Deformed wings in the bumblebee *Bombus terrestris* (Linnaeus, 1758) in Luxembourg

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Abstract. Deformed wing virus (DWV), a persistent pathogen in European honeybees contributing to colony losses, also affects wild bees, including bumblebees, and a wide range of other insects. Bumblebees play a crucial role in crop production in temperate and cold ecosystems. A case of wild buff-tailed bumblebee workers *Bombus terrestris* (Linnaeus, 1758) showing visible clinical symptoms associated with overt, acute DWV infection is documented in Luxembourg, the first report for this bumblebee species widely distributed in the country. Further investigation is needed in Luxembourg to understand the impact of infectious diseases on wild pollinators and the role of managed pollinators in pathogen spillover.

Key words: pollinators, deformed wing virus, pathogens, bumblebee, *Bombus terrestris*, Luxembourg.

1. Introduction

RNA viruses associated with honeybees are known to infect a wide range of flower visitors, including wild bees, wasps, beetles, hoverflies, and butterflies (e.g., Evison et al. 2012, Levitt et al. 2013, Ravoet et al. 2014, Bailes et al. 2018, Pislak Ocepek et al. 2022). Many of these insects play vital roles as pollinators, with bumblebees (*Bombus*) particularly noteworthy for their substantial contribution to the economic value of crop production in temperate and cold ecosystems of the northern hemisphere (Kleijn et al. 2015). The buff-tailed bumblebee, *B. terrestris*, is one of the most widely distributed and abundant bumblebee species in the West-Palaearctic (Rasmont et al. 2008). It has been successfully bred in captivity since the 1980s and has been widely commercialized to supply greenhouse pollination services (Dafni et al. 2010).

Deformed wing virus (DWV) is a persistent pathogen in European honeybees, significantly contributing to overwintering colony losses (Wilfert et al. 2016). DWV is a multi-host virus, also widespread among wild bee populations and several other insects (Martin & Brettell 2019). The shared use of flowers is a recognized pathway for disease transmission within and between insect species (Manley et al. 2015, Alger et al. 2019, Dalmon et al. 2021). DWV spillover and prevalence has been identified in several bumblebee species in various geographical areas, including wild and commercial colonies of *B. terrestris* (see Nanetti et al. 2021 for a comprehensive review). Research indicates that DWV decreases longevity in *B. terrestris* individuals (e.g., Graystock et al. 2016).

The occurrence of DWV and other viruses have been widely documented in honeybees in Luxembourg (Clermont et al. 2015). However, except for a first observation of acute symptoms of DWV in a red-tailed bumblebee queen (*Bombus lapidarius*) in Frisange, on the Luxembourg-French border

(Cantú-Salazar 2019), there is currently no published information on the occurrence or prevalence of this virus in wild pollinators in the country or its potential impact on their populations. A case of severe symptoms of DWV is documented here in wild *Bombus terrestris* workers, the first report for this widely distributed species in the country.

2. Materials and methods

The observations reported here were fortuitous. The first author had been regularly visiting a bumblebee nest detected beneath a warehouse in the urban area of Belvaux, in the south of Luxembourg (Lon/Lat WGS84: 5.94374 E | 49.50698 N; Luref: 63748 E | 63607 N). Specimens presenting clearly deformed wings were spotted on the 9th of June 2022 and on the 4th of July 2022. These were collected for microscopic examination, preparation, and deposition in the collection of the National Museum of Natural History Luxembourg (MNHNL). The dry collection of wild bee specimens (including bumblebees) of the MNHNL was examined afterwards to investigate if unpublished records of bumblebees showing acute clinical symptoms associated to DWV had been deposited.

Since workers from the white-tailed bumblebee complex (particularly *B. terrestris* and *B. lucorum*) cannot be reliably separated based on morphological characters (Rasmont et al. 2021), their identities were confirmed using DNA sequencing at the MNHNL. DNA was extracted from one middle leg by an ammonium acetate-based salting-out procedure (Miller et al. 1988) and quantified using a Drop-Sense 16 spectrophotometer (Trinean, Gentbrugge, Belgium). A fragment of the 421bp region within the mitochondrial cytochrome c oxidase subunit I gene (COI) was amplified by polymerase chain reaction (PCR) using the primers BF2 (GCHCCHGAYATRGCHTTYCC) and BR2 (TCDGGRTGNCCRAARAAYCA) (Elbrecht & Leese, 2017). Each PCR contained 12,5 uL Promega Gotaq, 0.5 µM of each primer and 15-30 ng DNA in a final volume of 25uL. PCRs were run in a Mastercycler nexus (Eppendorf, Hamburg, Germany). Post PCR, the samples were handled as described in Christou et al. (2023) and final sequences were quality checked, edited, and trimmed in Geneious R6 version 6.1.8 (Kearse et al. 2012). For DNA-based taxonomic annotation the sequence was blasted against COI reference sequences from the Barcode of Life Data system (BOLD; Ratnasingham & Hebert 2007).

3. Results

The first specimen (MNHNL81755), spotted emerging from the bumblebee nest, presented visible and unmistakable clinical symptoms associated to DWV (described by Genersch et al. 2006, de Miranda & Genersch 2010, de Miranda et al. 2013, Cilia et al. 2021), consisting of crumpled wings and small patches of discolouration (Fig. 1A). The second specimen reported here (MNHNL81775) was collected in the immediacies of the same nest on the 4th of July 2022, also showing visible but asymmetric wings deformity and damage (Fig. 1B). DNA sequencing confirmed that both bumblebee worker specimens were *Bombus terrestris*.

