Resources (altitude 170 m) is near the town of Gembloux on cultivated land within 1 km of livestock (cattle, horses, pigs). About 2.6 km from the site, there is a meteorological station (CRA-W) from which we obtained data on temperature, rainfall and wind speed (measured at 2 m high). The Libramont trap belonging to the Department of Farming Systems (altitude 480 m) is also located outside the town and surrounded by an industrial zone, a coniferous forest and pastures grazed by cattle and sheep. The Libramont meteorological station (Pameseb, a public sector body) is next to the trap.

2.3. Identification of *Culicoides*

The arthropod samples were sorted and all *Culicoides* were identified to species level, using the morphological key devised by Delécolle (1985), who himself was involved in identifying some specimens in this study. Males were identified by their genitalia. Females of the species *C. obsoletus* and *C. scoticus* could not be distinguished and in this paper are counted together.

All the female specimens were examined and their physiological status categorized as follows:

- 'nulliparous': abdomen transparent, not pigmented and empty (without any blood or eggs);
- 'blood': abdomen pigmented (small rosy drops) or engorged with blood;

- ‘blood + eggs’: abdomen pigmented (small rosy drops) with blood and bearing eggs;
- ‘eggs’: abdomen bearing only eggs;
- ‘parasitised’: with long nematode in the abdomen;
- ‘damaged’: without abdomen.

BTV transmission between its ruminant hosts occurs only through the bites of vector species of *Culicoides* and the virus needs to be introduced elsewhere for a bluetongue outbreak to occur, either by the movement of infected hosts or by infected midges being carried on the winds (Mellor et al., 2000). This explains why the physiological status of wind-borne *Culicoides* is relevant to disease transmission, as the unfed midges (=nulliparous) are unable to transmit the virus but females with abdomen bearing blood or eggs are likely to do so.

3. Results

The RSTs at Gembloux and Libramont caught 664 *Culicoides* specimens belonging to 19 species and 97 *Culicoides* specimens belonging to nine species, respectively. Table 1 gives an inventory of the species that were present in the samples from the RSTs at the two sites. Six definite subgenera (*Avaritia*, *Beltranmyia*, *Culicoides*, *Monoculicoides*, *Silvaticulicoides* and *Wirthomyia*) and one group without a precise subgenus affiliation were represented. The subgenera *Avaritia* and *Culicoides* contained the species known or suspected as bluetongue

Table 1
Inventory of the *Culicoides* species captured by the Rothamsted suction traps (RSTs) at the two sites

Subgenus	Species	GE ^a	LI ^b
<i>Avaritia</i>	<i>Culicoides chiopterus</i> (Meigen, 1830)	+	+
	<i>Culicoides dewulfi</i> Goetghebuer, 1936	+	+
	<i>Culicoides obsoletus</i> (Meigen, 1818)	+	+
	<i>Culicoides scoticus</i> Downes and Kettle, 1952	+	+
<i>Beltranmyia</i>	<i>Culicoides circumscriptus</i> Kieffer, 1918	+	–
	<i>Culicoides salinarius</i> Kieffer, 1914	+	–
<i>Culicoides</i>	<i>Culicoides deltus</i> Edwards, 1939 ^c	+	–
	<i>Culicoides lupicaris</i> Downes and Kettle, 1952 ^c	+	+
	<i>Culicoides newsteadi</i> Austen, 1921 ^c	+	–
	<i>Culicoides pulicaris</i> (Linnaeus, 1758)	+	+
	<i>Culicoides punctatus</i> (Meigen, 1804)	+	+
<i>Monoculicoides</i>	<i>Culicoides stigma</i> (Meigen, 1818)	+	+
<i>Silvaticulicoides</i>	<i>Culicoides achrayi</i> Kettle and Lawson, 1955 ^c	+	+
	<i>Culicoides pallidicornis</i> Kieffer, 1919	+	–
	<i>Culicoides subfasciipennis</i> Kieffer, 1919	+	–
<i>Wirthomyia</i>	<i>Culicoides minutissimus</i> (Zetterstedt, 1855)	+	–
Without a precise subgenus affiliation	<i>Culicoides kibunensis</i> Tokunaga, 1937	+	–
	<i>Culicoides festivipennis</i> Kieffer, 1914	+	–
	<i>Culicoides vexans</i> (Stæger, 1839)	+	–

(+): Presence. (–): absence.

