Association between land cover and *Culicoides* (Diptera: Ceratopogonidae) breeding sites on four Danish cattle farms

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Biting midges of the genus *Culicoides* are vectors of bluetongue virus. Their larval habitats are poorly known in Northern Europe. Three classes of the CORINE land cover index, found within 300 m of four farms in Denmark, were used to stratify sampling sites for a total of 360 soil core samples from 30 sampling points. Soil samples were set up in emergence chambers for hatching adult *Culicoides*. Two species of *Culicoides* (*C. punctatus* and *C. pulicaris*) emerged from nine of 12 soil samples from a wet, grazed field with manure. Seventy-two other samples from similar land cover on the three other farms were negative. Seven sampling points from pastures were incorrectly classified by CORINE. The remaining 23 sampling points were classified correctly. The visually observed land use was not sufficiently detailed to adequately predict *Culicoides* breeding sites in this study. The CORINE index failed to identify pastures in which *Culicoides* breeding sites were found.

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1. Introduction

Biting midges of the genus *Culicoides* are vectors of bluetongue virus that infects ruminants. The virus has caused a costly epidemic in large parts of North-West Europe since its introduction in 2006. *Culicoides* breeding ecology is poorly understood but important for virus research (Kettle 1977, Wilson & Mellor 2008, Zimmer *et al.* 2008).

Larvae of *Culicoides* have a general preference for the top layer of permanently non-submerged humid environments (Blackwell & King 1997, Uslu & Dik 2006). Conte *et al.* (2007) stated that although up to 90% of *Culicoides* spp. breed in waterlogged habitats, the subgenus *Avaritia* (comprising the bluetongue vector *C. obsoletus* complex) favour drier, semi-moist habitats. Zimmer *et al.* (2008) found that larvae of the *C. obsoletus* complex were abundant in maize silage in Belgium, and Nielsen *et al*. (1998) demonstrated that members of the subgenus *Culicoides* (e.g. vectors *C. punctatus* and *C. pulicaris*) breed in a heavily polluted marsh area in Denmark. The public available digital CORINE index provides information about the land cover in Europe at a spatial resolution of 25 m based on remote sensing data from satellite images (EEA 2000). Thus,

