Biting midges

and the bluetongue outbreak in Northwestern Europe

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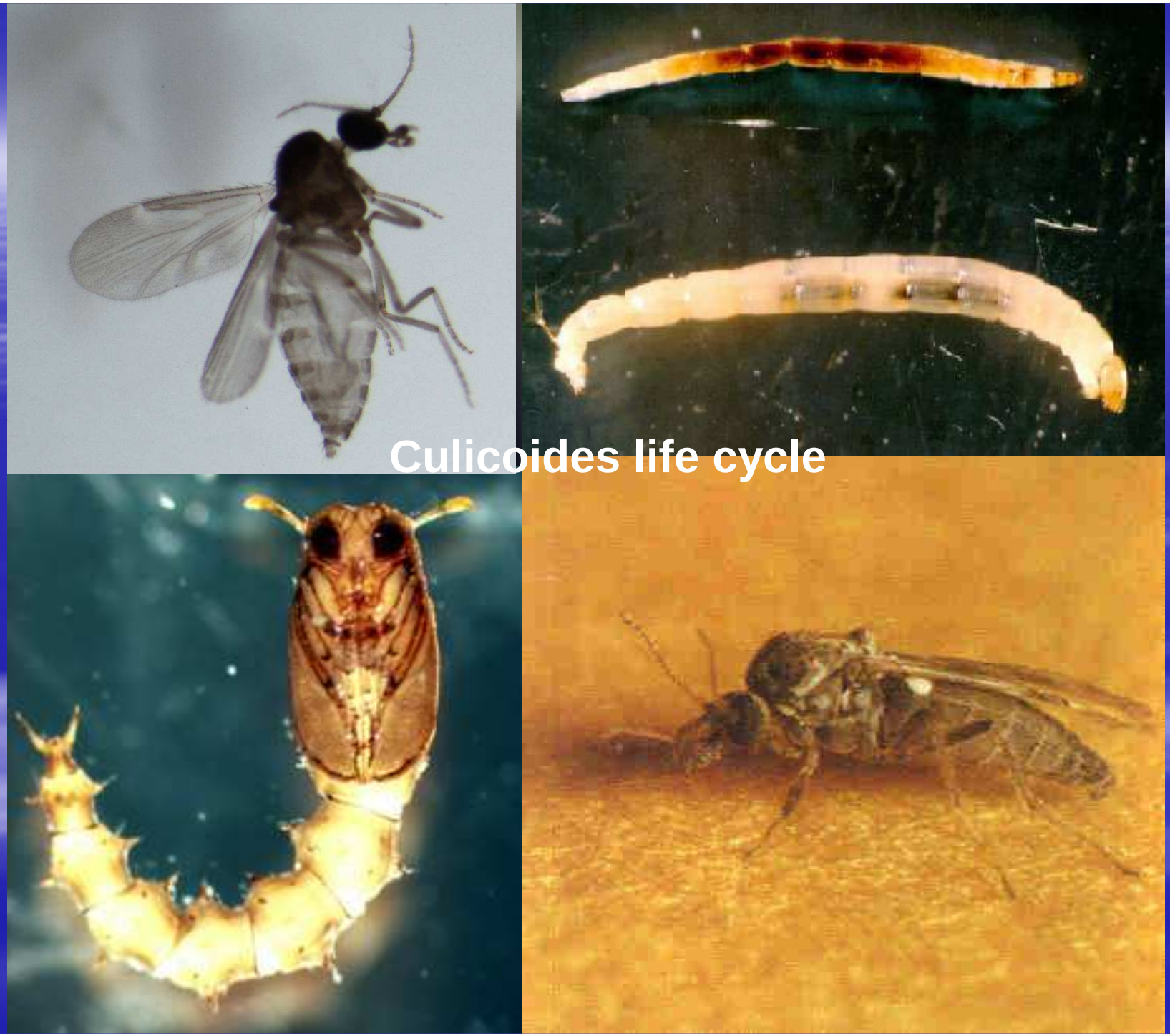
Contents

- Biology of Ceratopogonidae and Culicoides
- Biting midges as disease vectors
- Bluetongue epidemics in Europe
- Methods and results of the vector monitoring in Belgium/Europe

Biology

- Family Ceratopogonidae (± 60 genera, 4000 species)
- small (1 to 3 mm) diptera belonging to the suborder nematocera
- All members of the family have an egg, 4 larval instars, a pupal and an adult stage.
- In the northern regions the third or fourth instar may experience a diapause in order to survive the winter (vector free period for *Culicoides*-borne diseases),
- Larval habitats very variable, larvae feed on decaying vegetation and mushrooms, mosses and algae or are predaceous on nematodes or insect larvae,
- Both males and females feeding on nectar (pollinators) but females of some species feeding on insects or vertebrates


Culicoides life cycle

Adult females of some Ceratopogonids feeding on flying insects, will pierce the abdomen of the mating male and suck out its contents.

Such females may later be found with the dried male genitalia still attached to their own abdomen.

A scant consolation for the male these remainders represent a chastity belt (an effective mating plug) to the female

Haematophagous Ceratopogonidae

Haematophagous species are found within the genera *Culicoides* (1340 spp, worldwide), *Austroconops* (1), *Leptoconops* (80) and in the subgenus *Lasiohelea* (50) from the genus *Forcipomyia*.

Man probably not a preferred host but the following species occurring in Belgium are known to feed on men: *C. impunctatus* (Scotland), *C. pulicaris* (Wetteren), C. riethi (Ghent), *C. vexans*

Culicoides bites

In persons more allerqic to midges

4 days feeding interval for *C.obsoletus* in Southern England

Breeding sites

C. festivipennis

C. chiopterus
C. dewulfi

***C. obsoletus* s.l.**

Lake edge	Temporarily humid	Swamp	Puddles or mud	Mud + dung	Dung	Ensilage	Halophilic
achrayi duddingstoni	*pictipennis* *vexans*	*punctatus* *pulicaris*	*festivipennis* *kibunensis*	*nubeculosus*	*dewulfi* *chiopterus*	*scoticus* *obsoletus*	*halophilus* *circumscriptus* *newsteadi* *riethi*

Example of variability biting midges habitat

Hotel resort in Jamaica

Surrounded by beautiful mangroves, breeding place of *Culicoides barbosai* and *C.furens* (crepuscular activity)

Mangrove forest cut and replaced by a sandy beach.

Breeding ground for *Leptoconops becquaerti*, diurnal activity

Beach now mostly replaced by grass

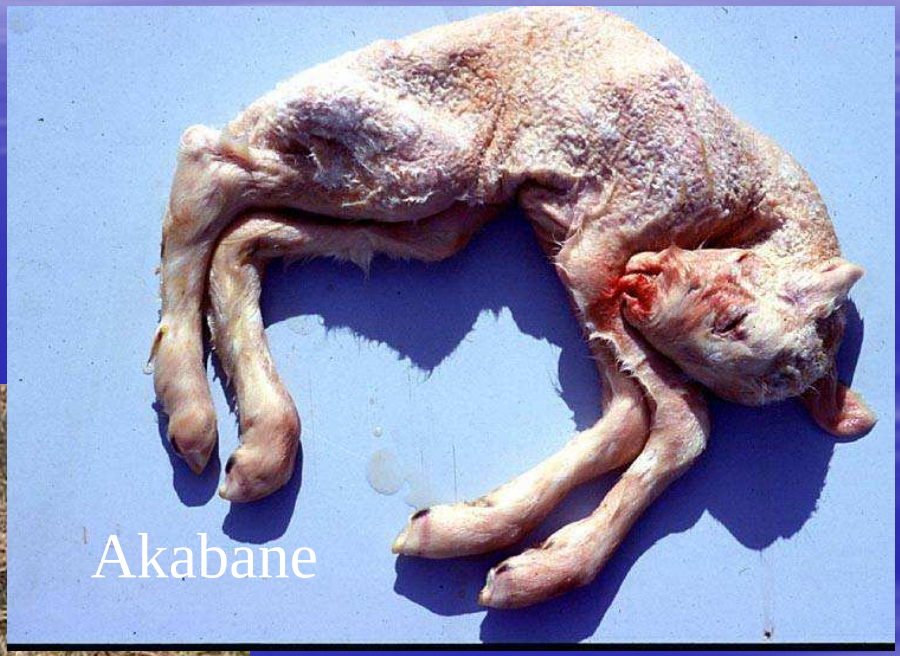
Culicoides and wildlife

Five species of biting midges use faeces of Loxodonta spp as breeding place and …

spent most of their life behind the elephant's ears feeding.

Biting midges as disease vectors

Bluetongue



Akabane

African Horse sickness

Worldwide biting midges are known to transmit 66 viruses, 15 protozoa and 26 filarial nematodes

Blue tongue virus and disease

non contagious viral disease, transmitted by midges of the genus ***Culicoides*** (family of Ceratopogonidae), virus replication in host as well as in vector

The virus (24 or 25? serotypes) infects wild and domesticated ruminants but essentialy sheep, less frequently goats, cattle, camelidae and antilopes.

