The Asian wasp Vespa velutina nigrithorax: Entomological and allergological characteristics

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Abstract

The yellow-legged or Asian wasp (Vespa velutina nigrithorax) has spread rapidly across Europe since its first introduction in France, in 2004. Originally from South-East Asia, it is considered an invasive species outside its native region. Apart from the ecological and economic implications of its presence, it may cause health problems to humans due to the toxic and allergenic components of its venom. Vespa velutina nigrithorax has become the most prevalent cause of anaphylaxis due to Hymenoptera venom in some regions of Spain. Although sIgE against both antigen 5 (Vesp v 5) and A1-phospholipase (Vesp v 1) has been detected in these patients, only Vesp v 5 may be considered a dominant allergen. Interestingly, Vesp v 1 appears to be a glycosylated allergen different from A1-phospholipases from other species. Inhibition studies suggest that Vespula spp venom could behave as primary sensitizer. Besides, changes in sIgE and sIgG4 during Vespula venom immunotherapy in patients with anaphylaxis due to V. velutina support the use of Vespula venom extracts to treat these patients. The purpose of this review is to explore the biological behaviour of V. velutina and to summarize the current knowledge of the allergic reactions provoked by this wasp.

KEYWORDS allergy, anaphylaxis, Hymenoptera, Pol d 5, Ves v 5, Vesp v 1, Vesp v 5, Vespa velutina nigrithorax

1 INTRODUCTION

The Asian wasp, also known as the Asian predatory wasp or the Asian hornet (Vespa velutina, of the Vespa genus, Vespidae family and Hymenoptera order), was first described in 1836 by the French entomologist Amédée Louis Michel Lepeletier. This species of wasp originated from South-East Asia, in particular the region between Northern India and the Indochinese Peninsula, Taiwan and Indonesia. Due to its extreme success in colonizing new areas, V. velutina is now widespread in other continents and countries where it is considered an invasive species.1–10

Although V. velutina Lepeletier 1836 is a well-defined wasp, up to 12 different colour variants geographically distributed throughout Asia have been identified.11 These colouration patterns and their geographic variations are determined by genes, as have been proven with specific mitochondrial and microsatellite markers; however, the influence of developmental constraints related to crypsis, thermoregulation and aposematism has also been suggested.11 In general, variations in colouration are a genuine visual aposematic signal that species employ to warn potential predators as a defence against being attacked or eaten. The warning signals displayed by wasps include the colour patterns of the various body segments, using light and black stripes on the abdomen, with yellow, red, black and white the most effective colours. The coloration of V. velutina varies among populations from almost entirely yellow or orange to extensively black.12,13
northern Mediterranean coasts having higher risk. The coastal Europe had a high probability of invasion, with the Atlantic and Switzerland and the Netherlands in 2017. In 2011, Villemant sain tree) trade products from China in 2004. The first objec - founder queen hibernating in pottery or other horticultural (bon - have been the result of an accidental importing of at least one

The yellow-legged or Asian wasp (Vespa velutina nigrithorax) is an invasive species in Europe. It may cause health problems to humans due to the toxic and allergic components of its venom. Two allergens have been identified: Vesp v 5 (antigen 5, dominant allergen) and Vesp v 1(A1 phospholipase).

The V. velutina colour form nigrithorax Du Buysson is the Asian wasp variant accidentally introduced into South Korea, Europe and Japan. This variant was first observed outside its native range in 2003 in South Korea. The incursion into Europe appears to have been the result of an accidental importing of at least one founder queen hibernating in pottery or other horticultural (bonsai tree) trade products from China in 2004. The first objective record of the presence of V. velutina nigrithorax in France was in Lot-et-Garonne in 2005. Once in France, V. velutina nigritho - rax took advantage of river valleys and major roads to spread at a rate of 60 km per year, reaching Spain in 2010, Portugal in 2011, Belgium in 2011, Germany in 2014, England in 2016 and Switzerland and the Netherlands in 2017. In 2011, Vilmelant et al. published an interesting model for the future invasion of V. velutina nigrithorax, predicting that many countries of Western Europe had a high probability of invasion, with the Atlantic and northern Mediterranean coasts having higher risk. The coastal areas of the Balkan Peninsula, Turkey and the Near East also appeared suitable and potentially colonized. Other parts of the world that show high climatic suitability for this species might also be potentially threatened by this insect, given that the scenario of entry through international trade could reoccur.

