First record of deformed wings in the red-tailed bumblebee, *Bombus lapidarius* (Linnaeus, 1758), in Luxembourg

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Abstract. A red-tailed bumblebee queen (*Bombus lapidarius* (Linnaeus, 1758)) showing a high degree of wing deformity and discolouration, which are the visible clinical symptoms associated with overt, acute Deformed Wing Virus (DWV) infection, is reported for the first time in Luxembourg. While in Luxembourg the occurrence of DWV and other pathogens have been clearly documented in honey bees, there is no information on their occurrence or prevalence in wild bees or other wild pollinators. Disease distribution and prevalence, pathogen virulence and their impacts as possible drivers of pollinator population decline, need to be addressed as research priorities in Luxembourg.

Key words. *Bombus lapidarius,* deformed wing virus, pathogens, pollinators,

1. Introduction

Pollinators provide a key ecosystem service to agricultural crops and wild plants and their importance for food security is widely acknowledged. The majority of European crops (84%) and of temperate wildflowers (78%) benefit from insect pollinators, in particular bees and hoverflies (Klein et al. 2007, Potts et al. 2015). Besides being directly crucial to the maintenance of plant diversity, pollinators also provide enormous added value by indirectly supporting an immense range of other organisms, from microbes and parasites, to specialist predators, herbivores, fruit- and seed-eating animals, among others (Ollerton 2017). The maintenance of pollinator abundance and diversity is thus critically important for both ecological function and agricultural services. However, evidence of the loss of pollinators is clear-cut: wild pollinators are declining at local, regional, and global scales, in both diversity and abundance and numerous species are threatened with extinction, primarily as consequence of human activities (Biesmeijer et al. 2006, Potts et al. 2010, Lebuhn et al. 2013, Potts et al. 2016, Ollerton 2017). Declines are frequently attributed to multiple interacting causes (Vanbergen et al. 2013), with emerging and re-emerging infectious diseases having the potential of causing profound impacts on both managed and wild pollinators (e.g., Schroeder & Martin 2012, Fürst et al. 2014). This is a serious cause for concern because pollinators are an integral part of healthy ecosystems and their precipitous decline presents a crisis for food security and human wellbeing.

Among the several pathogens that affect bees, recent studies have suggested that Deformed Wing Virus (DWV) is a main long-term stressor, strongly implicated in the loss of millions of Western honey bee colonies (Martin et al. 2012, de Miranda et al. 2013). Detailed reviews of the historical and recent data on DWV and its relatives, including the genetics, pathobiology and transmission of this important pathogen is given by de Miranda & Genersch (2010) and McMenamin & Flenniken (2018). Briefly, overt DWV infections are strongly associated with typical pathological signs or visible clinical symptoms (Lanzi et al. 2006) both in honey bees and bumblebees

(Genersch et al. 2006), which in adults consist of crumpled and/or vestigial wings, sometimes associated with a bloated, shortened abdomen and discolouration (de Miranda & Genersch 2010, de Miranda et al. 2013). Infected adult bees emerging with deformed wings are not viable and die soon after emergence (Yang & Cox-Foster 2007), which explains why observations of bees exhibiting wing deformities, such as those reported here, are uncommon and usually restricted to managed colonies or observed during experimental inoculation studies (e.g., Genersch et al. 2006, Lucia et al. 2014). Such acute and lethal infections have been associated with the viral load being injected directly into the hemolymph of the developing pupae by an arthropod vector, such as the ectoparasitic mite *Varroa destructor* Anderson & Trueman, 2000 (Dainat et al. 2009, Forsgren et al. 2009, Gisder et al. 2009, de Miranda & Genersch 2010, Martin et al. 2012, Wilfert et al. 2016). Usually, though, infections by DWV result in inconspicuous morphological symptoms, especially when the infection occurs in the adult stage or when mites carry only low virus titres (Benaets et al. 2017). Nevertheless, seemingly healthy asymptomatic bees may contain extremely high DWV loads, at levels that would typically be associated with wing deformity (Schroeder & Martin 2012); in these asymptomatic individuals, DWV can affect sensory response, learning and memory in adults (Iqbal & Mueller 2007), causing serious impacts on long-term foraging and survival (McMahon et al. 2016, Benaets et al. 2017).

