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Short Communication

Deformed wing virus in western honey bees (*Apis mellifera*) from Atlantic Canada and the first description of an overtly-infected emerging queen

Geoffrey R. Williams [a], Richard E.L. Rogers [b], Abby L. Kalkstein [c], Benjamin A. Taylor [a], Dave Shutler [a,*], Nancy Ostiguy [c]

[a] *Department of Biology, Acadia University, Wolfville, NS, Canada B4P 2R6*
[b] *Wildwood Labs Inc., 53 Blossom Drive, Kentville, NS, Canada B4N 3Z1*
[c] *Department of Entomology, Pennsylvania State University, University Park, 16802 PA, USA*

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A B S T R A C T

Deformed wing virus (DWV) in western honey bees (*Apis mellifera*) often remains asymptomatic in workers and drones, and symptoms have never been described from queens. However, intense infections linked to parasitism by the mite *Varroa destructor* can cause worker wing deformity and death within 67 h of emergence. Ten workers (eight with deformed wings and two with normal wings) and three drones (two with deformed wings and one with normal wings) from two colonies infected with *V. destructor* from Nova Scotia, Canada, and two newly-emerged queens (one with deformed wings and one with normal wings) from two colonies infected with *V. destructor* from Prince Edward Island, Canada, were genetically analyzed for DWV. We detected DWV in all workers and drones, regardless of wing morphology, but only in the deformed-winged queen. This is the first report of DWV from Atlantic Canada and the first detection of a symptomatic queen with DWV from anywhere.

Deformed wing virus (DWV) was initially isolated in Japan from adult western honey bees (*Apis mellifera*) collected from *Varroa destructor*-infested colonies (Bailey and Ball, 1991). DWV has now been detected in other parts of Asia, Europe, Africa, and most recently, in the USA and western Canada (Bailey and Ball, 1991; Chen et al., 2004; Berenyi et al., 2007). DWV has likely been in North America for some time because infections can often be asymptomatic. The virus can be transmitted horizontally during trophallaxis between colony members (Yue and Genersch, 2005) or when *V. destructor* feed on bee hemolymph (Bowen-Walker et al., 1999), and vertically through infected eggs or semen (Chen et al., 2006; Yue et al., 2007). *V. destructor* can also activate virus replication (Shen et al., 2005) and suppress immunity of bee hosts (Yang and Cox-Foster, 2005). Symptomatic (overt) infections of DWV require, but do not necessarily result from, *V. destructor* co-infection (Yang and Cox-Foster, 2005), and can produce wing deformity and other visible morphological abnormalities in workers and drones (e.g. Bowen-Walker et al., 1999; Yue and Genersch, 2005). Because symptomatic individuals live <67 h after emergence (Yang and Cox-Foster, 2007), DWV and *V. destructor* likely act synergistically to negatively affect colony health (Martin, 2001).

DWV has been detected in all life stages of western honey bees (i.e., eggs, larvae, pupae, and adults), and all workers and drones with wing deformities harbor the virus (Chen et al., 2005a). DWV was also the most prevalent (100%) virus in 29 queens sampled from colonies in Maryland and Georgia, USA; however, all queens were asymptomatic (Chen et al., 2005b). To our knowledge, there are no published accounts of DWV-infected queens with symptoms of wing deformity, although Fyg (1964) described wing deformation that he attributed to genetic mutation or chilling during pupal development. Here, for the first time, we report DWV in two provinces of Atlantic Canada, and provide the first description of an infected and overtly-symptomatic queen.

Between June and August 2007, we collected 1331 worker/drone bees from two underbasket dead-bee traps (Accorti et al., 1991) that were checked daily at two colonies in Nova Scotia, Canada (Taylor, 2008). Both colonies had *V. destructor* populations below the recommended treatment threshold (unpubl. data). We also collected two newly-emerged queens from two *V. destructor*-infested commercial operations in Prince Edward Island, Canada, one of which emerged from a *V. destructor*-infested cell and had deformed wings (right fore-wing deformed, but >50% normal size; right hind-wing <50% normal size, but not thread-like) (Fig. 1).