Since its introduction in Europe, V. velutina has been consid - ered a threat to the ecosystem because of its effects on insect pollinators, which most flowering plants require to reproduce, being honeybees one of the main species of pollinators affected. Due to its predation of foraging honeybees, V. velutina has had a serious effect on the beekeeping industry in the invaded countries. The Asian honeybee, Apis cerana, has developed several mechanisms to defend against V. velutina based on changes in olfactory cues. In addition, certain honeybee species have developed a structural weapon against their sympatric predators, the so-called ‘heat-balling’ where hundreds of bees encapsulate the wasp, increasing the temperature and carbon dioxide concentration inside the ball, causing the wasp’s death. In contrast, the European honeybee, Apis mellifera, displays inefficient anti-predator behaviour. Using a laboratory assay to measure V. velutina olfactory attraction, Couto et al. showed that pollen, honey and geraniol (a honeybee aggregation pheromone) were, by far, the most attractive odors for V. velutina. In fact, the strength of olfactory stimuli is greater than that of visual cues for locating food sources; therefore, the strategy of following pollen and honey odours from beehives is more efficient than searching for individual honeybees in flight. Beehives are therefore easy targets for V. velutina, and its attacks can result in a significant reduction in the bee colonies, which in turn has resulted in a demonstrated change in bee colony behaviour, with less time spent feeding on nectar, leading to lower pollen transportation due to the fear of being caught by the wasp.

The ecological and economic implications of the invasion by V. velutina have prompted campaigns to reduce its population and its negative effects in several countries of the European Union. Certain measures have proven successful, such as the policies developed in Great Britain where nests have been rapidly identified thanks to the efforts of the National Bee Unit and Asian Hornet Action Teams, an organization responsible for euthanizing detected nests and making them nonviable, so that adult wasps can no longer live there. Similarly, a strategy based on combining various eradication methods (such as trapping, citizen-collected scientific data for detecting the nests and the active search for nests, followed by their removal using me - chanical methods) has been tested in the Baleares archipelago in the Mediterranean and found to be successful; the last Asian wasp found there was in June 2018. Sugar-based traps have been widely used for attracting social wasps, V. velutina among them; however, the main problem with such traps is that they also trap other species and have a negative impact on native species. In addition, the attractant employed and the season can affect the traps’ effectiveness, making it more difficult to combat this species without damaging other species. New technologies,
such as exposure to heat, are being implemented to destroy *V. velutina* nests. Wasps have been observed to die faster when the temperature was gradually increased than when instantaneously increased. Steam injection could therefore be a promising technique for destroying these nests, given its effectiveness and eco-friendliness. An innovative scanning harmonic radar has been employed in Italy to track the flight of *V. velutina*, a technique that appears to be effective in detecting *V. velutina* nests in open terrain; however, it does not help to detect nests in highly urbanized areas, woodland landscapes or areas with steep slopes. In general, the early detection of *V. velutina* nests is essential due to the nearly impossible task of removing the pest in those countries with widespread and uncontrolled colonization by the wasp.

3 | PHYSICAL APPEARANCE AND LIFE CYCLE OF VESPA VELUTINA NIGRITHORAX

*Vespa velutina nigrithorax* has a black head with an orange-yellow face. The thorax is dark brown, almost black, and the abdominal segments are delimited by a fine yellow band on the dorsal part, being less defined on the ventral part. The fourth segment is almost entirely orange-yellow. Characteristically, the legs are brown with a yellow end, which is why the insect is known as the yellow-legged Asian wasp (Figure 1A). *Vespa velutina nigrithorax* is larger than common wasps but shorter than *Vespa crabro* (European hornet) and *Vespa mandarinia* (Asian giant hornet).

