review article

Wien Klin Wochenschr (2008) 120: ■–■
DOI 10.1007/s00508-008-1073-7
Printed in Austria

Wiener klinische Wochenschrift
The Middle European Journal of Medicine

Bluetongue: Vector surveillance in Austria in 2007

Peter Sehnal[1], S■■ Schweiger[2], M■■ Schindler[3], F■■ Anderle[1], Y■■ Schneemann[1]

[1] Natural History Museum Vienna, International Research Institute of Entomology, Vienna, Austria
[2] Natural History Museum Vienna, First Zoological Department (Herpetological Collection), Vienna, Austria
[3] University of Vienna, Faculty of Life Sciences, Department of Evolutionary Biology, Vienna, Austria

Zusammenfassung. Die ursprünglich aus Südafrika bekannte Blauzungenkrankheit (bluetongue disease, BTD) ist seit Herbst 2006 durch Ausbrüche in den Niederlanden, Deutschland und Belgien ein zentrales Thema in Mitteleuropa. Von dieser Infektionskrankheit, von der Österreich bisher verschont blieb, sind vor allem Schafe, Rinder, aber auch Ziegen und Wildwiederkäuer betroffen. Überträger des Bluetonguevirus (BTV, Familie Orbiviridae), das in verschiedenen Serotypen auftritt, sind Gnitzen (Diptera: Ceratopogonidae) der Gattung *Culicoides*. Fünfzig Arten sind als Vektoren für Krankheiten bekannt.

Seit Juni 2007 gibt es zwischen mit dem Bundesministerium für Gesundheit, Familie und Jugend, der Agentur für Gesundheit und Ernährungssicherheit und der Dipteren-Sammlung des Internationalen Forschungsinstitutes für Insektenkunde am NHM eine Forschungskooperation zur Vektor-Überwachung in Österreich. Dafür wurden an fünfzig Standorten in ganz Österreich Schwarzblau-Lichtfallen in der Nähe von Viehbetrieben installiert und von Juni bis Dezember 2007 einmal wöchentlich in Betrieb genommen.

Von den über 1,5 Millionen gesammelten *Culicoides* konnten 87,4% dem *C. obsoletus*-Komplex, 6,7% dem *C. pulicaris*-Komplex und 0,1% dem *C. nubeculosus*-Komplex zugeordnet werden. Aus diesen drei Komplexen sind potenzielle Vektoren für das BTV für Mitteleuropa bekannt. Ein Anteil von 0,3% des Materials waren *Culicoides*-Arten, die keinem dieser drei Komplexe zugeordnet werden konnten, 5,7% konnten nicht auf Artniveau bestimmt werden.

Die größten Individuenzahlen konnten im Juli und August festgestellt werden (allerdings war die Probennahme im Juni noch nicht in vollem Gange). Ab Oktober sanken sowohl die Gesamt- als auch die *Culicoides*-Zahlen beträchtlich.

Summary. Since the first outbreaks of bluetongue disease (BTD) were reported from The Netherlands, Germany, and Belgium in autumn of 2006, the disease is a main topic in Central Europe. The infectious disease, which originated in South Africa and from which Austria has been spared up to now, affects particularly sheep, cattle, also goats and wild ruminants – but never humans. Transmitters of the bluetongue virus (BTV, family Reoviridae, genus *Orbivirus*), which occurs in several 24 serotypes, are biting midges (Diptera: Ceratopogonidae) of the genus *Culicoides*. In Europe, *Culicoides imicola, C. obsoletus/C. scoticus, C. dewulfi*, *C. pulicaris* and, very recently, *C. chiopterus* have been implicated in BTV transmission.

In 2007, a project on vector surveillance in Austria was started between the Federal Ministry of Health, Family and Youth (Bundesministeriums für Gesundheit, Familie und Jugend; BMGFJ), the Austrian Agency for Health and Food Safety (Österreichische Agentur für Gesundheit und Ernährungssicherheit; AGES), and the International Research Institute of Entomology at the Natural History Museum Vienna. Fifty blacklight traps have been set up spread over the whole Austrian territory and activated once per week from June to December 2007.

Out of the more than 1.5 million collected *Culicoides* specimens, 87.3% were assigned to the Obsoletus complex, 6.7% to the Pulicaris complex, and 0.1% to the Nubeculosus complex. From these three complexes potential vectors for BTV in Central Europe are known. A percentage of 0.2% was assigned to species not belonging to any of these complexes, and 5.7% were not able to be determined to complex or species level.

