Journal of Apicultural Research 53(4): 466-468 (2014)
DOI 10.3896/IBRA.1.53.4.11

NOTES AND COMMENTS

First detection of deformed wing virus in *Xylocopa augusti* larvae (Hymenoptera: Apidae) in Argentina

Mariano Lucia[1,3,*], Francisco J Reynaldi[2,3] Guillermo H Sguazza[2] and Alberto H Abrahamovich[1] *

[1]División Entomología, Museo de La Plata, Universidad Nacional de La Plata, Paseo del Bosque s/n, 1900FWA, La Plata, Argentina.
[2]Cátedra de Virología. FCV-UNLP, La Plata, Argentina.
[3]CCT- CONICET La Plata., Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

Received 22 August 2013, accepted for publication 27 December 2013.

*Corresponding author: Email: mlucia@fcnym.unlp.edu.ar

Keywords: large carpenter bee, pollinators, South America, viruses, pathogens

Bees play an important role as pollinators of wild and cultivated plants, pollinating approximately 70 % to 80 % of the crops (Nabhan and Buchmann, 1997). The genus *Xylocopa* Latreille (Apidae: Xylocopini) comprises approximately 470 described species which are distributed worldwide, most occurring in tropical and subtropical areas (Michener, 2007). These bees are commonly known as carpenter bees because they build nests in dead wood, hollow internodes of bamboo stems, culms of herbaceous plants and structural timbers (Hurd and Moure, 1963). In general, the internal structure of the nests can be branched or linear, according to the nesting substrate, and with a single entrance connected to a system of tunnels. The brood is reared in individual and isolated cells until adult emergence. The female builds a linear series of cells throughout tunnels, each provisioned with a mixture of pollen and nectar ("bee bread"), then places the egg above the mass and finally, closes the cell with a partition made of a mixture of sawdust and saliva.

Several natural enemies are associated with large carpenter bees, including predators, parasites and parasitoids (Hurd, 1978). However, little is known about pathogens that affect *Xylocopa* species. Singh *et al.* (2010) reported the presence of two virus species affecting adults of *Xylocopa virginica* in the USA. Specimens collected from flowering plants, were positive for deformed wing virus (DWV) and black queen cell virus (BQCV). Here we present evidence of the first detection of DWV in the carpenter bee *Xylocopa augusti* Lepeletier in Argentina.

This virus was originally classified as a Picorna-like insect virus, but nowadays belongs to the genus *Iflavirus,* family *Iflaviridae*, order *Picornavirales.* DWV is currently distributed worldwide in honey bees, and even more, it is well established that millions of honey bee