^a GE = Gembloux 11/05/2006–31/12/2006.

^b LI = Libramont 11/09/2006–13/10/2006.

^c New species for Belgian fauna.

Table 2

Most abundant *Culicoides* species caught using the Rothamsted suction traps (RSTs) at the two sites

<i>Culicoides</i> species	GE ^a		% ^d	LI ^b		% ^d
	Number ^c			Number ^c		
	♂	♀	♂	♀		
<i>C. (Avaritia) obsoletus</i> ^e	68	345	63.4	2	56	63.9
<i>C. (Avaritia) scoticus</i> ^e	8			4		
<i>C. (Avaritia) chiopterus</i>	26	62	13.3	4	4	8.2
<i>C. (Avaritia) dewulfi</i>	16	23	5.9	0	3	3.1
<i>C. (Culicoides) pulicaris</i>	7	2	1.4	3	4	7.2
<i>C. (Culicoides) punctatus</i>	14	44	8.7	5	2	7.2
<i>C. (Monoculicoides) stigma</i>	10	0	1.5	6	2	8.2
<i>C. kibunensis</i>	2	11	2.0	–	–	–

^a GE = Gembloux 11/05/2006–31/12/2006.^b LI = Libramont 11/09/2006–13/10/2006.^c Number of specimens of *Culicoides* caught using the RST.^d Percentage of the total number of *Culicoides* caught at the site.^e ♀ not distinguished.

vectors: *C. obsoletus*, *C. scoticus*, *C. dewulfi* and *C. pulicaris*. All the species identified in the autumnal sampling at Libramont were also detected in the samples from Gembloux that had been collected before and during the bluetongue epidemic of 2006.

The results for the most abundant species caught at the two sites are presented in Table 2. At Gembloux the most abundant species were *C. obsoletus* and *C. scoticus* with 63.4% of the total catch, *C. chiopterus* (13.3%), *C. punctatus* (8.7%), *C. dewulfi* (5.9%), *C. kibunensis* (2.0%), *C. stigma* (1.5%) and *C. pulicaris* (1.4%); other species accounted for 0.6% or less. At Libramont, where the trapping time was much shorter and later in the season, the most abundant species were *C. obsoletus* and *C. scoticus* (63.9%), *C. chiopterus* and *C. stigma* (8.2%), and *C. punctatus* and *C. pulicaris* (7.2%); the remaining species caught accounted for 3.1% or less.

The overall ratio of males:females for all *Culicoides* together was 1:3 at both Gembloux (23.3%:76.7%) and Libramont (24.7%:75.3%). However, this ratio varied when the captures were considered at the species level: for example, *C. obsoletus* and *C. scoticus* together had an average ratio of 18.1%:81.9% at Gembloux over the period studied, and in autumn at Libramont the ratio for the same group was 9.7%:90.3%.

The overall physiological status (as previously defined) of females in a good state and not parasitised (479 individuals) caught with the RST is summarised in Fig. 2 for the five most abundant species at the Gembloux site. For these species (*C. obsoletus* and *C. scoticus* together, *C. chiopterus*, *C. punctatus* and *C. dewulfi*) the range in physiological status was: nulliparous (abdomen empty): 29–51%; abdomen with blood (traces or bag): 24–34%; abdomen with blood and eggs: 19–43%; abdomen with eggs only: 0–3%.

Considering the relatively low number of insects, these data give only an idea of the physiological status of the biting midges flying at a height of 12 m. The most important findings relate to *C. obsoletus* and *C. scoticus* which were more nulliparous (50.6%) in flight than biting midges bearing blood (27.6%) or blood with eggs (18.6%). A notable factor common to all species was the very low number of *Culicoides* in flight at 12 m bearing only eggs. Parasitised individuals (probably by mermitids) represented 3.7% of the females (none was found in the Libramont samples).