The highest numbers of individuals were recorded in July and August (not all traps, however, were activated in July). As from October the total amount of insects as well as the numbers of *Culicoides* decreased considerably.

Key words: Bluetongue Disease, Ceratopogonidae, *Culicoides*, Vector Surveillance, Austria.

Introduction

Bluetongue disease (BTD) was detected in Northern Europe for the first time in August 2006. Mainly sheep, but also cattle and goats, may suffer from this infection disease, whose causative organisms, bluetongue viruses

Correspondence: Peter Sehnal, Curator of the Diptera Collection, International Research Institute of Entomology, Natural History Museum Vienna, Burgring 7, 1010 Vienna, Austria,
E-mail: peter.sehnal@nhm-wien.ac.at

(BTV) of the family Reoviridae, genus *Orbivirus*, are transmitted by biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae). BTD originated in South Africa, and although small outbreaks now and then occurred in the Mediterranean region, the disease has been epidemic in this area since 1998 [1]. The affected countries in the outbreak of 2006 were The Netherlands, Belgium, Germany, northern France [2] as well as Luxembourg [3]. Thus, at that time, Austria was located in a kind of "sandwich position".

Ceratopogonidae are small biting midges (Insecta: Diptera: Nematocera) comprising about 5,000 species in 120 genera. In four genera (*Culicoides*, *Leptoconops*, *Forcipomyia*, and *Austroconops*) there are hematophagous females, which need vertebrate blood for the oogenesis [4]. In the genus *Culicoides* more than 1,400 species have been identified, of which 96% are obligatory bloodsuckers in mammals [5]. Fifty species are known to act as vectors for human and animal diseases, approximately 30 *Culicoides* species act as vectors for BTV worldwide. In Europe, *C. imicola, C. obsoletus/C. scoticus, C. dewulfi, C. pulicaris* and, very recently, *C. chiopterus* have been implicated in BTV transmission [6].

In 2007, a project on vector surveillance in Austria was started by order of the Federal Ministry of Health, Family and Youth (Bundesministerium für Gesundheit, Familie und Jugend; BMGFJ) and with the Austrian Agency for Health and Food Safety (Österreichische Agentur für Gesundheit und Ernährungssicherheit; AGES) as contractual partner. Preliminary, the time frame has been set from 1 June 2007 to 31 December 2007. Fifty sampling sites – farms with at least ten individuals of cattle, sheep or goats or with mixed livestock – were selected (Fig. 2); localities near water bodies and at low sea levels as well as farms not practising fixed transhumance were preferred. Samplings were carried out once a week with blacklight traps. Sampling sites – besides a power source – required a weather-proof area outside the stable, where the trap could be mounted close to but out of reach of the kept animals.

Objectives for the entomological surveillance

- recording findings of possible vectors of BTV throughout Austria and at each sampling site, respectively;
- survey of further *Culicoides* species occurring in Austria;
- acquisition of basic ecological data (minimum/maximum temperature, subjective estimation of weather condition, wind force and degree of sky cover) at the sampling site.

Before starting the project, information on biting midges in Austria was scarce. Eleven species (represented by only few dried specimens) of *Culicoides* collected from Austrian locations are stored in the Diptera collection of the Natural History Museum Vienna (NHM) (see list below; W = Vienna, Bgld = Burgenland, NÖ = Lower Austria, OÖ = Upper Austria, Slbg = Salzburg, Stmk = Styria, K = Carinthia, T = Tyrol, V = Vorarlberg):

Culicoides albicans (Winnertz, 1852): Austria: Alte Sammlung; NÖ: Mödling, Hainburg – *C. chiopterus* (Meigen, 1830): Slbg: Gastein; NÖ: Zwettl; OÖ: Kremsmünster – *C. fascipennis* (Staeger, 1839): Austria: Alte Sammlung; OÖ: Hammern; W. – *C. minutissimus* (Zetterstedt, 1855): NÖ: Baden, Weißenbach bei Mödling, Brühl; OÖ: Hammern; W. – *C. nubeculosus* (Meigen, 1830): NÖ: Hainfeld; OÖ: Hammern, Freistadt; Bgld: Neusiedlersee – *C. obsoletus* (Meigen, 1818) NÖ: Hainfeld; OÖ: Hammern, Linz, Freistadt; T: Achental; W. – *C. pictipennis* (Staeger, 1839): Austria: Alte Sammlung; T: Jenbach, Obladis, Schlunderbach; OÖ: Kremsmünster. – *C. pulicaris* (Linnaeus, 1758): NÖ: Hainfeld, Pernitz; OÖ: Gallneukirchen; T: Obladis; Bgld: Eisenstadt; W.

