Toxicon, Vol. 24, No. 9, pp. 950–954, 1986.
Printed in Great Britain.

0041-0101/86 $3.00+ .00
Pergamon Journals Ltd.

HORNET VENOMS: LETHALITIES AND LETHAL CAPACITIES

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(*Accepted for publication* 14 *May* 1986)

J. O. SCHMIDT, S. YAMANE, M. MATSUURA and C. K. STARR. Hornet venoms: lethalities and lethal capacities. *Toxicon* **24**, 950–954, 1986.—The i.v. LD_{50} values to mice of pure venoms of *Vespa mandarinia japonica, V. simillima xanthoptera, V. tropica deusta* and *V. l. luctuosa* were, respectively, 4.1, 3.1, 2.8 and 1.6 mg/kg. The LD_{50} value of 1.6 mg/kg distinguishes the venom of *V. luctuosa* as the most lethal known wasp venom. To measure the absolute lethality of a single sting, a new index, called lethal capacity, based on the amount of venom possessed by an individual and its lethality is presented. *V. mandarinia* and *V. tropica* are the most venomous known insects, with a lethal capacity of one sting from *V. mandarinia* delivering an LD_{50} (i.v.) dosage of venom to 270 g of mouse. The lethal capacity for an entire hornet colony, called colony lethal capacity, for *V. tropica* is 84 kg of mouse/colony.

HORNETS in the genus *Vespa* are the largest stinging insects in the world. These often conspicuously colored species attract both lay and scientific interest, are sometimes serious predators of honey bees (MATSUURA and SAKAGAMI, 1973; ISHAY *et al.*, 1967) and their stings can induce allergic reactions in hypersensitive individuals (ORI and HIYAMA, 1977; ABE, 1985; see SCHMIDT, 1986*b*, for general discussion). Extensive literature pertaining to the genus *Vespa* (SPRADBERY, 1973; EDWARDS, 1980; MATSUURA and YAMANE, 1984) and specifically to the four species of *Vespa* used in this study (MATSUURA, 1984; KOJIMA and YAMANE, 1980; KOJIMA, 1982) is available. With a few exceptions (e.g. KAWAI *et al.*, 1980; ABE *et al.*, 1982; ABE and KAWAI, 1983), little is known about the venoms of the hornets of eastern Asia and their venom lethalities are unknown. We report here the results from four diverse East Asian species and determine their capacities as defensive agents.

Worker hornets of *V. mandarinia japonica* Radoszkowski, *V. simillima xanthoptera* Cameron and of *V. tropica deusta* Lepeletier and *V. l. luctuosa* de Saussure were collected, respectively, 11–13 August 1980 in Hitachi Daigo and Mito, Japan, 17 August 1980 in Yuasa, Wakayama Prefecture, Japan, and 1 June 1985 in Inang, Sorsogon Province, Philippines. Immediately upon collection the workers of all species were placed on ice. Venom was extracted from cold-anesthetized live hornets or from live frozen (−10° to −20°C) individuals. All venoms were collected as pure fluids expressed from the sting tip into 5 or 10 μl microcapillary tubes. The microcapillary tube contents were then emptied

TABLE 1. VENOM YIELDS FOR FOUR SPECIES OF HORNETS

Species	Mean workers/ mature colony	Mean μl venom/ individual	% dry wt in liquid venom	Venom dry wt/ worker (mg)
V. mandarinia japonica	250	4.1	26.8	1.10
V. simillima xanthoptera	500	~2	21.2	~.42
V. tropica deusta	300	~3	~25.6	~.77
V. luctuosa luctuosa	200	~1.5	28.8	~.43

into 250 μl polyethylene Eppendorf centrifuge tubes, freeze dried and stored desiccated in the dark at −20°C until used. To obtain values of per cent dry matter in the liquid venom, known quantities of liquid venom were dried and weighed. LD_{50} values at 24 hr to Swiss white mice (six mice per dose) were determined by the method of REED and MUENCH (1938) with 95% confidence intervals calculated by the modification of PIZZI (1950) and means compared by the method described in WOOLF (1968).