Asian wasps are eusocial insects that live in colonies with a caste-based social system consisting of queens, workers and males. While males are haploids (only one copy of the species’ chromosomes is in their cell nucleus), females are diploids. At the end of the summer, each nest produces several queens who are then mated. Once a queen has mated with one or more males, it stores the sperm cells in a container in its abdomen. At the appropriate time, the queen lays as many fertilized and unfertilized eggs as possible, with a single queen laying thousands of eggs and, consequently, producing thousands of new individuals.

The founding mated queen emerges during the first warm days of spring and builds the primary nest for the incipient colony by collecting wood pulp and leaves, among other materials (Figure 1B). This primary nest is usually small and round and located in a protected place where food is readily available, particularly honeybees because they constitute an indispensable part of the Asian wasp larvae’s diet (Figure 1C). After four to five weeks, the first female workers emerge and help the queen. At this stage, the wasps often abandon the primary nest and build a larger secondary nest on the tops of trees. During the summer, the colony undergoes expansion, with the number of individuals reaching the thousands. New mated queens leave the nest to found another place to start a new colony. Although the abandoned nest is likely to disappear in winter, a recent study showed the presence of living individuals of all castes, as well as broods and eggs, inside the nests. It is therefore recommended to remove secondary nests in winter to get rid of the residual colony due to the possible re-growth during the following spring.
Asian wasps, as with other social Hymenoptera, have glands and a sac with venom in their abdomen. Their venom is a complex mixture of low-weight molecules, peptides and proteins, which can act as pheromones, toxins and allergens. These compounds are synthesized, stored and secreted by the venom gland in response to various stimuli.

### 4.1 Pheromones

Pheromones are volatile substances that wasps use to, among other things, regulate colony behaviour in order to warn their counterparts should the colony be threatened or to mark a place where food is available. Some of these pheromones are released by females to attract male counterparts favouring species-specific reproduction. In general, the pheromones of *V. velutina* workers are chemical molecules, particularly ketones, released through the stinger and are not dangerous for humans per se.

### 4.2 Toxins

Many peptides and proteins have been identified in the venom of *V. velutina*. The transcriptome of *V. velutina* venom was first described by Liu et al. in 2015 and later confirmed by Tan et al. in 2020. The former study identified a total of 293 putative toxin-encoding sequences grouped into eight classes. Of these, the haemostasis-impairing toxins and neurotoxins were the two most dangerous groups of toxins. Haemostasis-impairing toxins belong to two families (A and B in Figure 2). Family A includes nattering-4 (kininogenase activity), metalloprotease, phospholipase A2, vascular-endothelial growth factor toxin (all of them represented in Figure 2) and venom plasminogen activator, snaclec, lectoxin-Enh4 and fibrinogenase brevinase. Family B includes toxins that participate in the blood coagulation cascade (BCC in Figure 2) such as factor V activator, oscutarin-C, ryncolin-3/4, veferolin, coagulation factor, thrombin-like enzyme and venom prothrombin activator. Some of the toxins that participate in the blood coagulation cascade are common to snake venoms but are rarely reported in other Hymenoptera. This distinct haemolytic effect is probably the major lethal factor of the multiple organ failure produced by *V. velutina* stings. In terms of neurotoxins, up to 3 different groups have been identified depending on their mechanism of action, which can induce varying degrees of nerve degeneration and paralysis.