Ten species of Culicoides in Austria are listed by Franz [7]

Culicoides albicans: NÖ: Mödling; Hainburg – *C. fascipennis*: ÖO: Hammern bei Freistadt / NÖ: Lunz, Parkteich; W – (*C. winnertzi)* = *C. festivipennis*: NÖ: Lunz, Sulze im Kaminboden – *C. nubeculosus*: OÖ: Hammern; Freistadt; Schanz; Molln / NÖ: Hainfeld; Ramsau bei Hainfeld / Stmk: Koralpe – *C. obsoletus*: OÖ: Linz; Freistadt; Kremsmünster / NÖ: Umgebung Seitenstätten und Amstetten; Lunzer Untersee; Hainfeld / Stmk: Hohlweg des Lichtmessberges vereinzelt; Lichtmessgraben und Leichenberg bei Admont; Scheibelteich bei Admont; W / *C. pictipennis*: OÖ: Kremsmünster / NÖ: Lunz, Forellenteich Nr. 5 – *C. pulicaris*: OÖ: Freistadt; Hammern / NÖ: Lunzer Untersee; Pernitz; Hainfeld / Stmk: Scheiblteich bei Admont; Admont, Stiftmauer; Admont, Stiftgarten / W – *C. stigma*: NÖ: Lunzer Almtümpel; Lunzer Mittersee – *C. subfascipennis*: T: Jenbach in N-Tirol – *C. vexans*: NÖ: Lunzer Rotmoos, in der Tintenlacke und unteren Lacke im vorderen Rotmoos; Baden.

Recent data were not available.

Material and methods

The type of electric driven blacklight traps was used which proved of value in South Africa and Italy. The traps (Fig. 1) are produced by the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (IZS) in Teramo, Italy, as well as by the ARC-OVI, Onderstepoort, South Africa, and are currently called "Onderstepoort-type blacklight-traps". They were mounted as close as possible to the animals, but out of their reach, in a weather-proof area outside the stable and were activated from 1 June to 31 December 2007 every Monday from sunset to sunrise (see 8). The collection beaker contained a liquid composed of water and few drops of soap sud in order to break the surface tension. When operating the trap in nights with minus degrees, water was substituted for 75% ethanol. The sampled material was transferred into a plastic transport beaker containing 75% ethanol and forwarded to the Diptera collection at the Natural History Museum Vienna.

Simultaneously with operating the trap, basic meteorological data – minimum/maximum temperature as well as subjective estimation of weather condition (wind force and degree of sky cover) – of each sampling site were collected.

In order to spread the sampling sites in Austria as uniformly as possible the whole area was divided into grid cells of 40 km x 40 km, one farm per grid cell was selected as trap location. Sampling criteria: more than ten individuals of cattle,

Fig. 1. Trap mounted in a weather-proof location outside the stable

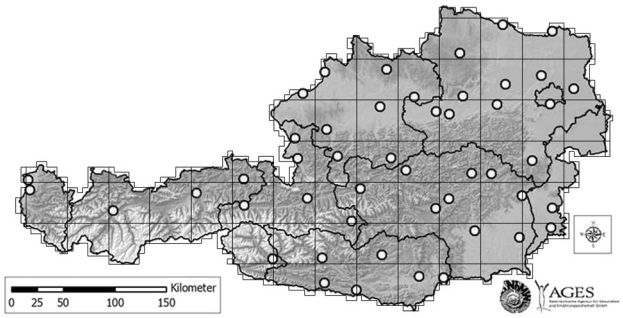

Fig. 2. Grid cells covering Austrian territory, bright spots indicating sampling sites

sheep or goats, a moderate altitude and the willingness of the farmer to cooperate. Grid cells covering areas of Austrian territory only partly and those covering alpine areas were not considered in site selection. Selected districts are listed in Table 1.