When we disturbed the nests of *V. mandarinia*, defending workers flew towards us and when near hovered and made loud clicking sounds with their mandibles. These aposematic clicks were warning threats that usually preceded actual physical attacks. The nest from Hitachi Daigo contained the queen and 51 workers, from which 62 μl clear venom and 8 μl milky venom were obtained. When only those workers with full reservoirs were considered (18), an average yeild of 3.5 μl per worker with a maximum of 7 μl was obtained. The nest of *V. mandarinia* from Mito contained the queen plus 161 workers which could be separated into groups of: (a) 104 good venom-yielding individuals with a mean venom content of 4.7 μl and a maximum of 12.5 μl per individual; (b) 19 failures that for one reason or another yielded no venom; (c) 16 teneral adults that produced no venom; (d) 19 milky venom-yielding individuals with a mean venom content of 4.7 μl per individual; (e) 3 individuals crushed during the collecting. Excluding teneral and destroyed individuals, the overall average was 4.1 μl venom per worker (Table 1).

Venom yields from *V. simillima* were smaller and less consistent than from *V. mandarinia*. The best extraction of *V. simillima* venom was from 21 individuals with a mean of 1.4 μl per individual. This value is undoubtedly low and was probably caused by the hornets spraying venom during the collecting and handling processes. The maximum individual venom yielded for *V. simillima* was 4 μl and we estimate the mean to be about 2 μl.

Actual venom yields per hornet for *V. tropica* were not obtainable, due to the hurried and primitive conditions in the field. In qualitative terms they yielded large amounts of venom and an estimate of 2 − 4 μl per individual would be reasonable. The collection and transport procedures for *V. luctuosa* entirely precluded quantitative venom measurement (85 hornets were required to produce 40 μl venom). Under normal conditions we would expect about 1 − 2 μl venom per individual from these small hornets.

The dry weights of 25 μl fresh venom were 6.69 and 5.31 mg, respectively, for *V. mandarinia* and *V. simillima* and for 20 μl of venom from *V. luctuosa* 5.75 mg. The *V. tropica* were wet when milked and some water was collected with venom from the sting tip, hence the value of 29.0 mg dry weight for 200 μl venom is unrealistically low. In Table 1 the mean value of 25.6 for the three other venoms was used for *V. tropica* to allow estimated calculations of other values.

TABLE 2. LETHALITIES AND LETHAL CAPACITIES OF HORNET VENOMS TO MICE

Species	Injection route	LD_{50} (mg/kg) 24 hr (95% CI)	Lethal capacity (g/sting)*	Colony lethal capacity (kg/colony)†
V. mandarinia japonica	i.v.	4.1 (2.2 – 7.6)	270	68
	i.p.	6.1 (3.4 – 11.2)	180	45
	s.c.	24.8 (14.5 – 43.4)	44	11
V. simillima xanthoptera	i.v.	3.1 (2.0 – 5.0)	135	68
	i.p.	3.8 (2.4 – 6.1)	110	55
V. luctuosa luctuosa	i.v.	1.6 (1.0 – 2.6)	270	54
V. tropica deusta	i.v.	2.8 (2.0 – 3.8)	280	84

*Lethal capacity (LC) = wt (g) of mouse which would receive 1 LD_{50} dosage of venom when stung by one insect.

$$LC = \frac{\text{mg venom/insect}}{LD_{50}\text{ in mg/kg}} \times 1000$$

†Colony lethal capacity = *LC* × number of individuals in an average mature colony.