### 4.3 Allergens

The potential allergenic components of *V. velutina nigrithorax* have been purified by chromatography from lyophilized *V. velutina* venom sac extract of individual wasps collected in Europe by Monsalve et al. Taking advantage of the publicly available transcriptomic data previously identified by Liu et al., the coding sequences for *V. velutina* can be extracted for comparison. Using serum samples from patients who had experienced allergic reactions, a number of studies were able to describe the first allergenic component: Vesp v 5, which corresponds to antigen 5 and was named following the recommendations of the World Health Organization and the International Union of Immunological Societies Committee. The degree of similarity with respect to antigen 5 from the common wasp (*Vespula vulgaris*) and the European paper wasp (*Polistes dominula*) is 77% and 75%, respectively. The second allergen to be recognized was A1-phospholipase and was named Vesp v 1. An interesting feature of Vesp v 1 is its glycosylated nature, given that similar A1-phospholipases in the venom of other Hymenoptera are not usually glycosylated. Despite this, the degree of similarity with respect to A1-phospholipase from *Vespula vulgaris* and *P. dominula* is 76% and 71%, respectively. Lastly, 2 hyaluronidase isoforms (Vesp
v 2A and Ves v 2B) have been partially purified and characterized as potential allergens, although specific immunoglobulin (IgE) against these isoforms has not yet been demonstrated. 44

5  CLINICAL TOXICITY OF VESPA VELUTINA VENOM

Vespa velutina is considered one of the most aggressive and feared species of Hymenoptera in China. Reports from China and South Korea show 54 and 49 deaths in a 2-year and 4-year period, respectively in both countries due to wasps. 50 Not to be outdone, the Asian giant hornet (Vespa mandarinia, the largest of all hornets) killed 42 people within a 4-month period in China in 2013. The majority of these deaths were due to the toxic properties of the hornet’s venom and the multiple stings inflicted. 50–54

Renal failure due to acute tubular necrosis in the setting of hae- morrhagic or rhabdomyolysis and due to direct nephrotoxicity is one of the most frequently reported causes of death in these cases. 51,52 Acute renal failure is more common after multiple stings but can also occur in the context of an allergic reaction. Similarly, toxic hepatitis and toxic myocarditis were reported as the most frequently observed clinical complication (46% and 45% of the patients, respectively) in more than 100 patients hospitalized after being attacked by at least 10 individuals of the Vespa mandarinia species in the province of Shaanxi (China). Acute interstitial nephritis, diagnosed in 25% of these patients, was the main cause of death. 55

Lastly, reversible ocular symptoms consisting of unilateral eye redness and pain sometimes associated with rhinitis, blurred vision and periorbital oedema were reported as a consequence of the projection of fluids by V. velutina when it felt threatened. No similar behaviour was observed in other Vespidae species. Although a favourable outcome is the rule in these cases, keratitis and neurogenic pain can present as complications. 56

6  ALLERGIC REACTIONS TO VESPA VELUTINA VENOM

6.1  An increasing problem

The first well-identified case of anaphylaxis due to V. velutina nigritorax in Spain was reported in Navarra (north of Spain) in 2014. 45 The first case of anaphylaxis in the northwest of Spain was detected in June 2015, closely followed by more cases in the subsequent 2 years. After 2018, however, the number of reported cases of anaphylaxis due to this Asian wasp exponentially increased and it currently represents three-quarters of new cases involving Hymenoptera anaphylaxis in this region of Spain. 46,49 Recognizing V. velutina is not difficult for these patients because of the government campaigns to identify this insect and its nests. Most of these patients also live and/or work in a rural environment, and they are used to identifying this wasp. 46