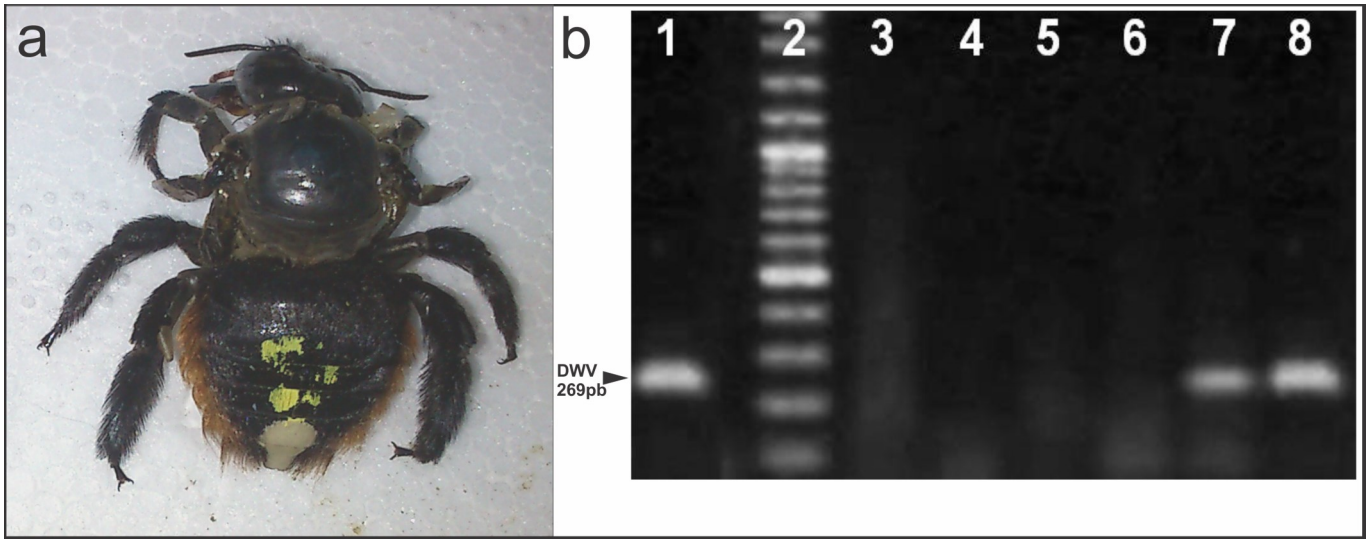

Fig. 1. Adult *X. augusti* with deformed wings and detection of DWV by PCR amplification in larvae. ***a.*** adult bee of *X. augusti* with clinical symptoms of DWV, ***b.*** 2.5 % agarose gel electrophoresis of PCR product of *X. augusti* larval samples, stained with ethidium bromide. Lane 1: DWV positive control; Lane 2: molecular weight marker (100bp); Lane 3: negative amplification control; Lanes 4-6: negative samples; Lanes 7-8: Positive samples.

colonies have been killed due to the global spread of the varroa mite and its inter-action with DWV (Martin *et al.*, 2012; de Miranda *et al.,* 2013). DWV has been frequently detected in other pollinators such as *Bombus terrestris* Linnaeus and *Bombus pascuorum* Scopoli (Genersch *et al*., 2006). Particularly, in South America it was detected in *Bombus atratus* (Reynaldi *et al*., 2013) and honey bees (Antunez *et al*., 2006; Teixeira *et al*., 2008; Reynaldi *et al*., 2010).

The present study was carried out in at the Unidad de Vivero Forestal area near to the experimental apiary of Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Buenos Aires, Argentina (34°54'39"S, 57°55'37"W, 18 m.a.s.l), from September 2012 to April 2013, where species of the genus *Xylocopa* are experimentally bred in artificial nests for biological studies. The artificial nests consist in internodes of canes (*Arundo donax*, Poaceae) closed at one end by the node and open at the other. Ten larvae and ten adults of *X. augusti* were processed in order to detect DWV. Three adult specimens presented deformed wings, a symptom compatible with presence of DWV (Fig. 1a). All samples were stored at -70ºC until they were processed. Briefly, each sample (adult or larvae) was crushed separately in stomacher bags with 1 ml of phosphate-buffered saline and clarified by centrifugation at 1,500 g for 15 min. Total RNA was extracted from 500 µl of the supernatant using 500 µl of trizol (Invitrogen) according to the manufacturer's instructions. The total RNA was resuspended in 50 µl of nuclease-free water. Then, 5 µl (approximately 3 µg) of total RNA was used for synthesis of complementary DNA (cDNA). This reaction was carried out using Moloney Murine Leukemia Virus retro-transcriptase (Promega) and 40 ng of a mixture of random hexamers primers. The PCR reaction was performed in a final volume of 25 µl using 5 µl of cDNA added to a reaction mixture containing 2.5 µl of 10X buffer (75 mM Tris-HCl pH 8.8, 20 mM $(NH_4)_2SO_4$, 0.01% Tween 20), 0.75 µl of 10 mM dNTp mixture, 1.5 µl of $MgCl_2$, 13.75 µl of RNase-free water, 0.5 µl (2.5 U) of Taq DNA polymerase and 0.5 µM of DWV-especific primers previously designed in our laboratory for the detection of this virus in honey bees (Sguazza *et al.,* 2013): DWVf: 5′ TGGTCAATTACAAGCTACTTGG 3′ and DWVr: 5′ TAGTTGGACCAGTAGCACTCAT3′ corresponding to conserved sequences of capsid proteins (VP2-VP1) of DWV. Negative PCR control were prepared by excluding the cDNA from the reaction and positive control were taken from positive samples to these viruses of honey bees detected in this laboratory. The PCR was carried out using a thermal protocol consisting of one denaturalization cycle of 5 min at 95ºC, followed by 40 amplification cycles [30 sec at 95ºC, 30 sec at 53ºC, extension of 60 sec at 72ºC] and a final extension of 5 min at 72ºC. The amplification product was analysed by 2.5% agarose gel electrophoresis and ethidium bromide staining. The PCR products were purified using a gel extraction kit (Wizard® SV Gel & PCR Clean Up, Promega) and sequenced (Biotechnology Resource Center, University of Cornell; Ithaca, USA). The sequences were analysed using the Basic Local Alignment Search Tool (BLAST) software.