A subset of 10 workers (eight with deformed wings and two with normal wings), three drones (two with deformed wings and one with normal wings), and the two queens underwent genetic analysis for DWV at Pennsylvania State University. We used a

* Corresponding author. Fax: +1 902 585 1059.
E-mail address: dave.shutler@acadiau.ca (D. Shutler).

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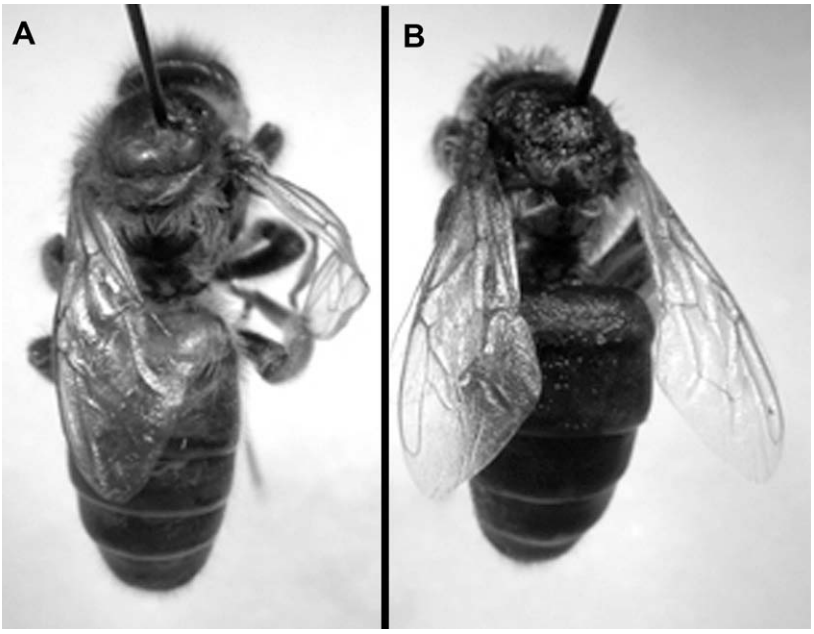

Fig. 1. A newly-emerged honey bee queen with wing deformity that had been exposed to *V. destructor* during development (A) and a newly-emerged normal-winged honey bee queen uninfected with *V. destructor* (B), both collected in July 2007 in Prince Edward Island, Canada.

modified version of the wing deformity ranking scale of Yang and Cox-Foster (2005) to categorize degree of wing deformity for each analyzed bee (Table 1). Using this system, our deformed-winged queen would be ranked 2, our deformed-winged workers (deformed-wing samples 1–3, 5–7, 9, and 10) would be ranked 5, 5, 4, 3, 3, 5, 4, and 5, respectively, and our deformed-winged drones (deformed-wing samples 4 and 8) would be ranked 4 and 3, respectively. All bees were individually frozen upon collection and stored at −80 °C. Just prior to shipping, each bee was thawed and crushed in 1.5 mL microcentrifuge tubes containing 1 mL of RNA*later* (Qiagen). At Pennsylvania State University, total RNA from each sample was extracted using TRIzol (Invitrogen) and resuspended in 20 µl of DEPC-treated water.