BT/EHD and wildlife

- Wildlife seems to be the feeding host of specific species of biting midges (e.g. in southeastern USA *C. lahillei* predominant species collected from deer during hemorrhagic disease epizootics in white-tailed deer instead of *C.variipennis*) (Smith,K.E. 1996)
- Studies in Germany showed that midges of the *C. pulicaris* and *C. obsoletus* groups prefer to feed on cattle even if other livestock or deer were nearby (Bartsch,S., 2009)
- BTV RNA was detected in experimentally infected red deer blood up to 100 days p.i. (Lopez-Olvera, 2010)
- Wildlife probably doesn't constitute a BT reservoir in Belgium since in 2008 overall seroprevalence in deer followed the decreasing seroprevalence in (vaccinated) cattle (as observed by Linden, 2010).

How does the virus survive vector-free periods ?

1. The virus can escape the defence mechanisms of the host while being fixed at the erythrocytes. It can be isolated from cattle up to 60 days after infection and demonstrated via PCR up to 160 days after infection.

2. Transplacental transmission towards calf occurs and transmission by ingestion of placenta is probable.

3. Viral RNA was found in offspring of infected midges.

4. Is there really a vector-free period in Belgium?

5. Overwintering of virus due to its persistence in T cells ?
(Takamatsu et al., 2003)
Skin fibroblasts increase longevity of (infected) T cells but when stimulated by insect bites may cause lysis of T cells and liberation of virus from T cells and by doing so stimulate transmission.

6. Via transplacental transmission

Case study: The Northern Ireland Incident

20 heifers imported from Holland were all PCR –ve before export and after import, 8 of them were cELISA +ve

Calving:

- One cELISA -ve/PCR -ve heifer delivered 1 calf
- Two cELISA +ve/PCR -ve heifers delivered 3 calves

Re-testing + confirmation at Pirbright:

- The cELISA -ve/PCR -ve heifer converted to PCR +ve
  Test results indicate early infection – only one stable Calf: cELISA +ve/PCR –ve (Colostrum)
- 3 calves cELISA +ve/PCR +ve

An in contact Scottish heifer converted also from PCR -ve to +ve

Infectious BTV isolated from 1 calf & the 2 PCR +ve heifers

Vector-free period – no vectors captured in the stable

P.S. In NL this transmission occurred in about 20% of the non-vaccinated cases and calves were PCR+ during at most 5 months (Santman 2011).

Distribution and spread of the disease

Africa, Asia, Australia and America but since about 50 years BT is observed sporadically in Southern Europe.

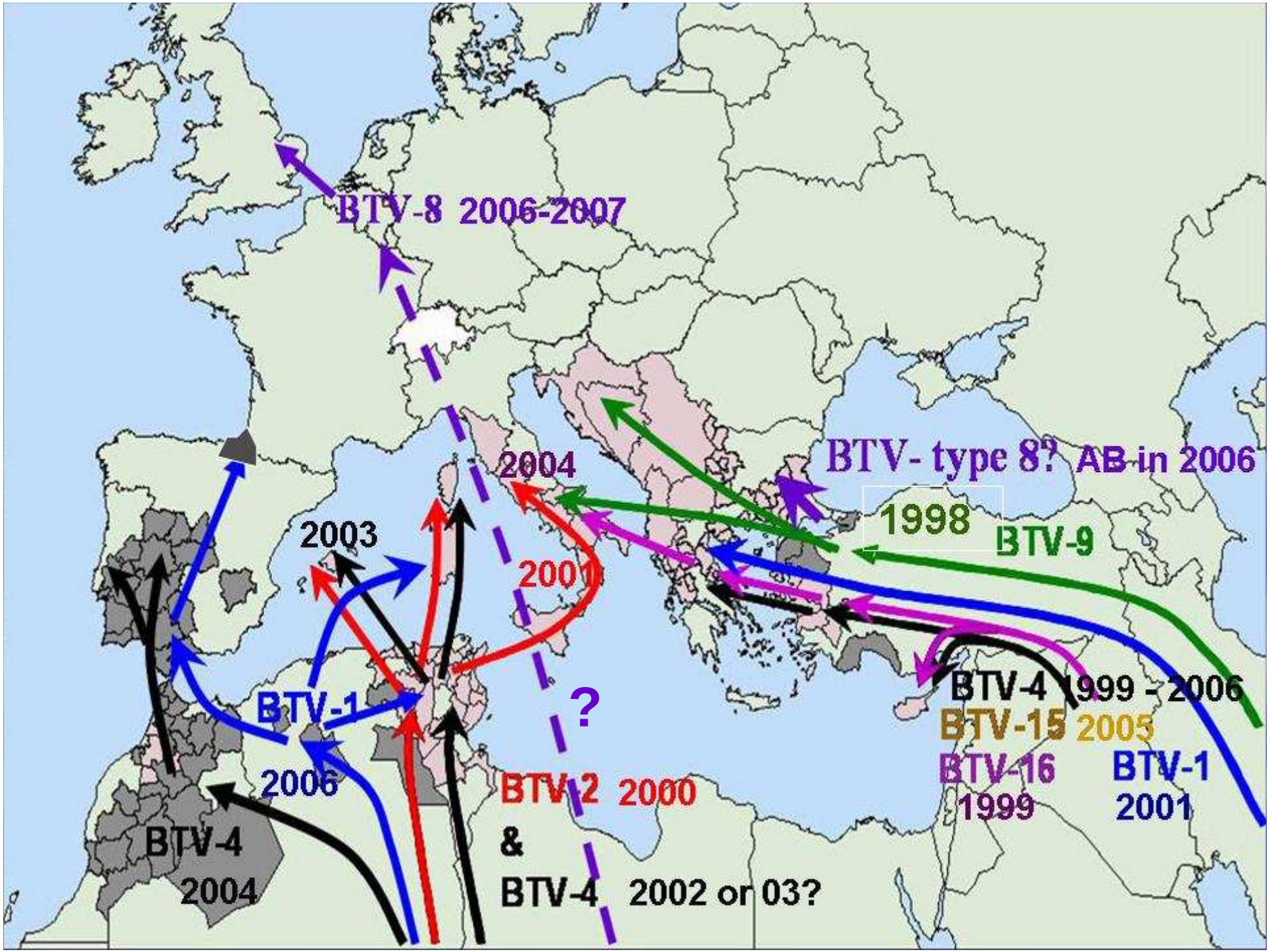
Since 1998 regular epizootics in the Mediterranean area (serotype 1,2,4,9 and 16) and since 2006 / 2008 also in Nord-Western Europe (serotype 8).

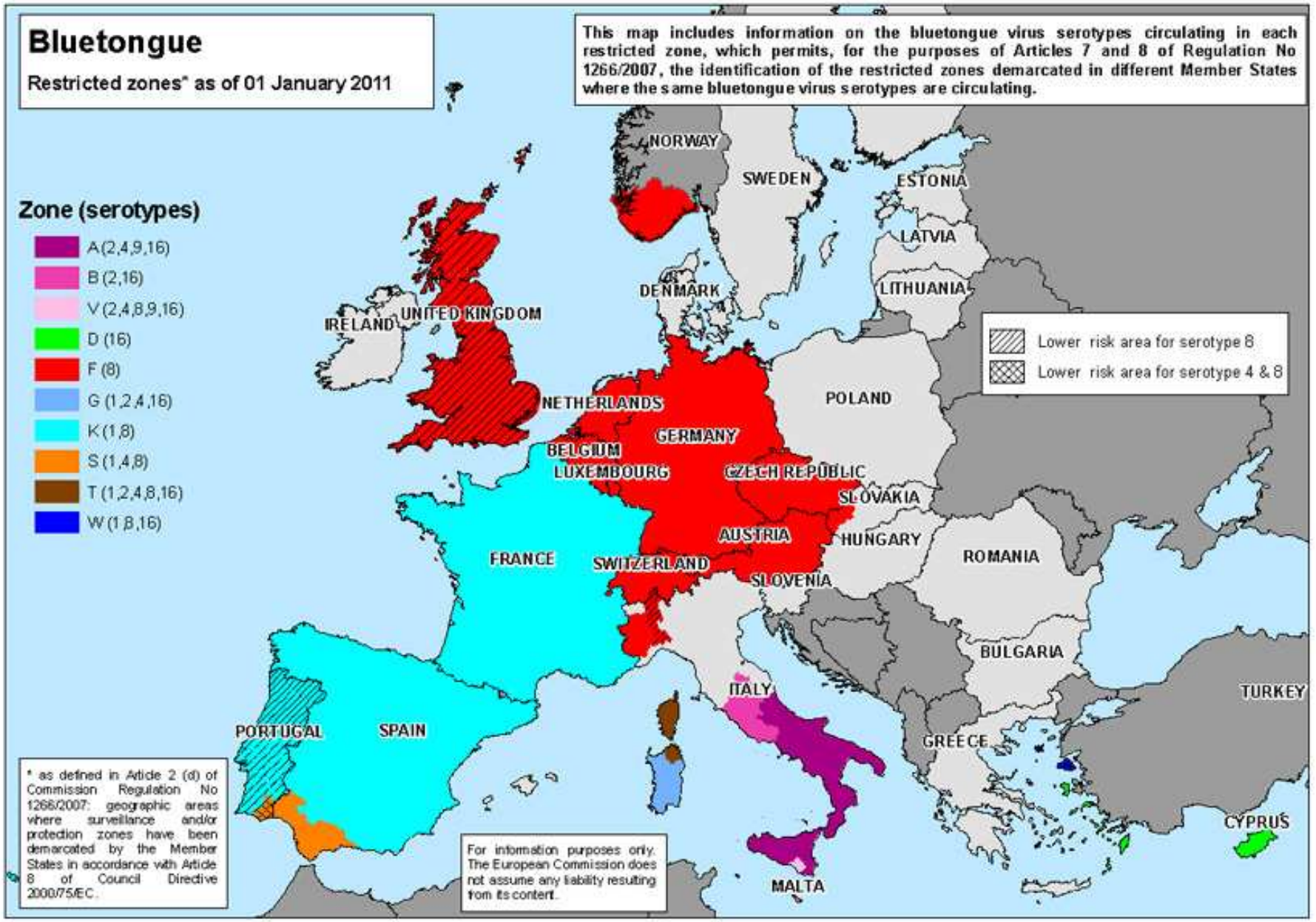
4 distinct routes towards Europe:

- from the East via Turkey / Cyprus
- via North Africa (Algeria, Tunisia) towards Italy, Corse, Mallorca
- via Morocco towards southern Spain and Portugal
- via an unknown route towards north-western Europe

+ utilisation of attenuated vaccines in some countries.