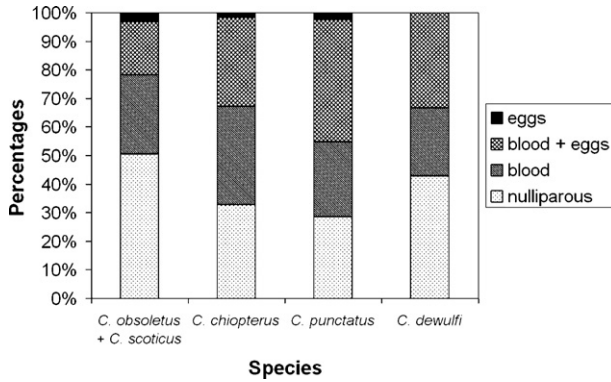


Fig. 2. Physiological status of females caught using the Rothamsted suction traps (RSTs) for the five most abundant *Culicoides* species at the Gembloux site.

The overall phenology of *Culicoides* in 2006 before and during the bluetongue epidemic at the Gembloux site can be determined on the basis of permanent trapping using the RST. Fig. 3 shows the general trend of the ‘monthly flight curve’ recorded for both males and females from 11 May to 31 December (last capture on 5 December), in relation to local meteorological data (i.e., monthly average temperature, average wind velocity and total rainfall). It is worth pointing out that trapping was not done throughout May and that the first case of bluetongue in Belgium appeared in August (see the BT arrow in Fig. 3).

The main flight peak was recorded in June when the average temperature was increasing at the same time as the wind and rainfall were decreasing. In July, the numbers of biting midges decreased when the temperature was very high. In August, when there was heavy and continuous rain, the flying populations decreased further. In September, when there was practically no rain,

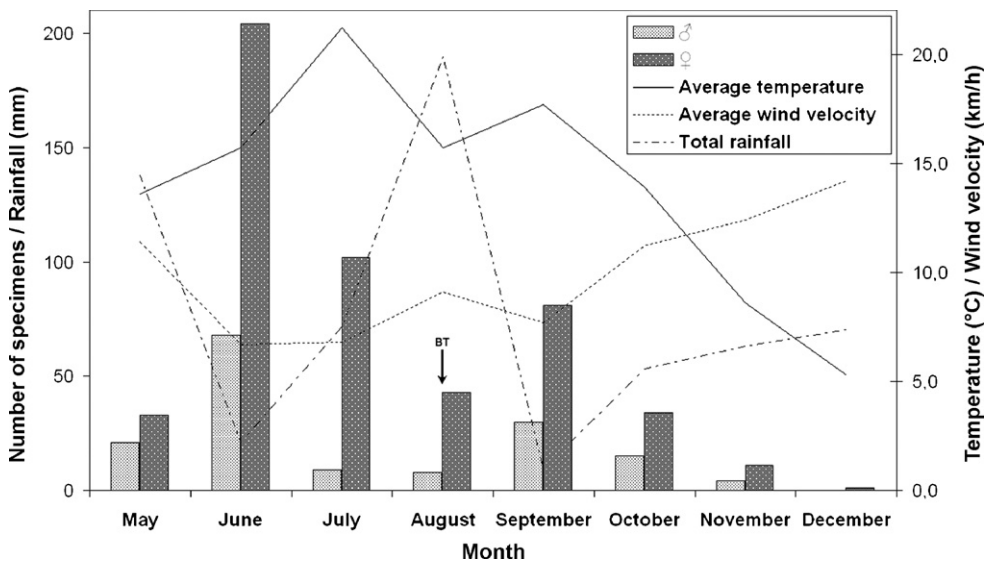


Fig. 3. Overall phenology of *Culicoides* spp. recorded from the Rothamsted suction traps (RSTs) before and during the bluetongue epidemic in 2006 at the Gembloux site. BT arrow = month of bluetongue outbreak in Belgium in 2006.

the temperature increased again and the midges became almost as numerous as they had been in July. Then, as the temperature decreased gradually and the rainfall and wind speed increased, the biting midge population fell from October through to December. Overall, the ‘flight curve’ for males and females was quite similar.