The lethalities of the four hornet venoms to mice by various routes of injection are listed in Table 2. Mice given twice an i.v. LD_{50} dose of *V. mandarinia* venom typically exhibited profound convulsions and died within a few minutes. Lower doses near the LD_{50} level induced, in order: extreme exophthalmia, convulsions, cyanosis, lethargy, shallow weak breathing and, finally, apneic convulsions and death. Mice given the venom by the i.p. route exhibited an initial withdrawal of the abdominal sides, giving a 'pinched-in' appearance, but otherwise exhibited no strong reactions and often died 6 – 18 hr later in a natural appearing position. All mice injected s.c. with venom from *V. mandarinia* exhibited immediate signs of great pain. Their conditions gradually deteriorated until death, which in all cases occurred in 10 – 19 hr. The other venoms caused similar reactions, except that with venom from *V. luctuosa* several mice injected i.v. passed blood in the urine.

The lethal capacity of each of the venoms is listed in Table 2. Lethal capacity is a measure of the weight of mouse that would receive an LD_{50} dosage of venom if all the venom in an average individual insect were delivered in one sting. The last column in Table 2 lists the colony lethal capacity, i.e. the weight of mouse that would receive an LD_{50} dosage of venom if all the members of the colony were to sting it.

Of all known insect venoms, only harvester ants of the genus *Pogonomyrmex* and a neotropical ponerine ant, *Ectatomma tuberculatum*, possess venoms more lethal than that of *V. luctuosa* (SCHMIDT and BLUM, 1978; SCHMIDT *et al.*, 1980). The other four tested species of *Vespa* (including *V. orientalis*; EDERY *et al.*, 1972) also possess very active venoms that are more lethal than those known from most bees and other wasps (SCHMIDT, 1986*a*).

To place the defensive capacity of hornet venoms in perspective, we can compare their lethal capacities to those of other insect species. Lethal capacity conveniently indicates the number of stings needed for a 50% probability of killing a potential predator of a given size, assuming the animal is as sensitive as mice to the venom. Hornets have the greatest lethal capacities known from insects. *V. mandarinia, V. tropica* and *V. luctuosa* all have i.v. lethal capacities ⩾ 270 g/sting. Although harvester ants possess far more lethal venoms than hornets, they have much less venom per individual and, hence, a lower lethal capacity. For example, the most lethal of these, *P. maricopa* from Willcox, Arizona, U.S.A, exhibits an i.v. LD_{50} of 0.125 mg/kg but produces only 25.0 ± 2.2 μg venom per ant (J. O. Schmidt and P. J. Schmidt, unpublished). Its i.v. lethal capacity is therefore 200 g/ant versus ⩾ 270 g/hornet for the above three species.

Hornets and many other social insects defend their colonies by attacking *en masse*. Therefore, to determine the effect and value of a venom, the combined lethal capacity of all the colony's defenders is important. The estimate, called the colony lethal capacity, is determined by multiplying the number of non-teneral workers in an average mature colony by the individual lethal capacity. By this measure, the colony i.v. lethal capacity for *V. tropica* is about 84 kg/colony. However, the most dangerous (and feared by humans) of all hornets is *V. basalis*, which constructs huge nests that when mature have average worker populations of approximately 5000 (YAMANE, 1977; S. Yamane, unpublished). Assuming these hornets are about the same size as *V. simillima* and have the same venom production and lethality, *V. basalis* has a colony lethal capacity of 675 kg/colony. But even this species may not have the record for greatest colony lethal capacity: *P. maricopa* from Willcox, with nests containing many thousands of individuals (at least 10,000 and probably many times that), probably has the greatest potential lethal capacity of any insect species (10,000 × 0.2 kg/ant = 2000 kg/colony).

Acknowledgments—We thank H. KAWANOBE and T. IKEDA for assistance in collecting *V. m. japonica*, DOMINGO ARCOS SR and other inhabitants of Inang for help in locating and collecting *V. t. deusta* and *V. l. luctuosa* and BILL NUTTING, DAVID BYRNE and FLOYD WERNER for manuscript reviews.

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