Exchange of genomic segments possible between different serotypes but also between vaccine and wild strains.


BTV-8 2006-2007
BTV- type 8? AB in 2006
2004
1998
BTV-9
2003
2001
?
BTV-1
2006
BTV-4
2004
BTV-2 2000
&
BTV-4 2002 or 03?
BTV-4 1999 - 2006
BTV-15 2005
BTV-16
1999
BTV-1
2001


Bluetongue
Restricted zones* as of 01 January 2011
This map includes information on the bluetongue virus serotypes circulating in each restricted zone, which permits, for the purposes of Articles 7 and 8 of Regulation No 1266/2007, the identification of the restricted zones demarcated in different Member States where the same bluetongue virus serotypes are circulating.
Zone (serotypes)
A(2,4,9,16)
B(2,16)
V(2,4,8,9,16)
D(16)
F(8)
G(1,2,4,16)
K(1,8)
S(1,4,8)
T(1,2,4,8,16)
W(1,8,16)
Lower risk area for serotype 8
Lower risk area for serotype 4 & 8
NORWAY
SWEDEN
ESTONIA
LATVIA
LITHUANIA
DENMARK
IRELAND
UNITED KINGDOM
NETHERLANDS
POLAND
GERMANY
BELGIUM
LUXEMBOURG
CZECH REPUBLIC
SLOVAKIA
AUSTRIA
HUNGARY
ROMANIA
FRANCE
SWITZERLAND
SLOVENIA
BULGARIA
ITALY
TURKEY
PORTUGAL
SPAIN
GREECE
CYPRUS
MALTA
* as defined in Article 2 (d) of Commission Regulation No 1266/2007: geographic areas where surveillance and/or protection zones have been demarcated by the Member States in accordance with Article 8 of Council Directive 2000/75/EC.
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Why the disease is spreading to the North?

1. Climatic changes ?

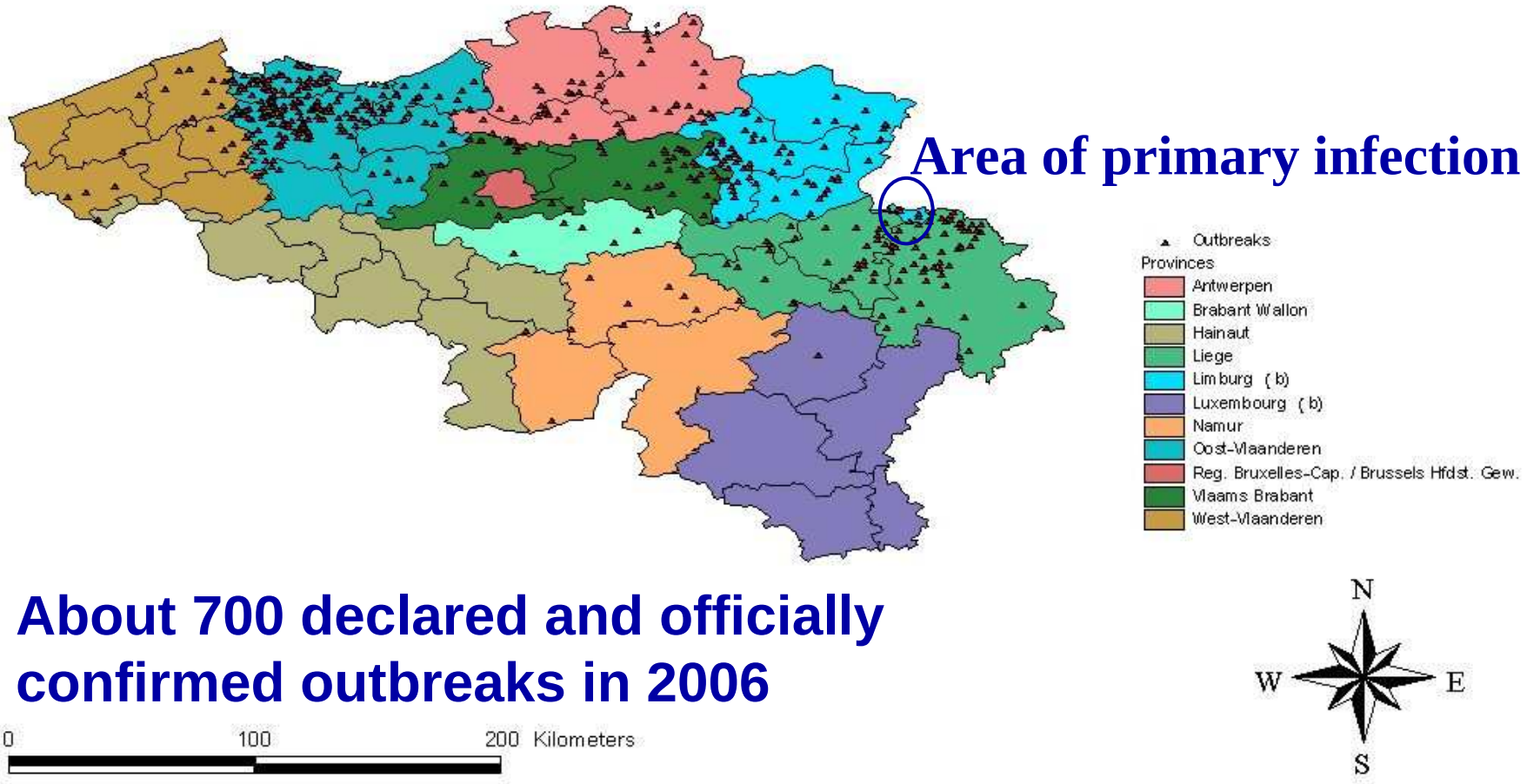
Global warming (more important for the higher latitudes, during winter and night) combined to increased rainfall, provokes:

- A larger distribution area for some vectors (*C.imicola*)
- An increased vectorial capacity or competence (*C.nubeculosis*)
- An increased activity, more frequent feeding and increased density of the vector (more transmission)

2. Transport ?

Active flight of the midges (small distance), via transport of infected animals or animal products, via transport of infected midges by car or plane or spread of midges with the wind.

Start of the 2006 BTV 8 epidemic



About 700 declared and officially confirmed outbreaks in 2006

In the past BT serotype 8 only observed in Africa, Asia or America

How BTV-8 could enter in the primary infection area?

1. Spread after importation of infected animals (Kerkerade NL – Gaia Park?), but in the area no (legal) importation between 1/01 and 18/08/06 of animals from countries other than neighbouring ones.

2. Importation of infected animal products: semen – only from Italy, Canada and USA .

3. Attenuated vaccine of SA = 3 bottles with different serotypes: A–1, 4, 6, 12 & 14 / B – 3, 8, 9, 10 &11 / C – 2, 5, 7, 13, &19