The weekly received samples were treated at the Natural History Museum Vienna according to the recommendations of the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (IZS) [8]: Initially, the sample was divided into *Culicoides* species and bycatch. In the case of samples with more than 500 specimens of *Culicoides* subsamples were taken, small samples were treated entirely [9]. The midges were assigned to species complex (Obsoletus, Pulicaris or Nubeculosus complex) and determined to species level, respectively. Since October 2007 males and females of *Culicoides* are recorded separately. Determination keys of Boorman [10], Goffredo & Meiswinkel [8], Goetghebuer & Lenz [11], and particularly of Delécolle [12] were used.

Results

In the course of the study 1,185 samples (with 4,457,387 insect specimens) were analysed, 56.83% (2,533,009 specimens) of which belonging to the genus *Culicoides*. Within this genus 87.3% (2,214,560 specimens) were assigned to the Obsoletus complex (= subgenus *Avaritia*), 6.7% (170,390 specimens) to the Pulicaris complex (= subgenus *Culicoides*), and 0.1% (2,941 specimens) to the Nubeculosus complex (= subgenus *Monoculicoides*). A percentage of 0.2% (7,334 specimens) was assigned to species not belonging to any of these complexes, and 5.7% (144,192 specimens) were not able to be determined to complex or species level (Fig. 3). Of all *Culicoides*

Table 1. List of provinces and districts containing sampling sites

Province	Grid cell	District	Altitude
Vienna	20	10. Bezirk	179
Burgenland	44	Oberwart	266
	56	Güssing	220
		Neusiedl am See	116
Lower Austria	3	Zwettl	594
	17	Amstetten	278
	18	Scheibbs	294
	13	Gänserndorf	149
	19	St. Pölten	299
	31	Neunkirchen	389
	4	Hollabrunn	425
	5	Mistelbach	183
	10	Melk	302
	12	Korneuburg	175
	11	Tulln	227
Styria	41	Knittelfeld	647
	28	Liezen	636
	29	Bruck/Mur	752
	30	Mürzzuschlag	662
	40	Liezen Gröbming	737
	41	Knittelfeld	647
	42	Leoben	569
	43	Hartberg	448
	54	Graz Umgebung	465
	55	Feldbach	352
Uper Austria	6a	Braunau	353
	7	Schärding	316
	15	Vöcklabruck	565
	8	Urfahr-Umgebung	682
	9	Perg	242
	16	Linz-Land	351
	27	Kirchdorf an der Krems	618
	26	Gmunden	504
Salzburg	14	Salzburg-Umgebung	569
	25	Hallein	450
	38	St. Johann	910
	39	Tamsweg	1190
	37	Zell am See	970
Tyrol	36	Schwaz	536
	24	Kitzbühel	673
	49	Lienz	669
	47	Imst	714
Vorarlberg	22	Bregenz	402
	32	Dornbirn	410

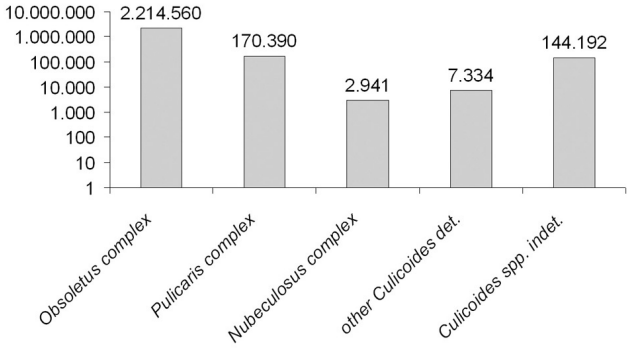

Fig. 3. Totals of Culicoides specimens

specimens collected from October to December 2007, 0.2% (190 specimens) were males.

Neither *C. imicola* nor *C. dewulfi* – both species proven to transmit the BT virus – could have been detected in the course of this study.

The percentage of *Culicoides* specimens in the samples varies considerably between the provinces. In every province, with the exception of Lower Austria and Tyrol, the quantity of bycatch outweighed the *Culicoides* specimens.

