PRELIMINARY STUDIES ON THE VENOM OF THE YELLOW-LEGGED HORNET *Vespa velutina nigrithorax*

Pantera B.[1], Tinti L.[2], Salvini L.[2], Pepiciello I.[1], Cervo R.[3], Cappa F.[3], Severino M.[1], Manfredi M.[4], Grossi V.[4], Macchia D.[5], **Francescato E.**[1] * info@entomon.it

[1]Entomon S.a.s., Firenze, Italy; [2]Fondazione Toscana Life Sciences, Siena, Italy ; [3]Dipartimento Biologia - Università Di Firenze, Firenze, Italy; [4]Laboratorio di Immunologia ed Allergologia, Ospedale San Giovanni di Dio, Firenze, Italy; [5]Allergologia Immunologia Clinica, Ospedale San Giovanni di Dio, Firenze, Italy

** In relation to this presentation, I declare the following, real or perceived conflicts of interest: I'm presenting these data in the role of Laboratory Manager of Entomon sas*

BACKGROUND

The Asian hornet *Vespa velutina nigrithorax* (VV) is an alien species in Europe. Since 2012 it was settled in West Italy where researchers of the Biology Dept of the University of Florence collected animals for these preliminary studies. From an allergological point of view it is important to understand even if patients stung from *V. velutina* and showing allergic reaction could be treated with the Hymenoptera commercially available extract *V. crabro* (VC) and *Vespula* spp.(Vspp) or need the specific therapy with VV venom extract.

OBJECTIVE

The aim of this work is to analyze and compare the three venom extracts from VC, VV and Vspp both from a structural point of view, using SDS-PAGE and mass spectrometry (MS), and from an allergological point of view with Immunoblot analysis and *in vitro* CAP-Inhibition of specific IgE for *Vespa crabro* (VCs-IgE).

METHODS AND RESULTS 1

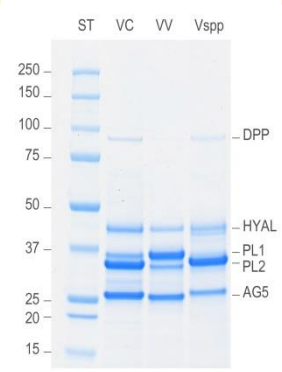

Fig.1 SDS-PAGE of 16µg of VC, VV and Vspp. venom obtained by Capillary Extraction (CEV). Analysis is performed on a 4-12% gradient gel with MES buffer.

Tab.1 Quantitative evaluation of the content of each band is performed using the software CLIQS (TotalLab Ltd.) and BSA standard as quantified control.

Tab. 1	HYAL (ng)	PL1 (ng)	PL2 (ng)	AG5 (ng)
VC	497	**429**	**1435**	1083
VV	207	**1262**	**325**	775
Vspp	323	**n.a.**	**1228**	424

SDS-PAGE comparison between the three samples shows differences between the relative abundance of different allergens, in particular of phospholipase (PL).

METHODS AND RESULTS 2

MS/MS analysis on the three whole venoms confirm the presence of the three major allergens hyaluronidase (HYAL), phospholipase (PL) and antigen5 (AG5) in all the extracts while dipeptidyl peptidase (DPP) is recovered only in the venom of VC and Vspp. Band digestion after SDS-PAGE of VV venom allows to determine the amino acid sequence of fourteen tryptic peptides from VV-PL which show a homology of 55% with VC-PL and of 36% with Vspp-PL. Vspp doesn't contain PL1 band maybe justifying the lower percentage of identity between the homologous protein in VV.

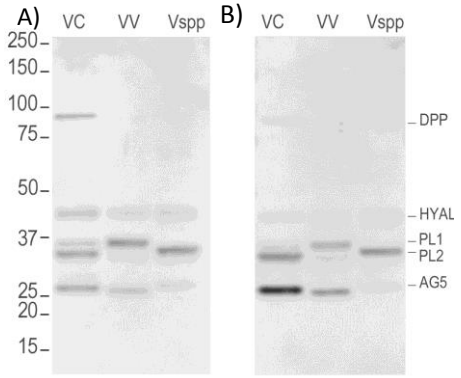

Fig.2 Immunoblot analysis of 4µg of VC, VV and Vspp. venom. Blotted PVDF membranes are incubated with sera from patients (A and B) with serum specific IgE for VC (VCs-IgE) >5kU/L

Immunoblot analysis shows different reactivity of the two sera to the three venom extracts respect to negative control. Serum A, in particular, shows reactivity to DPP in VC but no reactivity in Vspp; both sera A and B show higher reactivity to PL2 in VC and Vspp than in VV venom extract.

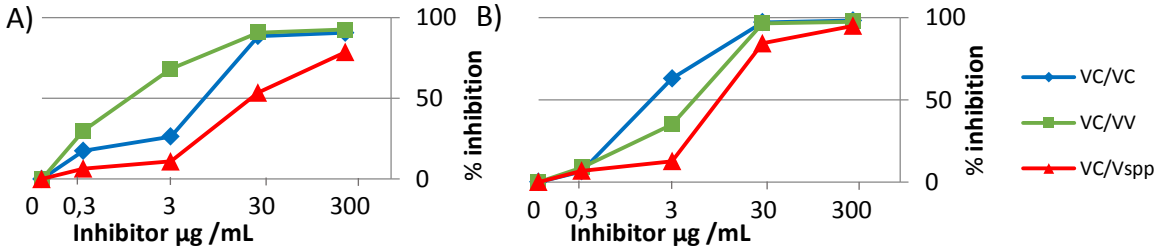

Fig.3 CAP-Inhibition studies (sera A and B): *Vespa crabro* specific IgE (VCs-IgE) inhibited by venom extract of VC, VV and Vspp. VC/VC: VCs-IgE inhibited by VC; VC/VV: VCs-IgE inhibited by VV; VC/Vspp: VCs-IgE inhibited by Vspp.

CAP-Inhibition studies confirm results from sequence analysis and Immunoblot assay: in serum (A) VC and VV venom inhibit VCs-IgE with the same trend, at lower concentration and reach a higher % of inhibition than Vspp; in serum (B), VC , VV and Vspp venom inhibit VCs-IgE with a similar trend but VC and VV start inhibiting at a lower concentration than Vspp.

CONCLUSIONS

The presence of the three major allergens HYAL, PL and AG5 in VV (**Fig.1**) shows homology of this venom extract with the counterparts of the phylogenetically closest European Vespids. In particular the homology of VV-PL with VC-PL, results of Immunoblot (**Fig.2**) and CAP-Inhibition experiments (**Fig.3**) suggest the possibility of treating allergic patient with VC venom extract. However, there are some evident differences that suggest the necessity of the specific venom extract against VV both for diagnostic and therapeutic purpose.