4. Importation of infected *Culicoïdes*

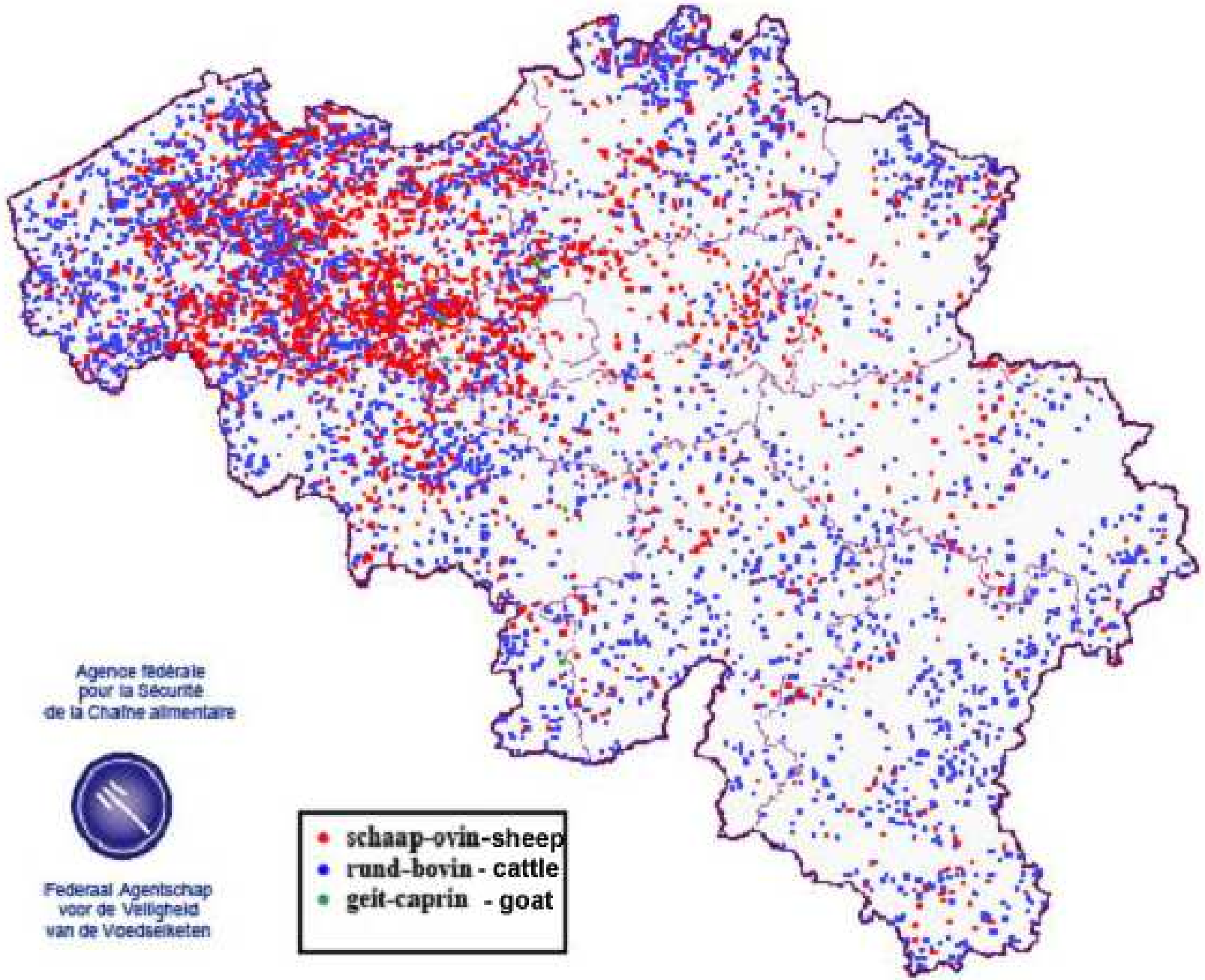
A) World Equestrian Games from 20/08 until 03/09/06 - 825 horses originating from 61 countries e.g. Mexico, Mauritius, South Africa and Argentina in the area of primary infection.

B) Daily tropical plants are imported from sub-Saharan Africa via the airport of Maastricht.

2007 Cross-sectional serologic study

- In January 2007 serologic survey in cattle
- Overall herd seroprevalence of 83%!
- True within-herd seroprevalences of 23.8 %
- BT seropositivity unevenly distributed throughout Belgium, with a gradient decreasing towards the south and the west of the country (Méroc et al, 2008).

Bluetongue outbreaks in Belgium in 2007



2008 Cross-sectional serologic study

- January to June 2008 survey in sheep
- Overall herd seroprevalence of 98%
- True within-herd seroprevalences of 77%
- BT seropositivity unevenly distributed with lower seroprevalences in the South East and the extreme West of the country.
- January-February 2008 survey in cattle
- Almost 100% seropositivity (Méroc et al, 2008).
- Serious problem of underreporting of the disease (in 2007 the % of farms reporting BT was on average 11.7%) + late reporting (time between infection and confirmed diagnosis often very long especially in cattle)

Vaccination against BTV8

Was in 2008 (-2010) vaccination of the whole Belgian herd necessary?

Obligatory vaccination of all sheep and bovines before end of year

In 2008 50 outbreaks/cases reported (3 ovine - 47 bovine) of which 27 were imported and 23 genuine cases (8 in W-Vl and 8 in Lux)

Registered vaccinations of cattle in 2008

PROVINCE	Situation on 23/10/08*	Situation on 22/01/09**
ANTWERPEN	41%	72%
WEST VLAANDEREN	21%	90%
OOST VLAANDEREN	53%	95%
HAINAUT	34%	89%
LIEGE	36%	91%
LIMBURG	37%	86%
LUXEMBOURG	35%	90%
TOTAL	40%	88%

% bovines having received *1 dose or ** 2 doses of vaccine (3 to 4 w)

Clinical symptoms in cattle

Pathogenesis: Tropisme for endothelial cells of blood vessels; provoking thrombosis and necrosis

conjunctivitis

Dermatitis

Lameness due to coronitis

Hyperaemia of the muzzle,

nasal discharge and salivation,

Abortions and malformed calves

Echymoses on the heart and hemorrhage at the base of the pulmonary artery

Inflammation, ulceration and necrosis

of the oral mucosa

Teats or udder

Diminished milk production and diminished fertility

Clinical symptoms in sheep

(more important pathology, higher mortality)

Inflammation,
ulceration and necrosis
of oral mucosa

Pneumonia
Pulmonary oedema
Mortality, abortions

Sometimes a blue, swollen tongue

Facial oedema

Hyperaemia of muzzle and lips

Lameness due to coronitis or muscle necrosis

Lesions of the udder

Vector monitoring

1) with UV traps

Ex.: John Hock (CDC trap) or OVI trap

UV light attracts midges

Ventilator sucks midges down

Connection towards battery

Midges fall in a solution which preserves the insects and/or virus for further analysis

2) By the use of emergence traps

To detect breeding sites, study their composition and the parameters influencing eclosion

3) By the use of immobile Rothamsted suction traps

- Insects trapped at a height of 12 m
- May give information on the physiologic or ecologic parameters which may stimulate the *Culicoïdes* to take flight and disperse
- Numbers of midges captured with these traps are low (1 to 10/day) but the diversity of captured Culicoides species is high and the trap catches continuously

4) By the use of CO^2 traps

Mosquito Magnet

Attraction by carbondioxide, warmth and humidity

Captures day and night (some *Culicoides sp* are diurnal)

Efficacy relatively good when outside temperatures low

Sampling of biting midges

A. Sampling at outbreak sites

OVI trap

Aim: to identify the *Culicoïdes* present at the outbreak sites and to examine the presence of virus in the vector.

- Culicoïde Virus PCR +: the vector may have fed on an infected animal
- Head of Culicoïde Virus PCR+: the virus has left the intestine and has reached the head
- Culicoïde + virus isolation : multiplying virus present in the vector
- Culicoïde + successful transmission : certainty that the *Culicoides* species is a competent vector

B. Longitudinal surveys

In order :

To study the phenology of the Culicoides spp or to determine the vector-free period (EU: less than 10 parous females/night/trap)

To correlate apparent density with ecological conditions

To ascertain the endophilic/exophilic behaviour of the *Culicoïdes spp*

To correlate apparent density with disease transmission rate

To study the physiology of the captured insects (nulliparous, parous, oviparous, fed)

From 2007 until last year mainly in sentinel dairy farms monitored by the FAFSC

Major questions

- Which biting midges are present in Belgium and which among them may play a role as vectors?
- What about a vector-free period?
- Is there a relationship between local vector presence / abundance and bluetongue incidence / prevalence?
- Can *Culicoides spp* presence and abundance be predicted by some ecological parameters?
- Can stabling of livestock or other strategies be recommended to reduce infection risk?

Results :

1. Species, activity, variations in abundance

Culicoides (essentially females) were captured all over the country. About 45 different *Culicoides spp* were captured in Belgium among them 5 known as vectors of bluetongue (*C. obsoletus/scoticus*, *C. dewulfi*, *C. chiopterus*, *C.pulicaris*)

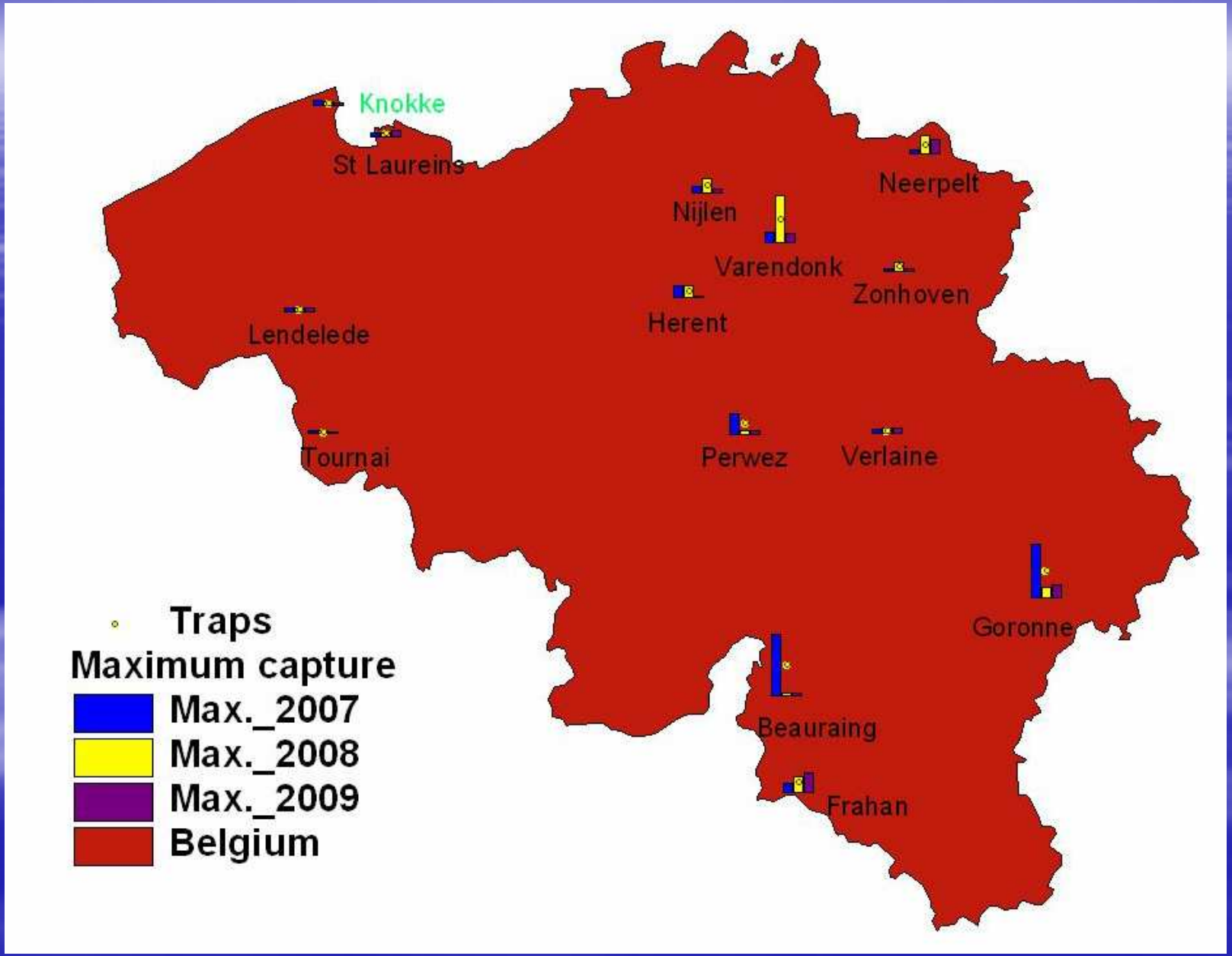
Culicioides obsoletus/scoticus constituted more than half of all captured midges and were present in all outbreak sites!