6.2  Specific IgE recognition to other Hymenoptera species in patients with allergy to Vespa velutina

Sensitization appears to have occurred through previous stings from Vespuca spp. or A. mellifera in most of the patients in the study of Vidal et al. 46 given that fewer than 25% of patients with an allergy to V. velutina recalled previous stings from the same insect. Accordingly, studies performed on more than 150 patients with anaphylaxis due to V. velutina have shown a very high predominance of Vespuca spp. allergic sensitization, with 100% of these patients presenting specific IgE (sIgE) against its venom and Ves v 5 the most frequently recognized allergen. 46,49,57 (Figure 3). In terms of the other Hymenoptera species, the sIgE of patients allergic to V. velutina also recognizes Dolichovespula maculata, P. dominula and its main allergen Pol d 5, Vespa crabro and A. mellifera. With respect to A. mellifera, the main allergen recognized in more than 50% of patients is Api m 5, the dipeptidyl peptidase IV, equivalent to Ves v 3 in Vespuca venom. 46,58 (Figure 3). Interestingly, the sensitization pattern among patients with and without previous V. velutina stings is similar, supporting the hypothesis that sensitization came through a different species. 46 In a different setting, Hirata et al. studied 27 Japanese patients who had been stung by Hymenoptera and presented sIgE to hornet venom. The authors did not specify the species responsible for the reaction nor the species of hornet used for the sIgE determination. Some 92.6% of these patients presented sIgE to rVes v 5. and the authors concluded that Ves v 5 allergens are the most potent allergens in hornet venom. 59 These results are not surprising, given that antigen 5 was proven to be a dominant allergen in vespid venoms other than that of V. velutina (not included in previous studies). 60–63 showing a varying degree of sequence homology. 65 The sequence identity described between Ves v 5 and Ves v 1 or V. velutina whole venom before being commercially available. The studies concluded that Ves v 5 is a dominant allergen for these patients because more than 85% of them had sIgE against Ves v 5. 46,49,57 In addition, levels of sIgE were higher against Ves v 5 than against Ves v 1 or V. velutina whole venom (measured by this customized model). Forty per cent of these patients presented sIgE against Ves v 1, which cannot therefore be considered a dominant allergen due to the fact that it did not reach the minimum 50% recognition threshold. 64,65 Since November 2020, a commercial immunoCAP against

6.3  Specific IgE to V. velutina and its allergen molecular components

Taken advantage of the isolation of Ves v 5 and Ves v 1 by Monsalve et al. 44 sIgE against these molecular components was measured after binding them to a high-capacity immunoCAP coupled to streptavidin (o212, Thermo Fisher Scientific) in patients allergic to V. velutina. A similar experiment was performed with V. velutina whole venom before being commercially available. The studies concluded that Ves v 5 is a dominant allergen for these patients because more than 85% of them had sIgE against Ves v 5. 46,49,57 In addition, levels of sIgE were higher against Ves v 5 than against Ves v 1 or V. velutina whole venom (measured by this customized model). Forty per cent of these patients presented sIgE against Ves v 1, which cannot therefore be considered a dominant allergen due to the fact that it did not reach the minimum 50% recognition threshold. 64,65 Since November 2020, a commercial immunoCAP against
V. velutina whole venom (U1223, Thermo Fisher Scientific) has been available for research purposes. Hymenoptera venoms are known to contain cross-reactive carbohydrates (CCDs) that could be involved in sIgE recognition or even provoke cross-reactions and false-positive results in sIgE determinations against whole venoms. However, this problem is solved with the use of most of their molecular components. As stated earlier, Vesp v 1 is a glycosylated A1-phospholipase. Thus, inhibition studies performed in patients with simultaneously positive sIgE to CCDs (MUXF3) and to Vesp v 1 showed extensive reduction (90%) of sIgE reactivity to Vesp v 1. Neither Vesp v 1 nor Vesp v 5 is commercially available at this time for the molecular diagnosis of V. velutina allergy. Possible interference with the sIgE to V. velutina whole venom should therefore be considered in those patients with positive sIgE to CCDs. The lack of Vesp v 1 and Vesp v 5 for diagnosis could be addressed by measuring sIgE to Ves v 1 and Ves v 5, given that a strong correlation has been found between them, and their respective counterparts in V. velutina, Vesp v 1 and Vesp v 5, and linear regression parameters might predict the levels of sIgE to Vesp v 5 given the values of Ves v 5 or even Pol d 5 (Figure 4).

