chemistry proceedings

MDPI

Proceeding Paper

Asian Hornet, *Vespa velutina* Lepeletier 1836 (Hym.: Vespidae), Venom Obtention Based on an Electric Stimulation Protocol †

Xesús Feás [1,*], M. Pilar Vázquez-Tato [2] and Julio A. Seijas [2,*]

[1] Academy of Veterinary Sciences of Galicia, Edificio EGAP, Rúa Madrid, No. 2-4, 15707 Santiago de Compostela, (A Coruña), Spain
[2] Departamento de Química Orgánica, Facultad de Ciencias, Universidad of Santiago De Compostela, Alfonso X el Sabio, 27002 Lugo, Spain; pilar.vazquez.tato@usc.es
* Correspondence: xesusfeas@gmail.com (X.F.); julioa.seijas@usc.es (J.A.S.)
† Presented at the 25th International Electronic Conference on Synthetic Organic Chemistry, 15–30 November 2021; Available online: https://ecsoc-25.sciforum.net/.

Abstract: The yellow-legged Asian hornet (AH) (*Vespa velutina* Lepeletier 1836 (Hymenoptera: Vespidae)) is naturally distributed in China, Southeast Asia, and India; however, it has been detected outside of its native area, confirmed as being established in South Korea, Europe, and Japan. Health risks and deaths caused by AH stings have become a public health concern. In the present work, a quick, straightforward, and inexpensive method for obtaining AH venom by electric stimulation is described. The availability of AH venom will lead to improved diagnostic and therapeutic methods, mainly by venom immunotherapy (VIT) in patients allergic to this invasive species.

Keywords: Asian hornet; *Vespa velutina*; venom; electrical; stimulation; allergy; stings; invasive species; venom immunotherapy (VIT); Hymenoptera

Citation: Feás, X.; Vázquez-Tato, M.P.; Seijas, J.A. Asian Hornet, *Vespa velutina* Lepeletier 1836 (Hym.: Vespidae), Venom Obtention Based on an Electric Stimulation Protocol. *Chem. Proc.* **2022**, *8*, 69. https://doi.org/10.3390/ecsoc-25-11723

Academic Editor: Roman Dembinski

Published: 14 November 2021

1. Introduction

Invasive alien species (IAS) are plants, animals, pathogens, and other organisms that are introduced and/or spread outside of their natural geographic range and which may cause severe ecological, economic, and social impacts on the invaded environments.

Several IAS insect species have migrated in the last decade to Galicia, located on the northwestern end of the Iberian Peninsula, and successfully colonized and spread, resulting in a broad range of consequences to recipient ecosystems and, thereby, human society [1]. Of these IAS, the yellow-legged Asian hornet (*Vespa velutina* Lepeletier 1836 (Hymenoptera: Vespidae)) was detected in Galicia in 2012. It was soon recognized as a pan-European threat after being detected in the province of Navarra, northern Spain (2010), in the north-western province of Minho in Portugal (2011), Belgium (2011), Italy (2012), Germany (2014), the Netherlands (2018), Majorca in the Balearic Islands (2015), as well as England and the Channel Islands (2016). The species has become a major concern to apiculture and pollination, given that the diet of these hornet colonies is predominantly based on honeybees and other insects [1,2].

However, *Vespa velutina* is not only a problem for beekeepers and their industrious flying insects, since other agricultural sectors, such as fruit production and viticulture, are also impacted; nevertheless, the medical-veterinary potential of *Vespa velutina* should also be outlined. Due to its habits, abundance, and wider distribution, the risk that the IAS *Vespa velutina* represents for human health is incomparable with other native species of Hymenoptera [3,4]. The medical community is requesting *Vespa velutina* venom extracts to aid diagnosis and treat allergy and/or anaphylaxis through immunotherapy [5]. Venom immunotherapy is the standard of care for people with severe reactions and has been shown to reduce the risk of future anaphylactic events and the risk of death [6–8]. In the present

work, a quick, straightforward, and inexpensive method for obtaining *Vespa velutina* venom by electric stimulation is described.