4. Discussion

As in the report of Cantú-Salazar (2019), it was not feasible to immediately deep-freeze the specimens to confirm the presence of the virus using RNA extraction methods. Nevertheless, the near 100% association between wing deformities and significantly elevated virus titers (de Miranda & Genersch 2010), supports the widely accepted understanding that this pathogen is the agent behind the malformed wings observed in emerging bees (Brettell et al. 2017).

Although DWV infections commonly affect *B. terrestris*, symptomatic wild bumblebee individuals with wing deformities are unlikely to be observed, as they are incapable of flying and typically die shortly after emergence (Yang & Cox-Foster 2007). This rarity in observations explains the infrequency of reported cases in wild specimens, such as the one presented here.

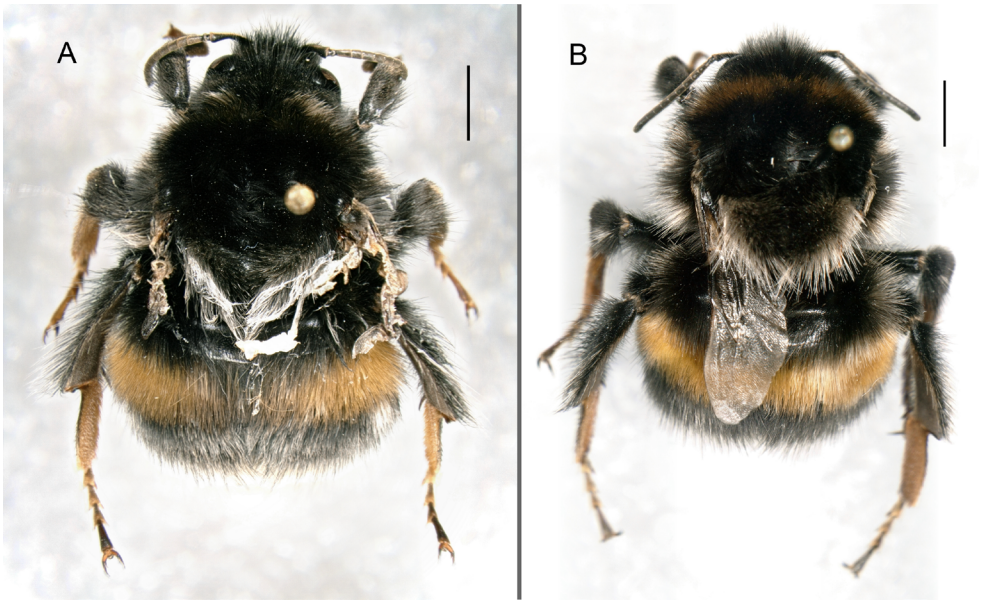


Fig 1. Wild buff-tailed bumblebee specimens (*Bombus terrestris*) collected outside a nest in Belvaux, Luxembourg. A: A specimen exhibiting a pronounced degree of bilateral wing deformity, characteristic clinical symptom related to acute Deformed Wing Virus infections. B: A second specimen showing a lesser degree of DWV symptoms, with unilateral wing deformity. Scale bar: 3 mm. Photos: MNHNL.

As noted in Cantú-Salazar (2019), anomalous wings in wild Hymenoptera in Luxembourg had already been reported. Schneider & Feitz (2003) documented rudimentary wings in two specimens of *Bombus hypnorum* (Linnaeus, 1758) and two of *Megachile willughbiella* (Kirby, 1802) that had fallen from nests situated under roofs, as well as in four cases of the potter wasp *Ancistrocerus nigricornis* (Curtis, 1826) found inside nests, all observed in Luxembourg between 1982 and 1999. While Schneider & Feitz (2003) attributed these observations to extremely low temperatures during insect development, it's also plausible that some cases could have been associated with DWV. Additionally, a third specimen of *B. hypnorum* with abnormal wings, collected by Nico Schneider in 2013, is also housed in the MNHNL collection. Apart from these and the *B. lapidarius* queen documented in Cantú-Salazar (2019), the authors could not locate other wild bees with anomalous wings in the MNHNL collection.

Wild pollinators are inherently exposed to native parasites and pathogens, yet infectious diseases can potentially inflict significant impacts on their populations (Potts et al. 2010, Fürst et al. 2014, Manley et al. 2015, Tehel et al. 2016). These impacts may become particularly pronounced when coupled with additional stressors, such as increased competition (e.g., with managed bees) for food resources and exposure to pesticides or other environmental pollutants (Vanbergen & the Insect Pollinators Initiative 2013, European Commission 2018). Although the generalist bumblebee species *B. terrestris* is globally expanding (Ghisbain et al. 2021), the reduction in Europe's ecological suitability for the other bumblebee species (Ghisbain et al. 2024) is a matter of great concern, as it has far-reaching consequences such as contributing to biodiversity loss and potentially jeopardizing food security.

Whilst DWV has become the most intensively studied insect pathogen globally (Martin & Brettell 2019), comprehensive information on the prevalence and pathogen load of this and other RNA viruses in wild pollinators remains largely insufficient (McMahon et al. 2015). In Luxembourg,

understanding the impact of infectious diseases on wild pollinator populations, and the potential role of managed pollinators in pathogen spillover, is an area that requires further investigation.

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