Outdoor trapping: below 5°C almost no activity. Sometimes midges (essentially nulliparous) captured outdoors in middle of winter. Indoors: *C.obsoletus* and *C. dewulfi* seek shelter in stables during winter and prolong their activity period by doing so. But, in summer also, midges may be numerous inside stables where animals are kept.

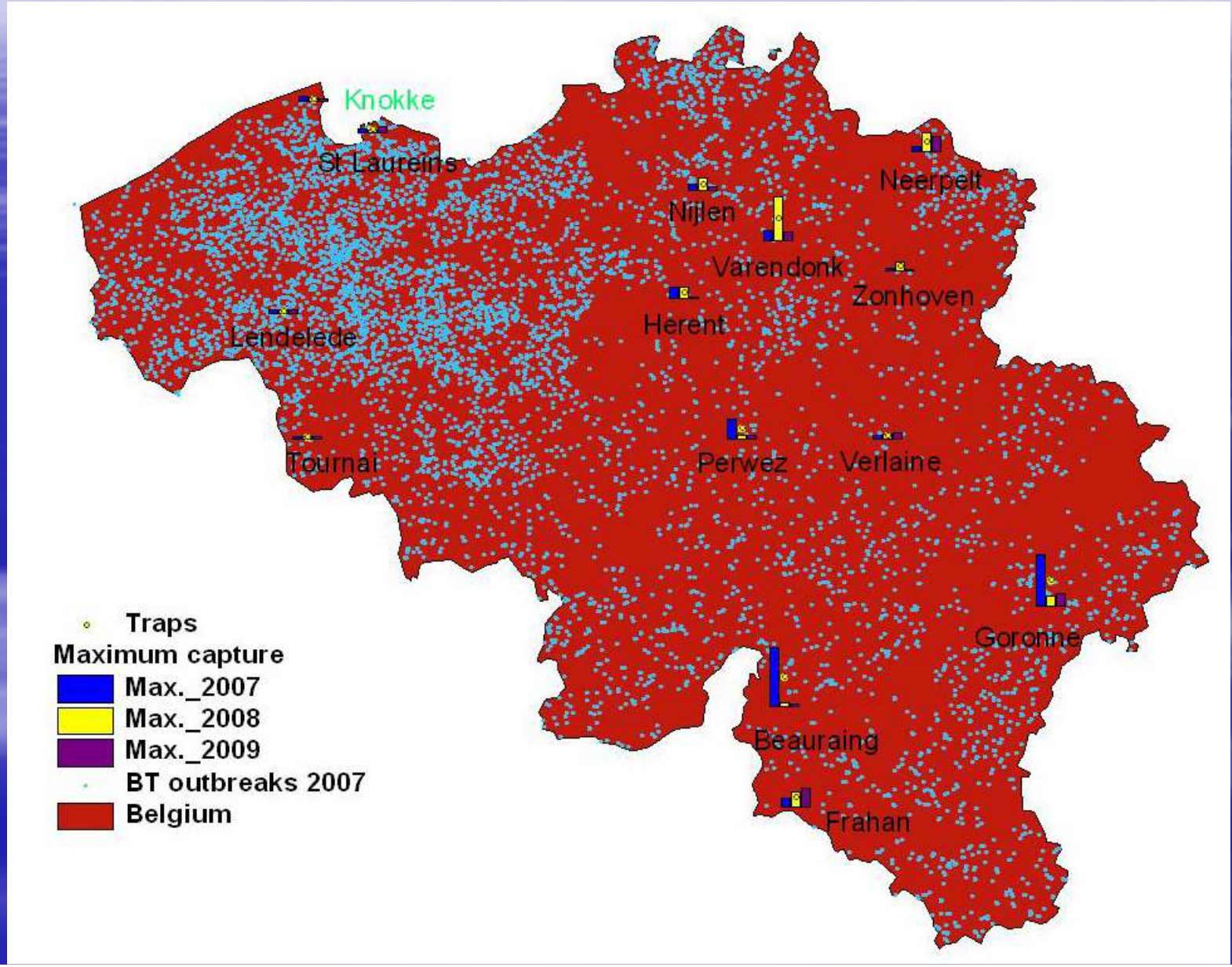
Lower apparent density of midges in Western Belgium corroborates lower seroprevalences observed in sheep from this regions during the national CERVA survey (01-06/2008) In the South East fewer infective midges may be present due to the colder environment?

Numbers of vectors captured varies considerably spatially (maximal capture/night: between 160 and 16000) and temporally (max.capture from April to October - but also large differences between years)

Maximum apparent densities in OVI traps at sentinel sites

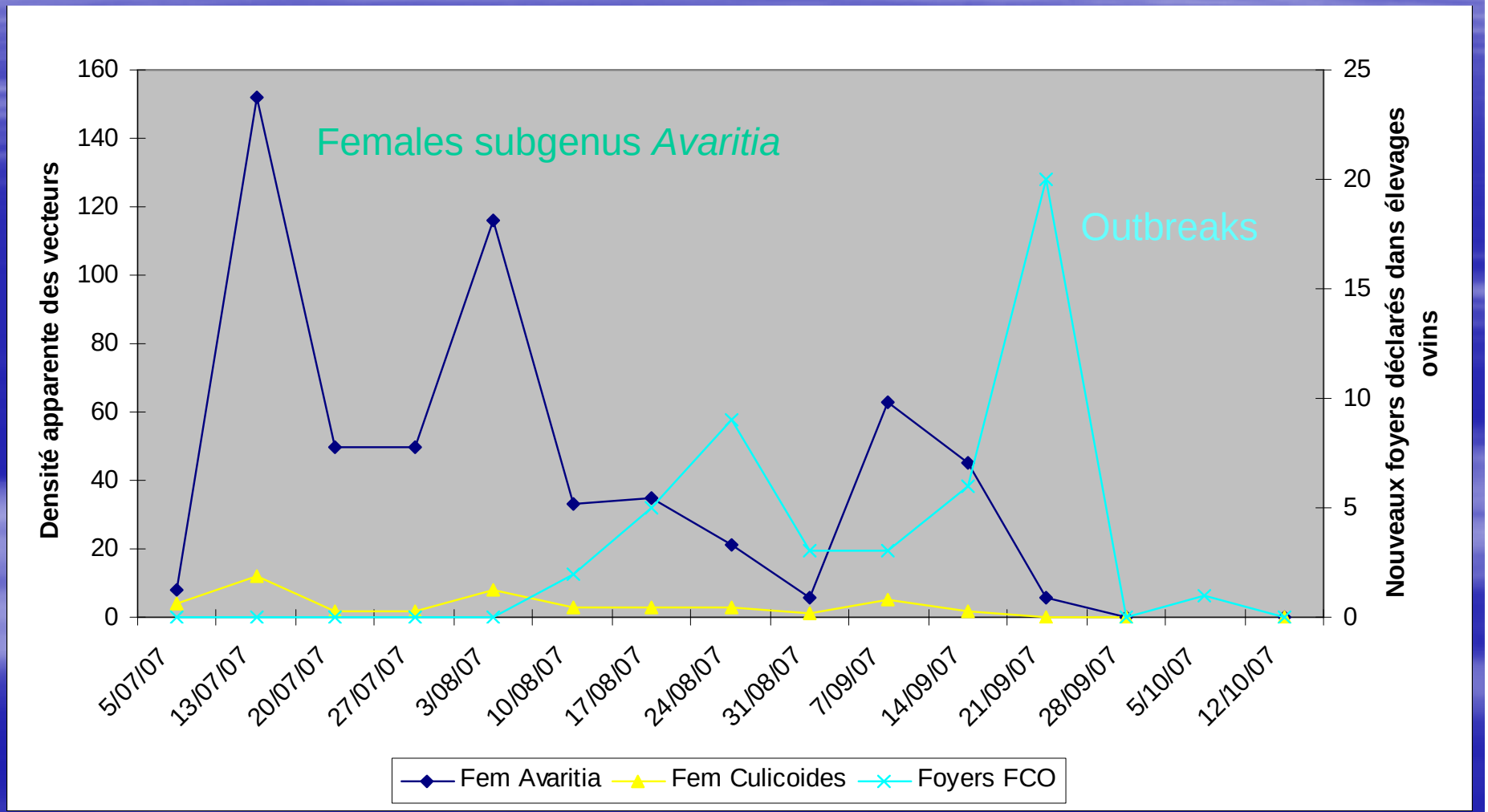


2. Relationship between local vector abundance and BT incidence?



2. Relationship between local vector abundance and BT incidence?

Comparison between Culicoides apparent density and outbreak occurrence within a radius of 5 km around the farm of Lendelede in 2007 (31 outbreaks in the 5km zone)



It is hard for the vector to get infected but once infected transmission is easy!