2. Methods

Adult female *Vespa velutina* specimens were obtained while using an active collecting net method at an apiary (Figure 1a). The field study was conducted during September 2017 in one apiary (43°37′15.2″ N 7°35′26.7″ W) located in San Pedro de Viveiro (municipality of Viveiro), in the Western Mariña in the province of Lugo (Galicia, Spain). An electrical venom collection device (IGK Electronics, Varna, Bulgaria) was used to harvest the insect venom (Figure 1b). Once transported to the lab, the dried venom was removed from the glass plate (Figure 1c). Insects were identified using their external morphological characteristics. In brief, *Vespa velutina* averages about 2 to 3 cm in length, its head is black with an orange face and mouthparts, the antennae are brown dorsally and orange ventrally. The thorax is dark brown, almost black. Metasomal terga are brown, with a thin yellow band on the first segment and a thin orange band on the second and third segments; the fourth metasomal segment is orange; the fifth and sixth metasomal segments are orange-brown. The legs are brown, with yellow tarsi, and the wings are brownish hyaline.

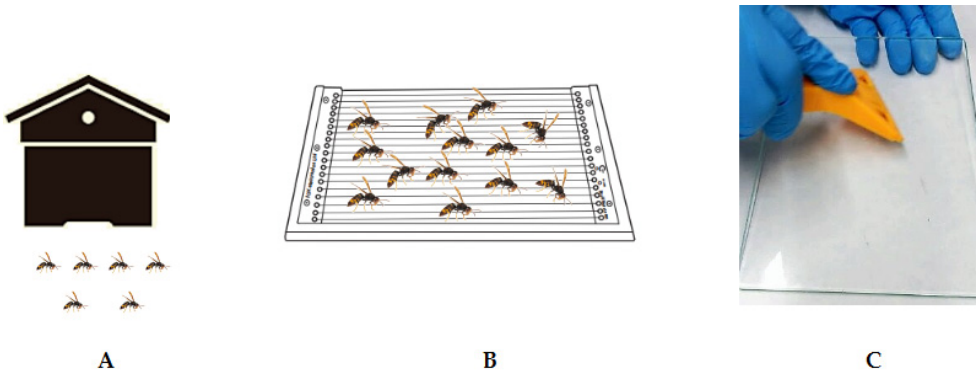

Figure 1. Sequential steps for obtaining *Vespa velutina* venom, based on an electric stimulation protocol: (**A**) Collection of *Vespa velutina* specimens in the apiary; (**B**) *Vespa velutina* electric stimulation; and (**C**) scraping of the *Vespa velutina* venom.

3. Results and Discussion

A prerequisite to studying the nature of the venoms is the development of methods for their collection. The distinctive *Vespa velutina* honeybee-capture behavior results in apiaries representing a suitable place to catch enough live specimens for posterior venom extraction. The target insect is not easily confused with any other hymenopteran species of hornet, bees, or wasps normally present at the apiaries in different areas of Europe, such as the European hornet (*Vespa crabro* Linnaeus, 1758); the Oriental hornet (*Vespa orientalis* Linnaeus 1771); *Bombus* spp., *Vespula* spp. and/or *Polistes* spp. The *Vespa velutina* can be clearly differentiated because of its unique dark color pattern, which is mostly black. Moreover, the *Vespa velutina* has the fourth abdominal segment almost entirely orange-yellow, is smaller than the native European hornet, and possesses yellow tipped legs.

At a lab-scale level, there are well-tested and widely accepted techniques for obtaining venom from several hymenopterans. They are based on dead, frozen insects, where the whole sting apparatus needs to be dissected, using microsurgical forceps, and the obtained venom sacs are basically (i) manually extracted from the separated venom sacs (i.e., by gentle squeezing), or (ii) homogenized and/or just pooled to collect the liquid fraction by centrifugation. The microdissection of the venom reservoir, usually by the precise manipulation of specialized needles, requires a high degree of operator skills. This makes the manual extraction of venom a tedious, laborious, and time-consuming task. In the present work, an electrical venom collection device designed to harvest honeybee venom was used. The device consists of a solid wooden frame with a removable glass plate under

the electrical wires. Once the venom harvesting is complete, the glass plate is removed and scraped with a razor at the lab for *Vespa velutina* venom obtention. Detailed information regarding all aspects required for finding, collecting, and properly handling *Vespa velutina* specimens, as well as apparatus and methods used for venom extraction, is described in [9].