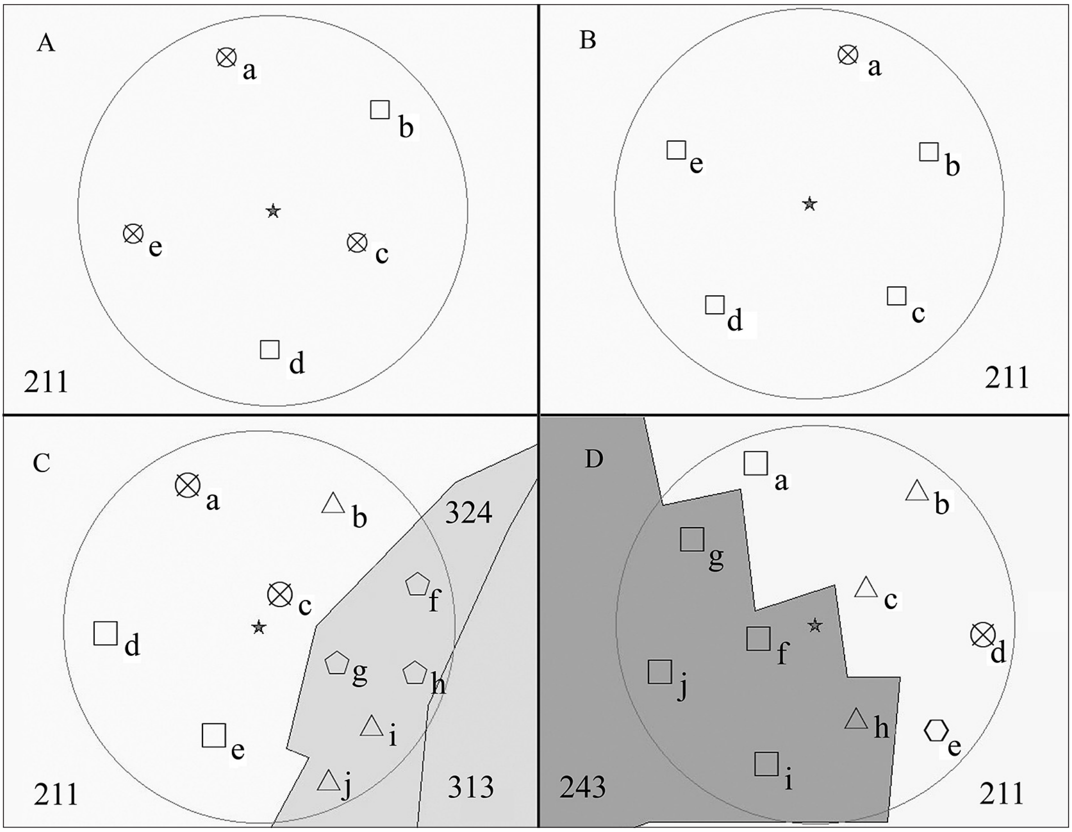

Fig. 1. The four locations with sampling points. In each sampling point, 12 soil samples were performed. Stars symbolize farms with light traps and the circles are their 300 m radius. Localities: A: Lille Skensved, B: Ølstykke, C: Randbøl, D: Fladen. Crossed circles = grazing; triangles = meadow; squares = crop fields (corn or grain); pentagons = forest area; hexagon = wetland. Numbers show the CORINE indices. The area with index 313 was omitted because of its small size. *Culicoides* spp. only hatched from sampling point *d* on location D. Graphics: Carsten Kirkeby.

the CORINE index could be useful as a shortcut to predict *Culicoides* breeding sites near farms, as an alternative to visiting each farm and visually inspecting the surrounding areas, an expensive and time-consuming operation. The objective of this study was to evaluate if land cover classification, including the CORINE land cover index, around farms may be used to identify *Culicoides* breeding sites.

2. Materials and methods

Four locations (cattle farms) in Denmark were selected for this study. The CORINE 2000 index shows land cover classes based on satellite photos with the resolution of 25 m. In Denmark, 31 CORINE classes are represented, of which four occurred within a 300 m radius of the four farms:

- Non-irrigated arable land (class 211)
- Land principally occupied by agriculture, with significant areas of natural vegetation (class 243)
- Transitional woodland/shrub (class 324)
- Mixed forest (class 313) (which was omitted due to its very small area within 300 m radius)

Before visiting each farm, five sampling points in each CORINE class within a 300 m radius were selected and plotted on the map, aiming at an evenly dispersed pattern, resulting in a total of 30 sampling points (Fig. 1). The 300 m distance was chosen on the basis of a study by Nielsen (1963)

Table 1. Numbers of dominant *Culicoides* species caught in light traps four weeks after sampling. Soil samples were taken on the 29th (B) and 31st (A) of July and the 13th (D) and 14th (C) of August, corresponding to week 1 (A, B) and 3 (C, D).

		Weeks after collection of soil samples			
Farm	Species	+1	+2	+3	+4
A	*C. obsoletus* complex	992	74	31	44
	C. punctatus	244	37	8	0
	C. pulicaris	132	2	4	2
B	*C. obsoletus* complex	102	18	26	157
	C. punctatus	78	21	11	29
	C. pulicaris	30	9	7	18
C	*C. obsoletus* complex	130	221	192	218
	C. punctatus	2	0	52	4
	C. pulicaris	13	3	92	43
D	*C. obsoletus* complex	197	181	0	9
	C. punctatus	374	631	0	21
	C. pulicaris	259	497	2	8

where the majority of *Culicoides* were caught within 300 m of the breeding site, suggesting that the midges have a short flight range.

On each farm, the geographical coordinates from the map were located using GPS, and the present land use in the points was visually observed (Grazing; Meadow; Crop fields (corn or grain); Forest area; and Wetland). Twelve soil core samples were subsequently collected along the perimeter of a 2 m radius around each sample point with 50 cm distance between each sample. Samples were collected during a two week period in July and August 2008, just before the 2008 outbreak of bluetongue in northern Europe began (Conraths *et al.* 2009). The soil cores were exhumed using a metal pipe (97 mm in diameter) and were up to 7 cm deep, depending on the hardness of the soil. The resulting 360 soil samples were set up in emergence chambers modified from Dyce & Marshall (1989), made of black painted plastic with a 13 mm funnel leading to a clear collection chamber. Emergence chambers were sealed with tape and kept in constant light at room temperature. For very dry samples (locations A and B) 5 ml of tap water per centimeter soil depth were added to facilitate a humid environment for hatching. Emerging *Culicoides* were identified to the species level. A Poisson distribution was fitted to the sample points from which *Culicoides* emerged (positive sampling points), and a Goodness-of-Fit test was performed.

To compare the species ratios from the emergence traps with the adult *Culicoides* population, *Culicoides* were caught in Onderstepoort Blacklight traps on the same four cattle farms (location A, B, C, D), once a week for four weeks after soil sampling on each farm. Light trap samples were examined morphologically and the dominant *Culicoides* specimens identified to species or species complex level after Kremer (1965) and Nielsen (1982).

3. Results

In total, 35 *Culicoides* specimens hatched from the emergence chambers (34 *C. punctatus* and one *C. pulicaris*). All *Culicoides* emerged between day 19 and 61 from the sample date. Other insects hatched from the emergence traps from day 5 to day 200 when the samples were discarded. *Culicoides* emerged only from a single sampling point (Location D, point *d*) occupied by grazing cattle. Six other sampling points on the three other farms were also occupied for grazing at the time of sampling, but none of these were positive. The only observed difference between the positive and the six other negative sampling points in grazing areas was wetter soil in the positive sampling point. All sampled grazed fields held a considerable amount of manure from the cattle. No *Culicoides* emerged from the 13 sample points collected from areas covered with crops; the three sampling points in areas with forest cover; the six sampling points in meadow areas; or from the single point in a wetland area.