Due to the close association with varroa infestation, DWV has currently a near global distribution, and is the most widespread of the currently described viruses that infect honey bees (Schroeder & Martin 2012), with Europe likely being an important source of and/or route for the global spread of emerging and re-emerging DWV strains (McMahon et al. 2016). In Luxembourg, the occurrence of DWV and other viruses has been clearly documented in honey bees (Clermont et al. 2015), but there is no information on their occurrence or prevalence in wild bees or other wild pollinators.

2. Observation of deformed wings in a *Bombus lapidarius* specimen in Luxembourg

On the 18th of July 2018, one red-tailed bumblebee queen (*Bombus lapidarius* (Linnaeus, 1758)) was found dead beside the herbaceous strip between the southern limit of the Park and Ride lot at Frisange and the neighbouring agricultural area, on the Luxembourg-French border (Lon/Lat WGS84: 6.19502 E | 49.50642 N; Luref: 81948 E | 63521 N). The specimen had the visible clinical symptoms associated with overt, acute DWV infections in individual bees: a high degree of wing

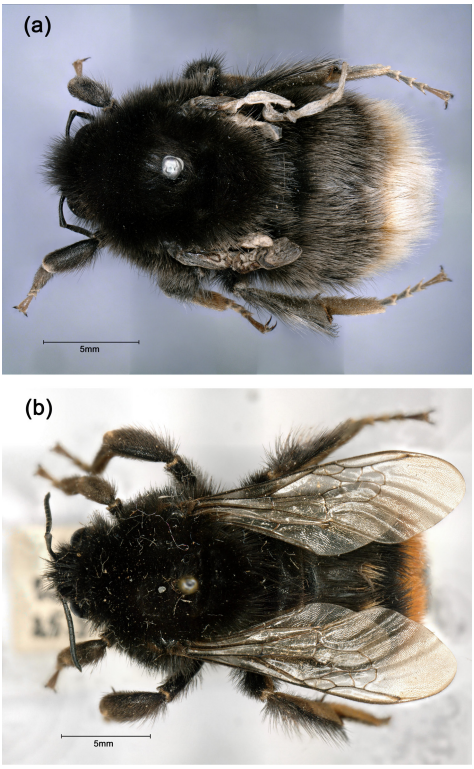


Fig 1. a) Red-tailed bumblebee queen (*Bombus lapidarius*) collected in Frisange, Luxembourg, showing a high degree of wing deformity and discolouration, the typical clinical symptoms associated with overt, acute Deformed Wing Virus infections. b) Asymptomatic *Bombus lapidarius* queen from Harprich (Moselle department, France), showing normal wings and the characteristically black body and orange tail (from the entomological collection of the Natural History Museum of Luxembourg).



Fig. 2. A closer view of the highly deformed wings of the *Bombus lapidarius* queen specimen collected in Frisange, Luxembourg (Fig.1a).

deformity, with both pairs of wings looking like a thread-like structure and discolouration (Figs. 1a & 2). While females of this species are typically distinguished by their black body with a distinctive orange tail (Fig. 1b), this specimen presented a largely discoloured abdomen with a whitish tail. As the individual was found in perfect condition and with its body parts still relaxed, it is likely that it had recently crawled out of the nest and died presumably not far from the nest location. The species identity was confirmed at the Natural History Museum of Luxembourg using DNA barcoding. *B. lapidarius* has a widespread distribution across central Europe and is a dominant crop pollinator, having been ranked as the second top bee species with the highest mean contribution to crop production value at the continental level (Kleijn et al. 2015).