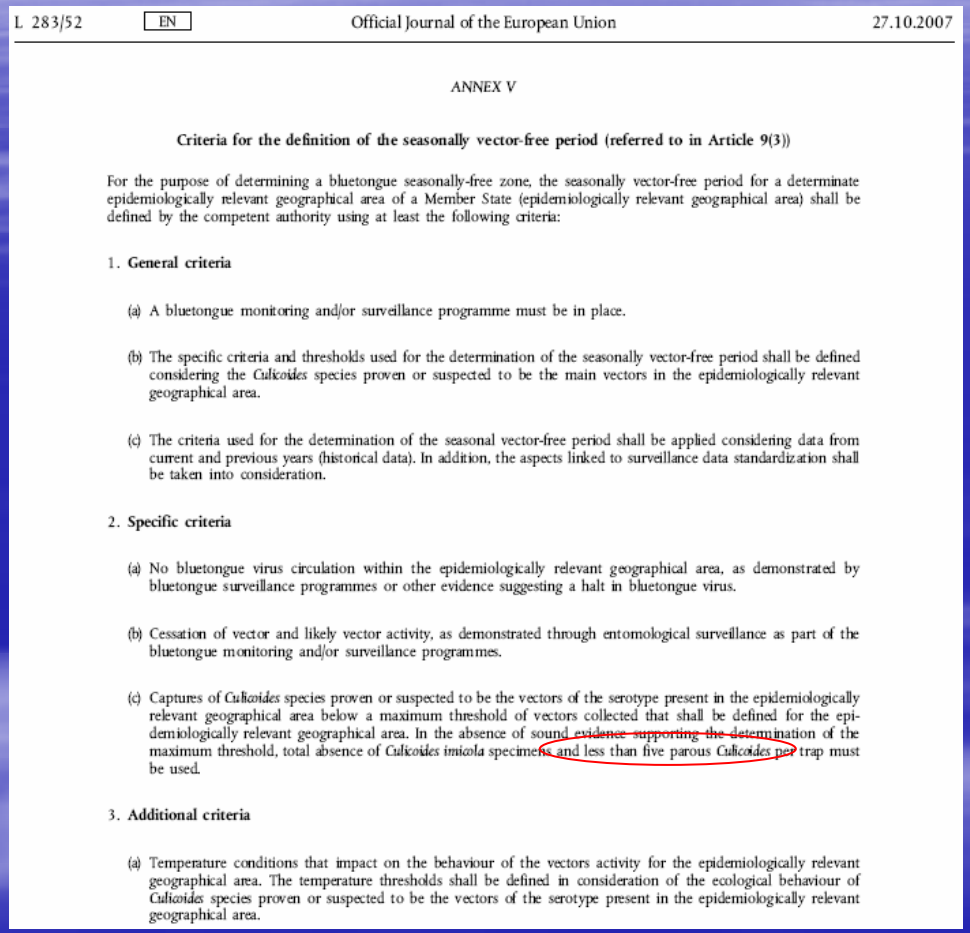
3. Analysis of ecologic parameters

- Aim: to examine if midge abundance / presence (species specific or total) is influenced by ecologic parameters (direct environment, climatic data, landcover in a radius of 5, 2 or 1 km around monitoring site, etc..)
- Influences on apparent abundance were recorded for temperature, rainfall, wind, distance from farm, soil type, cloudiness
- Influences on species composition were recorded for rainfall, temperature and landcover found in the area around the trap,

BUT!

- Probable bias on abundance because of relative high proportion of *C.obsoletus s.l.* in the catches and because all longitudinal monitoring had been carried out in dairy farms.
- Not all species have the same activity periods and thus not all species are captured as effectively with blacklight traps.

4. Vector Free Period (VFP)



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ANNEX V

Criteria for the definition of the seasonally vector-free period (referred to in Article 9(3))

For the purpose of determining a bluetongue seasonally-free zone, the seasonally vector-free period for a determinate epidemiologically relevant geographical area of a Member State (epidemiologically relevant geographical area) shall be defined by the competent authority using at least the following criteria:

1. **General criteria**

(a) A bluetongue monitoring and/or surveillance programme must be in place.

(b) The specific criteria and thresholds used for the determination of the seasonally vector-free period shall be defined considering the *Culicoides* species proven or suspected to be the main vectors in the epidemiologically relevant geographical area.

(c) The criteria used for the determination of the seasonal vector-free period shall be applied considering data from current and previous years (historical data). In addition, the aspects linked to surveillance data standardization shall be taken into consideration.

2. **Specific criteria**

(a) No bluetongue virus circulation within the epidemiologically relevant geographical area, as demonstrated by bluetongue surveillance programmes or other evidence suggesting a halt in bluetongue virus.

(b) Cessation of vector and likely vector activity, as demonstrated through entomological surveillance as part of the bluetongue monitoring and/or surveillance programmes.

(c) Captures of *Culicoides* species proven or suspected to be the vectors of the serotype present in the epidemiologically relevant geographical area below a maximum threshold of vectors collected that shall be defined for the epidemiologically relevant geographical area. In the absence of sound evidence supporting the determination of the maximum threshold, total absence of *Culicoides imicola* specimens and less than five parous *Culicoides* per trap must be used.

3. **Additional criteria**

(a) Temperature conditions that impact on the behaviour of the vectors activity for the epidemiologically relevant geographical area. The temperature thresholds shall be defined in consideration of the ecological behaviour of *Culicoides* species proven or suspected to be the vectors of the serotype present in the epidemiologically relevant geographical area.

- Purpose of legislation is to permit safe animal movement for as long as possible with a low risk of revocation
- One of the only examples where entomological surveillance is explicitly used as basis for policy

Definition of VFP

Based on UV light traps placed outside

- Complete absence of parous females of any species
- < 5 parous females of any species
- < 10 females of any species
- Complete absence of C. imicola
- < 5 parous females of C. imicola

Comparison of definitions for Belgium

	End vector low abundance			Start vector low abundance		
	≥ 5 parous ♀	≥ 10 ♀	> 0 parous ♀	< 5 parous ♀	< 10 ♀	0 parous ♀
Average 2007	13/04/07	15/04/07	11/04/07	4/11/07	22/10/07	19/11/07
nr traps	14	24	14	14	24	14
Average 2008	2/05/08	27/04/08	24/04/08	31/10/08	26/10/08	13/11/08
nr traps	11	22	12	10	15	10