In the positive sampling point, the 12 soil core samples yielded 14, 1, 0, 0, 4, 2, 3, 2, 0, 2, 5 and 2 adult *Culicoides*, respectively. The Goodness-of-Fit test showed that the 12 different yields were not significantly different from a Poisson distribution (p = 0.46). Thus, the variation between samples may be due to random variation. Based on a Poisson approximation, the mean yield per soil sample was 2.91±0.97 specimens. Each soil core sample covered 73 cm^2 corresponding to a mean yield of 400 ±132 adult *Culicoides* per m^2 in the positive sampling point.

None of the seven sampling points from pastures were classified as Pastures (class 231) in CORINE, instead they were classified as Non-irrigated arable land (211) (Fig. 1). The remaining 23 sampling points were all classified correctly.

Culicoides were collected from light traps in all farms (Table 1). The three most dominant species on the farms were *C. obsoletus* (complex), *C. punctatus* and *C. pulicaris*.

4. Discussion

The soil sampling on the first two locations took place in a drier period than the last two, which is likely to have affected the amount of *Culicoides* eggs laid. However, numerous other insects hatched from all locations.

Breeding sites of the *C. obsoletus* complex, presumed to be the main vector of bluetongue in northern Europe are previously demonstrated to breed in bog land, marsh and, to a lesser extent, swamps and mud (Kettle & Lawson 1952), middens, horse and cow dung (Campbell & Pelham-Clinton 1960), moist forests and muddy soil (Trukhan 1975, Mirzaeva *et al*. 1976). Some of these habitats are not found within 300 m from the sampled farms which could explain why no specimens of this complex were hatched in this study. Although middens, horse and cow dung were found within the 300 m limit, they were not included in the random sampling. Muddy soil was sampled, but no specimens from the *C. obsoletus* complex hatched. Kettle & Lawson (1952) found 97% of *C. obsoletus* specimens in bog land and marsh whereas only 3% were obtained from swamps and mud, which indicates that mud is not the preferred habitat of *C. obsoletus*.

The *C. pulicaris* complex breed in marsh, swamps and mud (Kettle & Lawson 1952), and forest leaf-litter and ponds (Trukhan 1975, Mirzaeva *et al.* 1976). Kettle & Lawson (1952) found 13–26% specimens of this complex in mud, which is the same type of habitat as where the specimens in this study also hatched.

The species composition on location D, was (*C. pulicaris*, *C. punctatus*, and *C. obsoletus* complex) 1:34:0 in the emergence traps and roughly 1:2:5 in the light traps in the following four weeks, suggesting that important breeding sites of *C. pulicaris* and *C. obsoletus* were not sampled in the area or that these species were attracted to the light traps from outside the 300 m radius. The *C. pulicaris* : *C. punctatus* emergence ratio is very close to the results from Nielsen *et al.* (1998), who obtained 32 *C. punctatus* and 1 *C. pulicaris* from 21 *in situ* emergence traps in a marsh polluted with organic material in Denmark during the summer of 1996. Thus, the positive sampling point in this study is quantitatively comparable with a eutrophic marsh area as a breeding site for *C. punctatus* and *C. pulicaris*.

Nielsen *et al.* (1998) found an emergence rate in the marsh area of up to 482.6 *Culicoides* per m^2 in 23 weeks. In this study, the estimated density in the positive sampling point was 400±132 *Culicoides* per m^2. The two obtained densities are not directly comparable and further experiments are needed to estimate the ratio between *in situ* and *in vivo* hatching, and quantification of the efficiency of the emergence traps.

Since the Goodness-of Fit test on the samples in the positive sampling point showed no significant divergence between the *Culicoides* data and a standard Poisson distribution, the data showed no evidence of clustering of the number of adult *Culicoides* within this sampling point.

Only one of seven sample points in grazing cattle fields (from all four locations) was found positive, indicating that the visually observed land use is not accurate enough to predict *Culicoides* breeding sites. Thus, other more detailed measures must be used for prediction of *Culicoides* breeding sites. Since none of the seven sampling points from pastures were correctly classified by the CORINE index, it failed to predict the present land use. This problem could be due to crop rotation or that the CORINE index is too coarse for this purpose.

Based on this study, we recommend that other measures are included in the future prediction of *Culicoides* breeding sites. Methods such as LIDAR (light detection and ranging) or SAR (synthetic aperture radar) can detect e.g. soil moisture, topography, biomass, canopy height, leaf area and would thus be interesting to include in the further research (Wagner *et al*. 2009).

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