4. Discussion

4.1. Effectiveness of the RST for trapping *Culicoides*

The interception of arthropods at a height of 12 m using the RST, initiated to monitor aphid populations, can also be used for *Culicoides*, a total of 761 specimens belonging to 19 species having been caught in 7 months with two RSTs at two sites in Belgium in 2006. This confirms that “*Culicoides* are capable of being dispersed passively as aerial plankton over much greater distances” (Hayashi et al., 1979) despite the short flight range they have, as most species disperse only a few hundred metres from their breeding sites (Mellor et al., 2000).

The number of *Culicoides* midges caught using the RST at Gembloux (664) over 221 days was relatively low compared with the large number of individuals sometimes trapped with black-light as an attractant (several hundred per night and per trap), but proportionally the diversity of species recorded (19) was high. During the monitoring survey in 2006, however, a total of 1959 *Culicoides* specimens belonging to 16 species were trapped at 36 sites during outbreaks over almost 140 nights, a low trapping rate explained by the livestock insecticide treatments (De Deken, 2008).

Service (1971) showed that in suction traps placed at different heights (up to 5 m) the number of adult *Culicoides* caught fell markedly with height, with very few specimens caught at 5 m. However, Holmes and Boorman (1987) demonstrated that trap type (light, unbaited suction or baited suction) and its location affected the daily total of catches (e.g., more individuals were caught in light traps at 5–6 m than at ground level).

The advantages of the RST, however, are undeniable:

- as a non-attractant trap it catches equally all the insects passing by and present in the ‘aerial plankton’;
- the trap works continuously and therefore the captures may be more representative of temporal *Culicoides* activity rather than point trapping with light or CO₂;
- its activity day and night also enables it to capture diurnal species;
- it is cost-effective as it needs only electricity to operate and can also be used for other purposes such as aphid monitoring.

Subject to the results using the RST in 2006 being confirmed, this kind of trap is less labour-intensive in monitoring *Culicoides* than other traps, as it catches a high number of species, albeit a low number of specimens. If a good correlation is found between the catches of the RST and the classic light traps regarding the date of first appearance of potential bluetongue vectors and the density of these vectors, then the RST may be a valid alternative for bluetongue vector monitoring.

4.2. Diversity and abundance of *Culicoides* species

The diversity of *Culicoides* species (19) recorded by only one RST is remarkable given that the total number of species listed for Belgium was 36 (Gosseries, 1991). With reference to the

'Catalogue of Palaearctic Diptera, Vol. 3: Ceratopogonidae' (Remm, 1988), the catalogues of Borkent and Wirth (1997) and Borkent (2007) and the most recent 'Catalogue of the Diptera of Belgium' (Gosseries, 1991), four species were new to Belgian fauna: *Culicoides achrayi*, *C. deltus*, *C. lupicaris* and *C. newsteadi*. In the Belgian catalogue *C. festivipennis* was named *C. odibilis* Austen, 1921, which is a synonym.

The *Avaritia* and *Culicoides* subgenera which include the '*C. obsoletus* complex' and the '*C. pulicaris* complex' with closely related species (Mellor and Tweddle, 2002), respectively, were well represented. These two complexes contain the main vector species of bluetongue. Belonging to the *Avaritia* SG, the species *C. imicola* Kieffer, 1913, another vector of bluetongue in some areas, including Africa, Asia and Mediterranean Basin (Mellor et al., 2000; Wittmann et al., 2001), was absent from the samples from both sites.