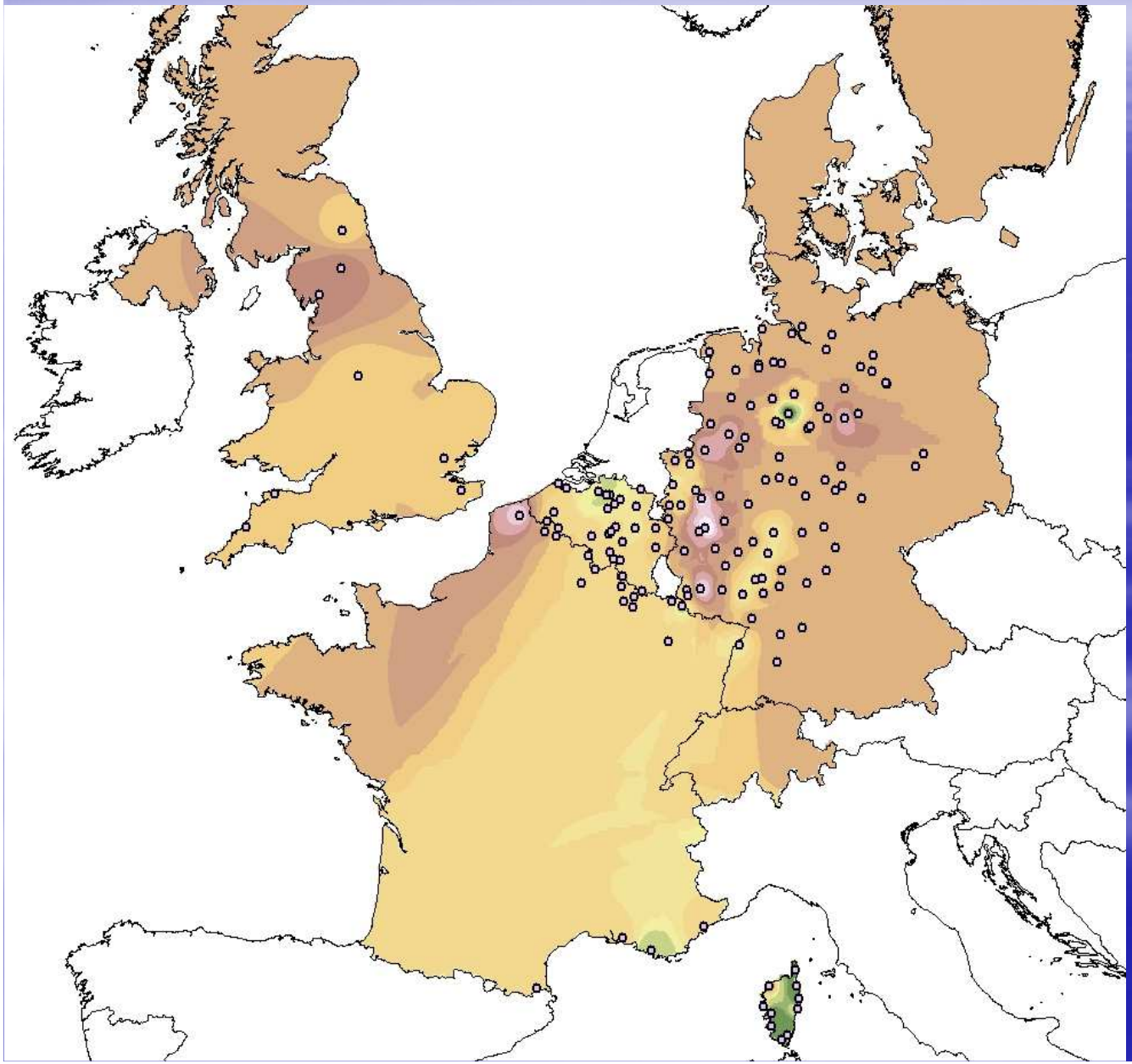
For Belgium no important differences between the three criteria when used to define the end of the period of low vector abundance but to define the start of this period the difference is almost 3 weeks according to the criterion used.

Interpolation studies to visualise the start/end of the Vector Free Period in Europe

To convert point data on the start/ end of the activity period for *C.obsoletus s.l.* collected in different European countries into surface maps an inverse weighted distance interpolation technique was used

Output raster limited to contours of France, UK, Germany, Belgium, Denmark and Sweden; output pixel size 10km^2

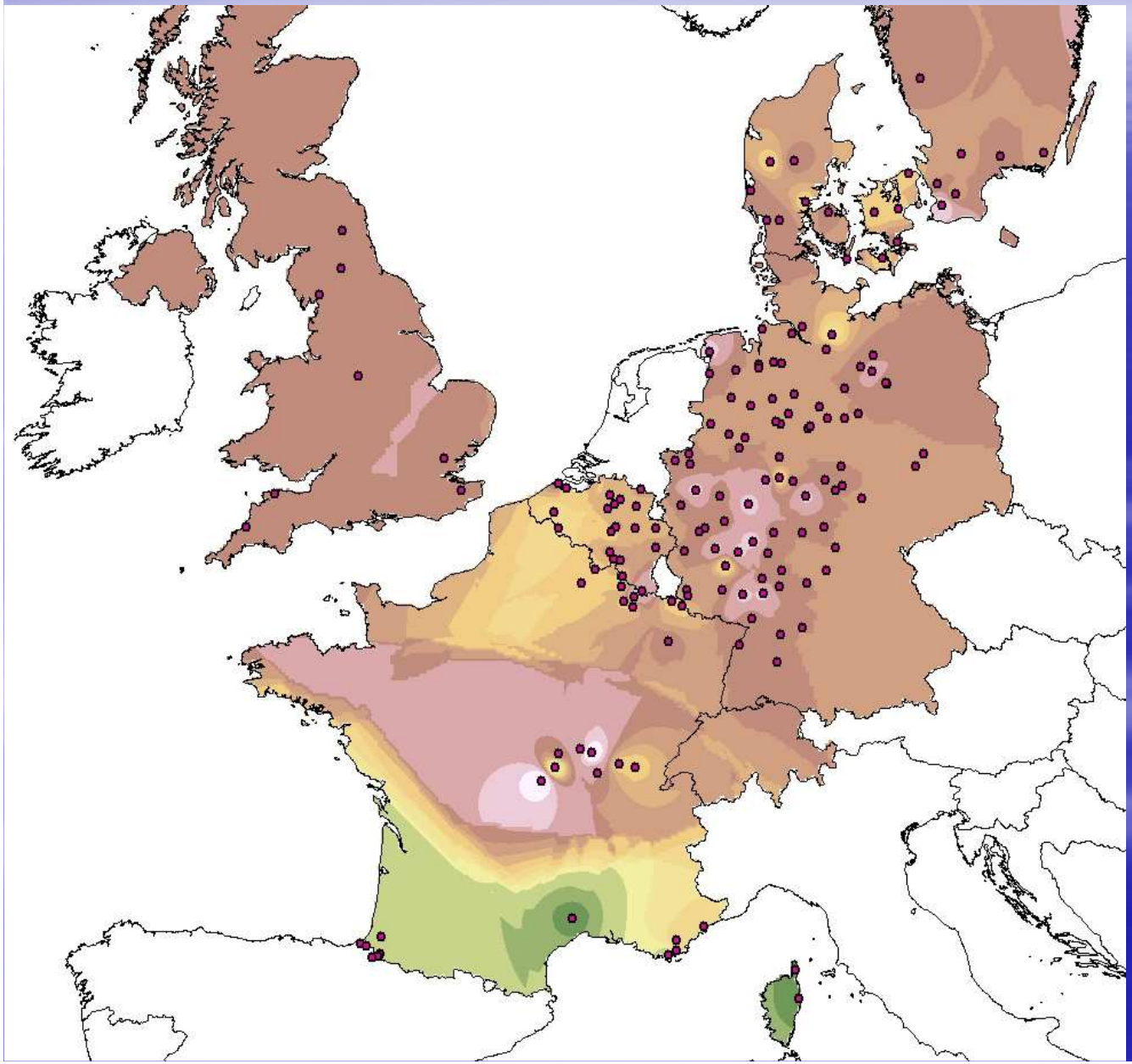
Start activity midges in 2007



No data Northern Europe

Exceptional warm spring → earlier activity in areas with sandy soils, in valleys (Rhine) and in the warmer south?