It was not possible to immediately deep-freeze the specimen to test for the presence of the virus using RNA extraction methods. However, while molecular methods are needed for accurate DWV diagnosis in asymptomatic bees, the overt symptoms of DWV are clear and it is currently accepted that this pathogen is the responsible for the malformed wings of emerging bees (Brettell et al. 2017), as several studies have confirmed that wing deformities show a nearly 100% association with highly elevated titers of the virus (de Miranda & Genersch 2010). Nevertheless, the possibility of other pathogens contributing to the visible symptoms at least to a certain extent cannot be totally excluded. Detail studies on virus diagnosis in wild bees and other pollinators in Luxembourg and neighbouring areas should be conducted.

3. Discussion

While this is the first observation of deformed wings in a *Bombus lapidarius* specimen in Luxembourg, it is not the first report of anomalous wings in wild Hymenoptera in Luxembourg. Schneider & Feitz (2003) mentioned four cases of reduced wings in wild bees (two cases in *Bombus hypnorum* (Linnaeus, 1758) and two in *Megachile willoughbiella* (Kirby, 1802)) and four in the potter wasp *Ancistrocerus nigricornis* (Curtis, 1826), all observed in Luxembourg between 1982 and 1999. Even though the authors attributed the observations to extremely low temperatures during the development of the insects, the possibility that some of the cases were related to DWV cannot be excluded.

Viruses originally thought to be honey bee-specific are now known to occur in and infect a wide range of wild bee species, ants and wasps (Evison et al. 2012, Ravoet et al. 2014). DWV has been detected in multiple bumblebee species and is known to replicate in *B. lapidarius*, as well as in *B. huntii* Greene, 1860, *B. impatiens* Cresson, 1863, *B. lucorum* (Linnaeus, 1761), *B. monticola* Smith, 1849 and *B. vagans* Smith, 1854, though symptomatic infection has been described in few species (e.g., *B. terrestris* Linnaeus, 1758 and *B. pascorum* (Scopoli, 1763) – see Manley et al. 2015 and Tehel et al. 2016 for comprehensive reviews). DWV has also been detected in some solitary bee species (Tehel et al. 2016). A possible indirect transmission path of pathogens has been described by means of contact with shared contaminated

flowers (Singh et al. 2010, Mazzei et al 2014), or when bumblebees rob honey from honey bee nests (Genersch et al. 2006, Evison et al. 2012). Moreover, such viruses are not restricted to Hymenoptera: some, including the DWV group of variants, have been detected in a range of insect hosts, such as beetles and hoverflies (e.g., Bailes et al. 2018, Levitt et al. 2013), which are also important providers of pollination services and regularly share flowers with bees.

While wild pollinators are naturally exposed to native parasites and pathogens, strong exposure to non-native species can make them more vulnerable to this stressor, especially when combined with other pressures like poor nutrition, pesticides and other pollutants (European Commission 2018). Indeed, the widespread and on-going spillover of diseases from managed to wild pollinators has been demonstrated and has shown to present a threat to some wild species (Fürst et al. 2014, McMahon et al. 2015). However, detailed knowledge on the prevalence and pathogen load is still lacking for the majority of infectious diseases in wild pollinators. This represents a most important gap in our understanding of the role that such diseases may play in the decline of their populations. In the case of social bees, the production of large numbers of non-viable bees, as well as workers with learning deficits or lost prematurely from the colony, may result in an obvious negative impact on colony fitness, as their input to colony performance is lost or jeopardized (de Miranda & Genersch 2010). For DWV, mounting evidence supports the idea that the virus spills over from Western honey bees to wild bee species, potentially reducing the fitness of individual wild bee hosts (Fürst et al. 2014, Tehel et al. 2016). Disease distribution and prevalence, pathogen virulence and the impacts of disease emergence as a possible driver of population decline, as well as the potential for infectious pathogens to spillover from managed bees to the potentially hundreds or thousands of other pollinator species coming into contact with honey bee viruses, need to be addressed as research priorities in Luxembourg.

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