Three larvae were positive for DWV by PCR (Fig. 1b) but none of the 10 adults analysed were positive for the virus, not even the three adults with crippled wings. The BLAST results confirmed the identity of the PCR-amplified sequence which showed 99% of homology with the DQ224309 sequence. Since the larva are reared in individual and isolated cells until adult emergence, the only contact with the environment during their development, is when the female provisions them with the bee bread. Due to this, we could hypothesize that either the female picks up viral particles on her body during foraging and introduces them in the nest, or the pollen used to make the bee bread is contaminated. Viral particles of DWV have already been detected in *X. virginica* in the USA and also in pollen, indicating that pollen itself may harbour infectious viruses (Singh *et al.,* 2010). It is known that presence of viral RNA in an individual does not necessarily indicate an active infection, even more DWV presence is strongly associated with typical pathological symptoms (Lanzi *et al.,* 2006). On the other hand, our findings show pathological symptoms without detection of DWV. The failure to detect DWV in adults of *X. augusti* with crippled wings could be due to an acute infection in the larval stage which produces the deformation of the wings, followed by a viral clearance in the adult stage. Further studies are needed in order to understand the possible existence of viral clearance for DWV in *X augusti* adults.

Acknowledgements

We thank the anonymous reviewers for their comments and suggestions which improved this manuscript. A special thanks to personnel of Unidad de Vivero Forestal of Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata for your help; and to Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina (CONICET) for continued support. This study was financed by PICT 2012/Nº 2112 (FONCyT) and PICT 0851 (2012-2014).

References

ANTÚNEZ, K; D´ALESSANDRO, B; CORBELLA, E; RAMALLO, G; ZUNINO, P (2006) Honey bee viruses in Uruguay. *Journal of Invertebrate Pathology,* 93(1): 67-70. http://dx.doi.org/10.1016/j.jip.2006.05.009

DE MIRANDA, J R; BAILEY, L; BALL, B V; BLANCHARD, P; BUDGE, G; CHEJANOVSKY, N; CHEN, Y-P; GAUTHIER, L; GENERSCH, E; DE GRAAF, D; RIBIÈRE, M; RYABOV, E; DE SMET, L VAN DER STEEN, J J M (2013) Standard methods for virus research in *Apis mellifera.* In *V Dietemann; J D Ellis; P Neumann (Eds) The COLOSS* BEEBOOK*, Volume II: standard methods for* Apis mellifera *pest and pathogen research. Journal of Apicultural Research,* 52(4): http://dx.doi.org/10.3896/IBRA.1.52.4.22

GENERSCH, E; YUE, C; FRIES, I; DE MIRANDA, J R (2006) Detection of deformed wing virus, a honey bee viral pathogen, in bumble bees (*Bombus terrestris* and *Bombus pascuorum*) with wing deformities. *Journal of Invertebrate Pathology,* 91: 61–63. http://dx.doi.org/10.1016/j.jip.2005.10.002