The majority of *Culicoides* specimens (87.4%) belongs to the Obsoletus complex. In most provinces it represents the species complex with the highest abundance during study period. In the districts of Favoriten (Vienna), Gänserndorf (Lower Austria), and Neusiedl am See (Burgenland), however, specimens of the Pulicaris complex outnumbered other species groups. Generally, specimens belonging to the Pulicaris complex come second concerning abundance (6.7%). Species of the Nubeculosus complex were only found occasionally (0.1%) in the districts of Güssing, Oberwart, Wolfsberg, Gänserndorf, Korneuburg, Melk, Neunkirchen, Scheibbs, St. Pölten, Tulln, Schärding, Kitzbühel, Knittelfeld, Leoben, Salzburg-Umgebung, St. Johann and Tamsweg. A surprisingly high amount of specimens of the Nubeculosus complex occurred in the districts of Neusiedl am See and Mistelbach.

During the study period the highest amounts of total individuals were recorded in July and August (Figs. 4–7). However, it has to be considered that only few traps were activated in June. As from October the total amount of insects as well as the numbers of *Culicoides* decreased considerably. The samples of 22 October 2007, however, showed a late peak of biting midges.

Discussion

Specimens of the Obsoletus complex dominated 48 of 50 sampling sites; in Favoriten (Vienna), Gänserndorf (Lower Austria), and Neusiedl am See (Burgenland) the specimens of the Pulicaris complex were the most abundant. Although these two sampling sites are located in the most eastern parts of Austria, we doubt this to be the crucial factor, as none of the other samples showed correlations to directions. We rather assume the lack of forests and the presence of many temporary puddles (accumulated water and liquid manure) close to grazing animals to be the most plausible explanation. The distribution patterns of the vector species in Austria show no correlation to the recorded abiotic factors – despite the heterogeneity of landscape. A more detailed characterization of the sampling environment is desirable.

The results show considerable seasonal fluctuations in *Culicoides*. However, the study only covered parts of the flight period and mainly reflects aspects of late summer and autumn activity. Thus, an extension of this project – in order to cover the entire period of *Culicoides* activity – has been started by 2008.

The methods concerning the record of the meteorological data has not proven satisfactory as uniformity of recorded data could not have been achieved. Assumed reasons are as follows:

- traps were mounted in inhomogeneous positions concerning exposure to sun and wind as well as vicinity to indoor stable areas;
- unreliable recording of temperature data due to the huge amount of involved farmers;
- recording of wind force and degree of sky cover depended on subjective estimation of the farmers.

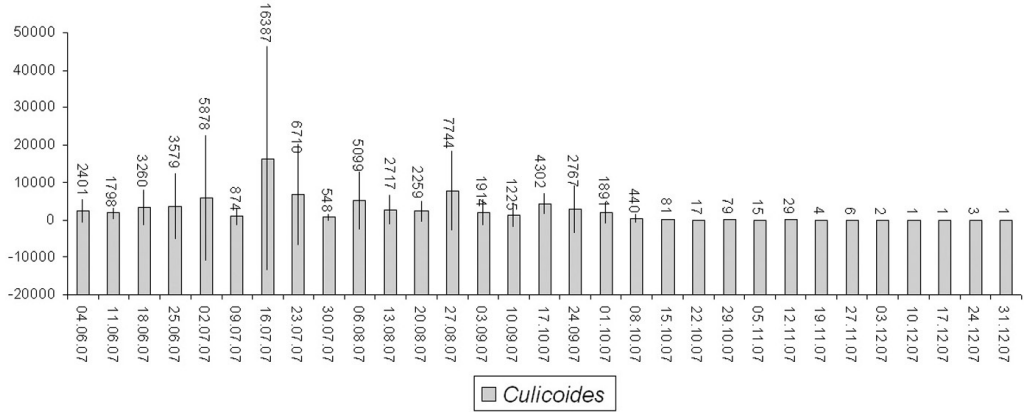

Fig. 4. Seasonal trends of Culicoides occurrence. Means of all sampling sites, with standard deviation