4. Conclusions

The *Vespa velutina* is an invasive alien species of medical importance in regard to the health of members of the public. We described a quick, straightforward, and inexpensive method for obtaining *Vespa velutina* venom, based on an electric stimulation protocol. The availability of *Vespa velutina* venom will lead to improved diagnostic and therapeutic methods, mainly by venom immunotherapy (VIT), in patients allergic to this invasive species.

Author Contributions: Conceptualization: X.F.; methodology: X.F., M.P.V.-T., and J.A.S.; writing—original draft preparation: X.F.; writing—review and editing: X.F., M.P.V.-T., and J.A.S. All authors have read and agreed to the published version of the manuscript.

Funding: The authors thank the Ministerio de Ciencia y Tecnología (Project MAT2017-86109P) for the financial support.

Institutional Review Board Statement: The Asian hornet, *Vespa velutina* Lepeletier 1836 (Hym.: Vespidae) is not a regulated invertebrate. Therefore, no ethical use approval is necessary.

Acknowledgments: X.F. thanks Jose María Vázquez for support when sampling at the apiary and Instituto de Salud Carlos III for a research contract (program number: RD16/0017/001). Thanks are also given to José Amoedo Montes and Carmen Cabadas Amoedo for providing the necessary facilities for this work, in San Amaro (Fornelos de Montes, Galicia).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Feás Sánchez, X.; Charles, R.J. Notes on the Nest Architecture and Colony Composition in Winter of the Yellow-Legged Asian Hornet, *Vespa velutina* Lepeletier 1836 (Hym.: Vespidae), in Its Introduced Habitat in Galicia (NW Spain). *Insects* **2019**, *10*, 237. [CrossRef] [PubMed]
2. Feás, X.; Vázquez-Tato, M.P.; Seijas, J.A.; Pratima, G.; Nikalje, A.; Fraga-López, F. Extraction and Physicochemical Characterization of Chitin Derived from the Asian Hornet, *Vespa velutina* Lepeletier 1836 (Hym.: Vespidae). *Molecules* **2020**, *25*, 384. [CrossRef] [PubMed]
3. Feás, X. Human Fatalities Caused by Hornet, Wasp and Bee Stings in Spain: Epidemiology at State and Sub-State Level from 1999 to 2018. *Biology* **2021**, *10*, 73. [CrossRef]
4. Feás, X.; Vidal, C.; Remesar, S. What We Know about Sting-Related Deaths? Human Fatalities Caused by Hornet, Wasp and Bee Stings in Europe (1994–2016). *Biology* **2022**, *11*, 282. [CrossRef]
5. Chugo, S.; Lizaso, M.T.; Alvarez, M.J.; Arroabaren, E.; Lizarza, S.; Tabar, A.I. *Vespa velutina* nigritorax: A New Causative Agent in Anaphylaxis. *J. Investig. Allergol. Clin. Immunol.* **2015**, *25*, 231–232. [PubMed]
6. Sahiner, U.M.; Durham, S.R. Hymenoptera Venom Allergy: How Does Venom Immunotherapy Prevent Anaphylaxis From Bee and Wasp Stings? *Front. Immunol.* **2019**, *10*, 1959. [CrossRef]
7. Vidal, C.; Armisén, M.; Monsalve, R.; González-Vidal, T.; Lojo, S.; López-Freire, S.; Méndez, P.; Rodríguez, V.; Romero, L.; Galán, A.; et al. Anaphylaxis to *Vespa velutina nigrithorax*: Pattern of sensitization for an emerging problem in Western countries. *J. Investig. Allergol. Clin. Immunol.* **2021**, *31*, 228–235. [CrossRef]
8. Seijas, J.; Feás, X.; Vázquez-Tato, M. Main allergens in European hornet (*Vespa crabo* Linnaeus, 1758) and Asian hornet (*Vespa velutina* Lepetelier, 1836): A comparative study of their structural properties. In Proceedings of the 24th International Electronic Conference on Synthetic Organic Chemistry, Basel, Switzerland, 15 November–15 December 2020. [CrossRef]
9. Feás, X.; Vidal, C.; Vázquez-Tato, M.P.; Seijas, J.A. Asian Hornet, *Vespa velutina* Lepeletier 1836 (Hym.: Vespidae), Venom Obtention Based on an Electric Stimulation Protocol. *Molecules* **2022**, *27*, 138. [CrossRef] [PubMed]