To detect DWV, primers were designed (DWV VP1a-F, CTCGTCATTTTGTCCCGACT; DWV VP1a-R, TGCAAAGATGCTGTCA AACC) using the DWV genome as reference (GenBank accession no. NC004830) to amplify 424 bp of the DWV capsid gene and sequenced to confirm specificity (Yang and Cox-Foster, 2005). For an internal control, 514 bp of the honey bee actin gene (primers actin-F, ATGAAGATCCTTACAGAAAG; actin-R, TCTTGTTTAGAGATCCAC AT) were amplified according to Yang and Cox-Foster (2005). cDNA was synthesized using M-MLV reverse transcriptase (Promega) according to manufacturer specifications, and PCR was carried out under the following parameters: an initial denaturing period at 94 °C for 8 min, followed by 35 cycles of 55 s denaturing at 94 °C, 55 s annealing at 51.5 °C, 1 min 25 s extension at 72 °C, and a final extension step for 10 min at 72 °C. A negative control lacking template DNA and a positive cDNA control from a sequenced honey bee sample were included in the PCR reaction. Five µL of each RT-PCR product were electophoresed in a 1.5% agarose gel, stained with SYBR Safe DNA gel stain (Invitrogen), and imaged using a Gel Doc XR (BIO-RAD).

Discounting deformed-wing sample 4 and normal-wing sample 3 because there was no amplification of the honey bee actin gene or DWV (possibly due to a failure during sample storage or preparation), DWV was detected in all 10 workers, the one remaining drone, and in the newly-emerged deformed-winged queen, but not in the newly-emerged normal-winged queen (Fig. 2). These data are consistent with previous work by Chen et al. (2005a) that identified DWV in all symptomatic individuals.

This is the first detection of DWV in bees in Canada outside of the west coast province of British Columbia (Berenyi et al., 2007), although symptomatic bees have become common throughout

Table 1
Proposed modifications to the Yang and Cox-Foster (2005) western honey bee (*Apis mellifera*) deformed wing ranking scale. Added criteria within a rank are in italics.

Rank	Description
0	All wings normal, without any noticeable deformity.
1	Only one tip of fore-wings with noticeable low-degree deformity; remainder of wings normal. *Tip defined as area distal to widest point of wing.*
2	Both fore-wing tips deformed; width of wings (measured at the widest position) slightly narrower than normal wings OR *one fore-wing deformed, but >50% normal wing width.*
3	Both fore-wings deformed, but >50% normal wing width OR *one fore-wing deformed, but <50% normal wing width and not thread-like.*
4	Both fore-wings deformed <50% normal wing width, but not thread-like OR *one fore-wing thread-like.*
5	*Both fore-wings thread-like.*

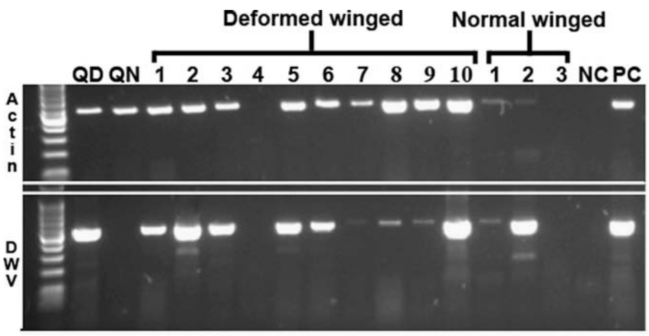

Fig. 2. SYBR Safe DNA stained 1.5% agarose gel of RT-PCR for the honey bee actin gene and deformed wing virus (DWV) in a deformed-winged queen (QD), normal-winged queen (QN), and 10 and three deformed-winged and normal-winged adult bees, respectively. Bands represent successful amplification. Positive (PC) and negative controls (NC) are included.

Canada and the United States in recent years and appear to be associated with *V. destructor* (R.E.L. Rogers and N. Ostiguy, pers. obs.). The most interesting finding is the detection of DWV in a symptomatic queen. Possibly, symptomatic queens have a reduced life expectancy and are less likely to be observed; for example, they may be killed by healthy newly-emerged sister queens before or shortly after emergence or they may have a reduced life expectancy, as demonstrated for workers by Yang and Cox-Foster (2005). Future experiments should investigate brood development of DWV-infected individuals, life expectancy and incidence of covertly- and overtly-infected queens, as well as the possible negative contribution of overtly-infected queens to the high incidence of queenlessness (Rogers and Williams, unpubl.) and colony mortality in recent years.

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