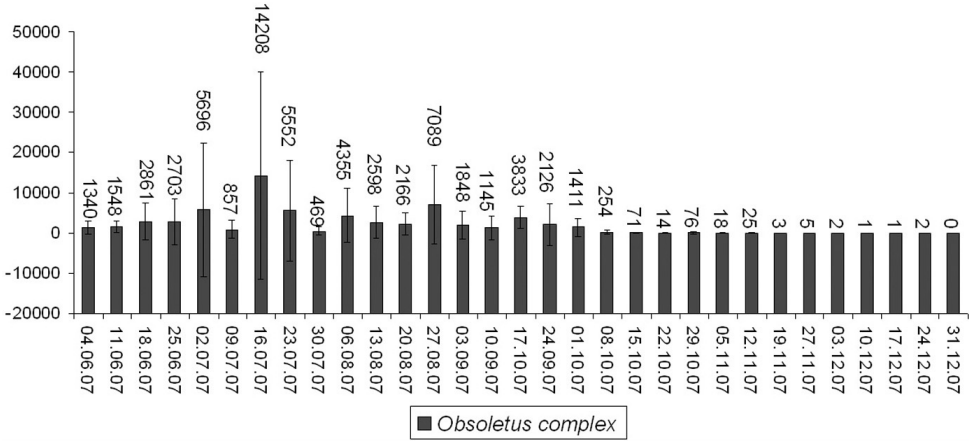

Fig. 5. Seasonal trends of Obsoletus complex occurrence. Means of all sampling sites, with standard deviation

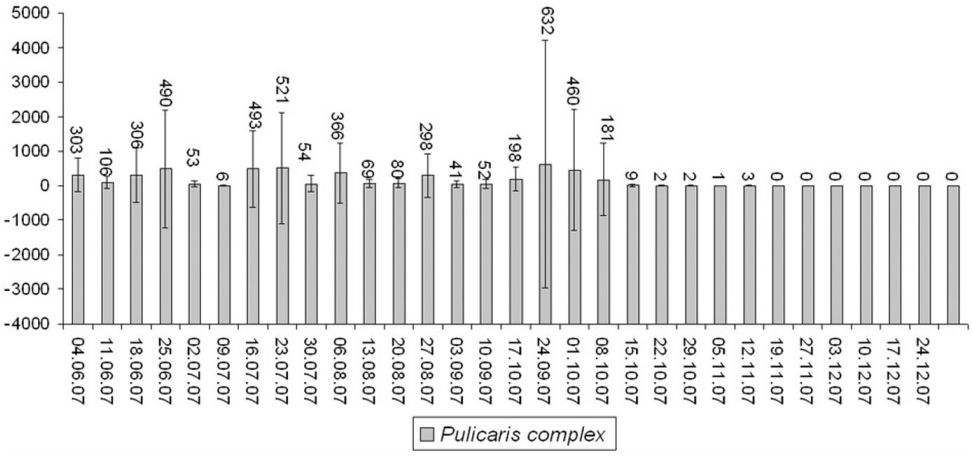

Fig. 6. Seasonal trends of Pulicaris complex occurrence. Means of all sampling sites, with standard deviation

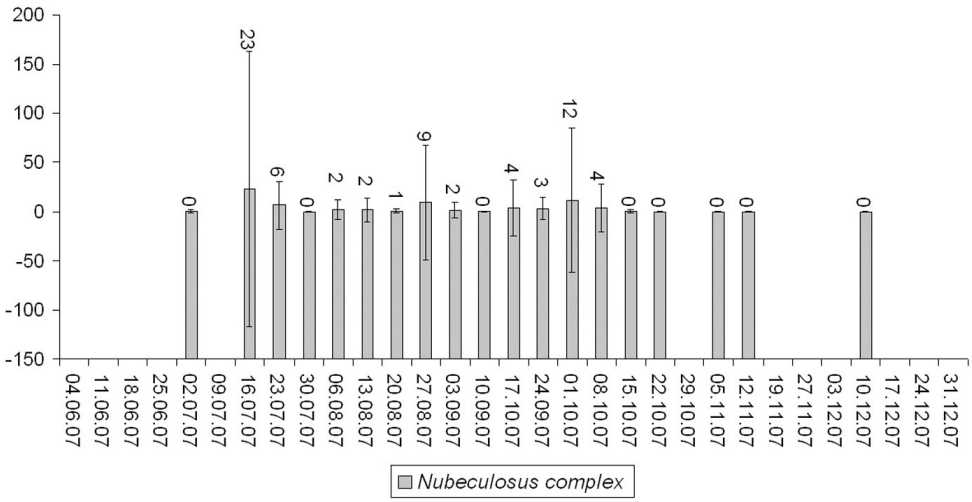

Fig. 7. Seasonal trends of Nubeculosus complex occurrence. Means of all sampling sites, with standard deviation

Due to the lack of uniformity, relationships between phenology and meteorological situation could not be evaluated statistically.

A correlation to altitude could not be verified, whereas strong wind force obviously has a negative influence on the activity of biting midges.

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