6.4 Biological activity of V. velutina venom by means of basophil activation tests

Basophils are often employed as target cells for ex vivo assays to detect the biologic activity of IgE sensitization. Markers such as CD63 and CD203c can be used to identify activated basophils, with CD63 the best clinically validated test. Basophil reactivity is useful for confirming IgE-mediated activation, and basophil sensitivity is more sensitive for detecting changes over time, as could be the case for allergen immunotherapy. In the study of new allergens, basophil activation tests (BATs) play a definitive role in confirming the allergic nature of the disease. In a group of 10 patients who had experienced V. velutina anaphylaxis, Rodriguez et al. demonstrated positive BAT reactivity against V. velutina whole venom (8/10) and its allergen components Vesp v 1 (5/10) and Vesp v 5 (7/10), all at very low concentrations. Thus, 1 µg/ml for V. velutina, 0.1 ng/ml for Vesp v 5 and 10 ng/ml for Vesp v 1 were enough to activate basophils in these patients. Only one patient showed no positive response against V. velutina or its components; however, a positive BAT was obtained with Vespula spp. venom in this particular patient. In general, despite needing higher concentrations, V. velutina whole venom had better performance than its allergen components. This finding, together with the easier availability of V. velutina whole venom, allows us to propose the use of this venom in clinical settings whenever it is needed to clarify the implication of V. velutina in an allergic reaction, as it has been proposed for other Hymenoptera venoms.

6.5 How patients allergic to V. velutina venom may be treated: the role of Vespula spp as sensitizing agent

The lack of specific tools for treating patients with allergy to V. velutina is a challenge for physicians. The general rule in allergic diseases is to target the genuine or primary sensitizing allergen; molecular diagnosis has helped in most of the cases. Patients with reported V. velutina allergy share a similar sensitization profile, with positive sIgE to Vespula spp. and Ves v 5. A strong correlation between sIgE to Ves v 5 and sIgE to Vesp v 5 has been
demonstrated, and a commercial determination of sIgE to *V. velutina* whole venom is now available. ImmunoCAP inhibition assays have proven useful for identifying genuine sensitizers. Thus, an inhibition assay of IgE reactivity to *V. velutina* whole venom, *Vespula* spp. and the inverted inhibition of *Vespula* spp. showed a lower inhibition activity over *V. velutina* and its allergens. These results suggest that *Vespula* spp. venom is the genuine sensitizer in these cases and support the idea that sensitization could have occurred after having been stung by *Vespula* spp.

According to these previous studies, *Vespula* spp. immunotherapy appears to be a wise option for patients with *V. velutina* allergy. In various settings, immunotherapy with *Vespula* spp. for patients with anaphylaxis to *Vespa orientalis* and *Vespa crabro* has proven to be efficacious. To date, 46 patients allergic to *V. velutina* and treated with monthly 100 µg *Vespula* spp. venom at the Allergy Department of Santiago de Compostela University Hospital in the northwest of Spain showed humoral changes in sIgE and sIgG4 at 6 and 12 months after initiating allergen immunotherapy. Specifically, a significant decrease in sIgE against *V. velutina* was observed in 76.1% of patients after 12 months of allergen immunotherapy, while a significant increase in sIgG4 against *V. velutina* was observed in 80.4% of them after 6 and 12 months. Although the amount of venom that is released during a sting varies in the different species of Hymenoptera, the administered dose of 100 µg seems enough since almost 30% of these patients suffered in field stings of *V. velutina* after a median of 9 months (range, 4–12 months) after starting immunotherapy with no systemic reactions.

7 | CONCLUSIONS

*Vespa velutina nigrithorax* is considered an alien species outside its native range in South-East Asia. Apart from the impact on the ecosystem, it causes relevant economic implications and health problems not only because of its potential toxicity in cases of multiple stings, but also because of severe allergic reactions.
Regarding allergic reactions: a) the main allergen components of *V. velutina* venom have been identified as Vespa v 1 (Phospholipase A1) and Vespa v 5 (Antigen 5); b) Vespa v 5 may be considered the dominant allergen recognized by most of the patients; c) both allergens share a high level of cross-reactivity with their counterparts in *Vespula* spp (Ves v 1 and Ves v 5); d) Vespa v 1 in contrast to other A1-phospholipases is glycosylated; e) even though sensitization could occur through different Hymenoptera species, inhibition studies suggest the role of *Vespula* spp venom as genuine sensitizer; and f) venom immunotherapy with *Vespula* spp venom could be useful in patients with systemic reactions to *V. velutina* venom.

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