Start activity midges in 2008

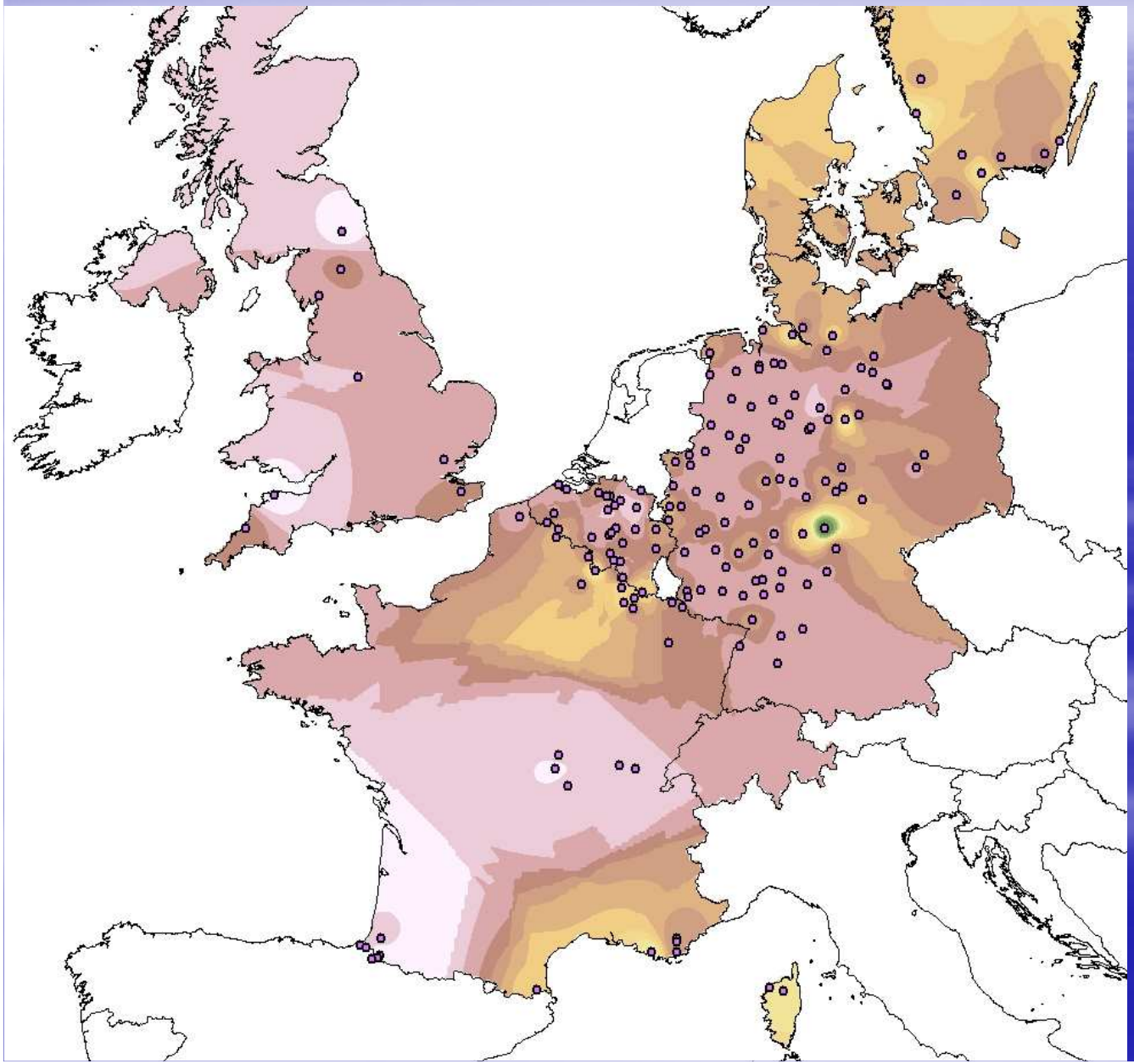


More traps set up in Central + Southern France

Start = later than in 2007

Altitude plays role

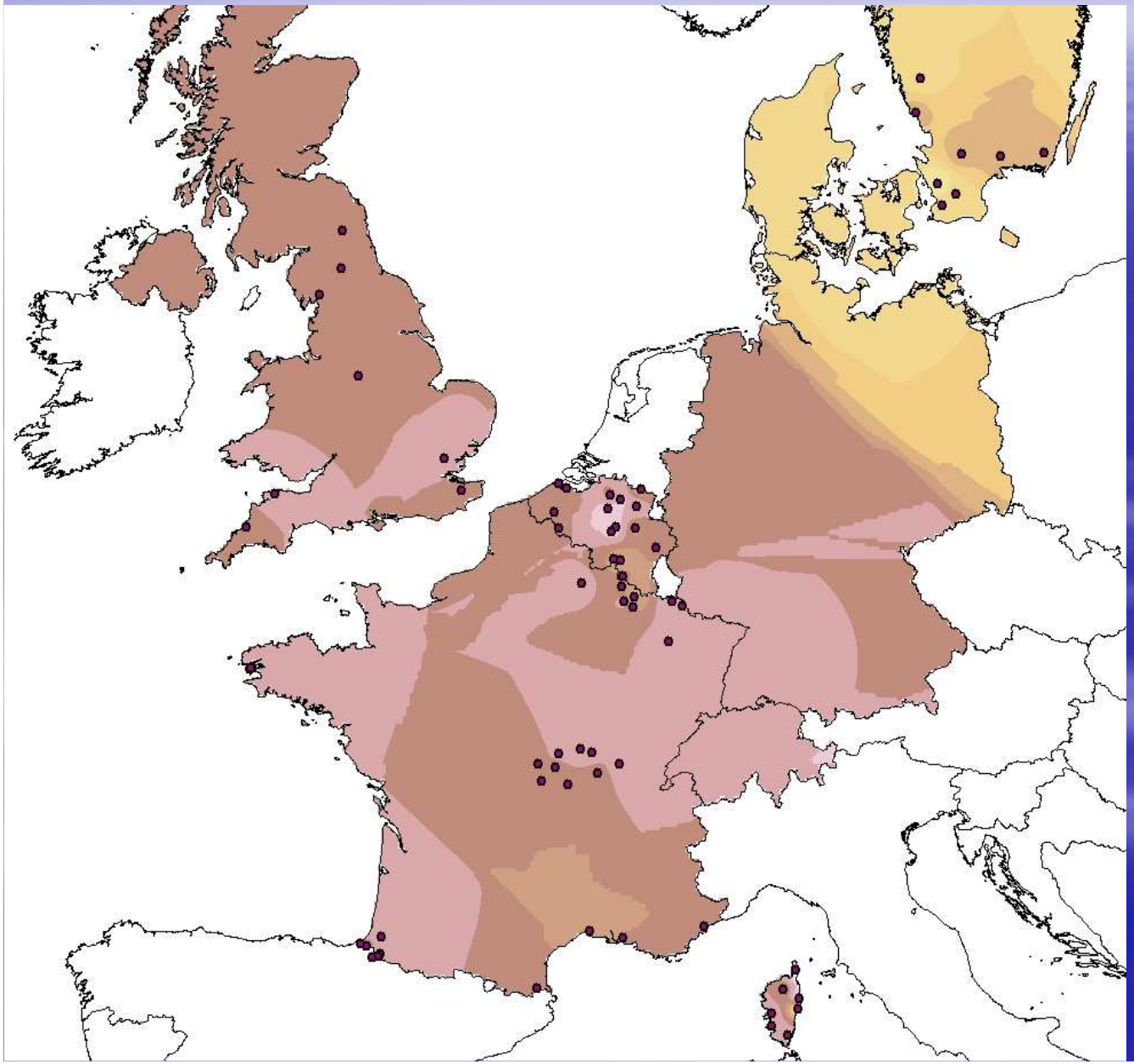
End activity midges in 2007



In South-West France and parts of UK midges until December (NAO+?)

End activity surprisingly soon in Mediterranean area

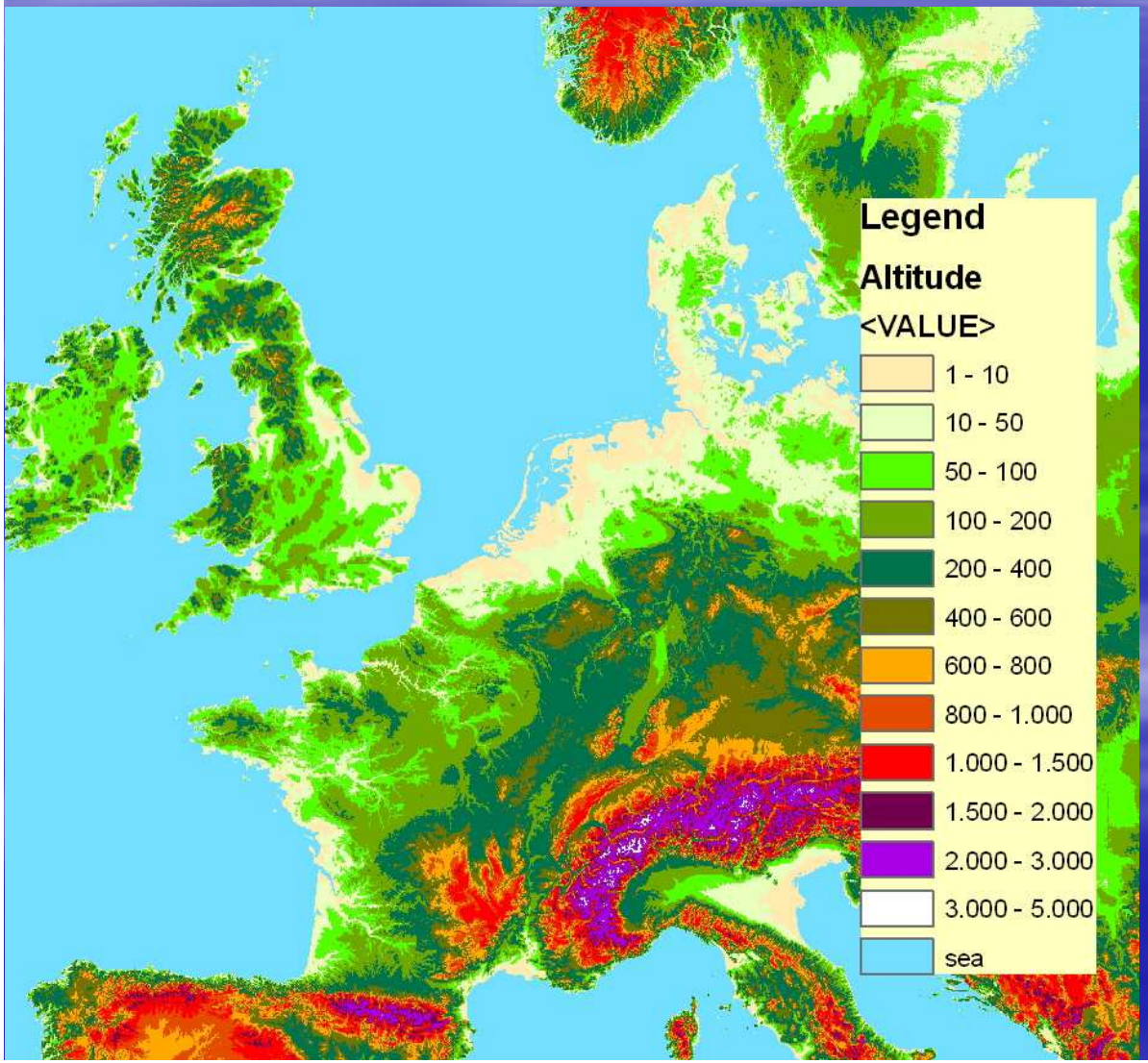
End activity midges in 2008



No data for Germany and Denmark

More uniform image when winter is harsh (NAO-)

Potential interesting parameters for VFP modelling



- Accumulated temperature or other temperature related data
- Latitude
- Distance from coast
- Distance from Mediterranean Sea
- Altitude
- Ecological zone
- Others?

Thanks for your attention

Be aware of bluetongue!