At the two trapping sites, *C. obsoletus* and *C. scoticus* together were the most numerous, with a rate of 63% at both sites and females being the most abundant (82% and 90%). *C. obsoletus* was shown recently (Mehlhorn et al., 2007) to have a vectorial capacity for BTV-8. Among the other most abundant species, *C. dewulfi*, recently identified as a vector of bluetongue in the Netherlands (Meiswinkel et al., 2007), was well represented in the samples from Gembloux (nearly 6%) and was also detected with three female individuals at the more southern site of Libramont. The data on the occurrence of *C. dewulfi* at Gembloux and Libramont shed new light on the Belgian distribution of this species. Another recognized BTV-vector, *C. pulicaris* (Caracappa et al., 2003; De Liberato et al., 2005), was also present at both sites, although in low numbers. This indicates that the RST can catch different suitable hosts for BTV transmission and could be used as a survey sentinel to monitor wind-borne *Culicoides* vectors annually at low cost.

4.3. Physiological status of *Culicoides*

Although there are temporal or specific variations, the overall data on the physiological status of the most abundant *Culicoides* caught with one RST show no particular prevalence of flying adults being nulliparous, or carrying blood or blood + eggs, although for *C. obsoletus* and *C. scoticus* together the proportion of nulliparous adults was higher. In contrast, the number of midges bearing eggs only (without blood) was very low or nil in all five species (*C. obsoletus* and *C. scoticus* together, *C. chiopterus*, *C. punctatus* and *C. dewulfi*). Holmes and Boorman (1987) reported that the percentage of gravid females was generally lower in the light traps placed at a height of 5–6 m rather than at ground level; they also mentioned that proximity of breeding sites to a trap may influence physiological factors and lead to bias in the catches. This questions the suitability of the RST for determining population parameters.

4.4. Phenology of *Culicoides*

The seasonal abundance in *Culicoides* midges has not been studied before in Belgium and the present data cannot be compared with previous data. In the UK, Holmes and Boorman (1987) studied the seasonal flight periods of *Culicoides* females using different types of traps over different years: the *C. pulicaris/punctatus* and *C. obsoletus* group, found in large numbers from April to October, had the most extended emergence periods, although some less abundant species had a much shorter flight period and appeared to be confined to the spring or summer months.

In this study, monthly *Culicoides* catches are compared against mean monthly weather variables to find some correlation between these parameters and to show the general trend during 2006. The maximum male and female flight activity of all the *Culicoides* species combined at the

Gembloux site occurred in June, corresponding to an increase in temperature, although the temperature did not reach its peak until July. This could be explained by a great emergence of biting midges in June after a cold winter and spring, corroborated by a peak of nulliparous (unfed) *C. obsoletus* and *C. scoticus* captured that month. The ‘monthly flight curve’ showed a progressive decrease until the end of the year, with a slack period in August probably due to the continuous rainfall. Low temperatures, heavy wind and rain are known to inhibit *Culicoides* activity and are probably responsible for the low catches in October and November until the population died out due to the low temperatures in December. The final catch was at the beginning of December. The flying activity is probably influenced by many factors and a future more in-depth analysis will try to pinpoint meteorological factors affecting the midges’ flight at species level for better understanding.

5. Conclusion

The RST which captures insects at a height of 12 metres and is generally used to monitor daily aphid activity, has proved to be an effective tool for monitoring dispersing *Culicoides* spp., some species of which are considered as potential vectors of the bluetongue virus (BTV). This trap is less labour-intensive than the classic light traps, as it catches a high number of species although the number of captured specimens is rather low. The continuous action of this non-attractant trap also enables temporal changes in the physiological status and flight curve of dispersing *Culicoides* to be monitored. Subject to a good correlation between the catches using the RST and the classic light trap being found in the future, the stationary Rothamsted suction trap may be a good alternative to monitor wind-borne *Culicoides* vectors at low cost.

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Conflict of interest

None of the authors C. Fassotte, J. C. Delécolle, R. Cors, T. Defrance, R. De Deken, E. Haubruge and B. Losson, has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the paper entitled “*Culicoides* trapping with Rothamsted suction traps before and during the bluetongue epidemic of 2006 in Belgium”.

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