LANZI, G; DE MIRANDA, J R; BONIOTTI, M B; CAMERON, C E; LAVAZZA, A; CAPUCCI, L; CAMAZINE, S M; ROSSI, C (2006) Molecular and biological characterization of deformed wing virus of honey bees (*Apis mellifera* L). *Journal of Virology,* 80(10): 4998 -5009. http://dx.doi.org/10.1128/JVI.80.10.4998-5009.2006

MARTÍN-HERNÁNDEZ, R; BOTÍAS, C; BAILÓN, E G; MARTÍNEZ-SALVADOR, A; PRIETO, L; MEANA, A; HIGES, M (2012) Microsporidia infecting *Apis mellifera*: coexistence or competition. Is *Nosema ceranae* replacing *Nosema apis*. *Environmental Microbiology*. 14(8): 2127-2138. http://dx.doi.org/10.1111/j.1462-2920.2011.02645.x

MARTIN, S J; HIGHFIELD, A C; BRETTELL, L; VILLALOBOS, E M; BUDGE, G E; POWELL, M; NIKAIDO, S; SCHROEDER, D C (2012) Global honey bee viral landscape altered by a parasitic mite. *Science,* 336: 1304-1306. http://dx.doi.org/10.1126/science.1220941

HURD, P D JR; MOURE, J L (1963) A classification on the large carpenter bees (Xylocopini) (Hymenoptera: Apoidea). *University of California Publications in Entomology,* 29: 1-365.

HURD, P D (1978) *An annotated catalogue of the carpenter bees (genus Xylocopa Latreille) of the Western Hemisphere*. Smithsonian Institution Press; Washington, DC, USA. 106 pp.

MICHENER, C D (2007) *The bees of the world (2nd Ed.).* Johns Hopkins University Press; Baltimore, USA. 992 pp.

NABHAN, G P; BUCHMANN, S L (1997) Services provided by pollinators. In *D Daily (Ed.) Nature's services: societal dependence on natural ecosystems.* Island Press, Washington, DC, USA. 133-150.

REYNALDI, F J; SGUAZZA, G H; PECORARO, M R; TIZZANO, M A; GALOSI, C M (2010) First report of viral infections that affect Argentinean honey bee. *Environmental Microbiology Reports*. 2 (6): 749-751. http://dx.doi.org/10.1111/j.1758-2229.2010.00173.x

REYNALDI, F J; SGUAZZA, G H; ALBICORO, F J; PECORARO, M R; GALOSI, C M (2013) First molecular detection of co-infection of honey bee viruses in asymptomatic *Bombus atratus* in South America. *Brazilian Journal of Biology.* 73 (4): 797-800. http://dx.doi.org/10.1590/S1519-69842013000400016

SGUAZZA, G H; REYNALDI, F J; GALOSI, C M; PECORARO, M R (2013) Simultaneous detection of bee viruses by multiplex PCR. *Journal of Virological Methods,* 194: 102-106. http://dx.doi.org/10.1016/j.jviromet.2013.08.003

SINGH, R; LEVITT, L A; RAJOTTE, E G; HOLMES, E C; OSTIGUY, N; VANENGELSDORP, D; LIPKIN W I; DEPAMPHILIS, C W; TOTH, A L; COX-FOSTER, D L (2010) RNA viruses in Hymenopteran pollinators: evidence of inter-taxa virus transmission via pollen and potential impact on non-*Apis* Hymenopteran species. *PLoS ONE,* 5(12): e14357. http://dx.doi.org/10.1371/journal.pone.0014357

TEIXEIRA, E W; CHEN, Y; MESSAGE, D; PETTIS, J; EVANS, J D (2008) Virus infection in Brazilian honey bees. *Journal of Invertebrate Pathology,* 99: 117-119. http://dx.doi.org/10.1016/j.jip.2008.03.014