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Pollination Ecology of *Vitex negundo* (Verbenaceae)

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At Visakhapatnam (17°42'N-82°18'E) *Vitex negundo* L. flowers twice a year, once during July-November and again during March to May. Auto-, geitono- and xenogamy pollinations produce fruit, but the xenogamy is superior. The flowers are zygomorphic and oblique. They open during 0830-1300 hr and are only visited by diurnally active insects. Some species commenced the activity by 6 AM well before the anthesis and were seen puncturing the buds for nectar collection. A total of 22 species of insects were found foraging at the flowers with *Apis cerana indica, Amegilla, Ceratina* and the wasps *Sphex* sp. *Delta conedus* and *Ropalidia spatulata* as the major pollinators.

Kew Words: Pollination, *Vitex*, Bees, *Apis, Amegilla*

Introduction

Considering the richness of flora and fauna in India the amount of information available on the pollination ecology of Indian plants is meagre. Even the work on economically important plants are not complete. Moreover, the biosphere is facing a serious threat of extinction of many valuable species. Hence, there is a need to understand their reproductive systems.

The present paper deals with the pollination aspects of *Vitex negundo*, a medicinally valuable plant of Verbenaceae, and the results are discussed from an ecological perspective.

Materials and Methods

Observations were made on the natural populations of *V. negundo* growing in vacant lands and along road sides at Chinawaltair (Site I), in the Harbour Park (Site II), Siripuram (Site III) and Research Scholars' Hostel (Site IV), all in Visakhapatnam. Pollen output per anther was determined from 25 flowers distributed over different trees (Subba Reddi & Reddi 1986). Similarly the pollen deposited on the stigmas was assessed at hourly intervals from the time of anther dehiscence (0830 hr) till the end of the day (1800 hr). Stigma receptivity and pollen viability were studied as per the method described by Subba Reddi & Reddi (1984). Nectar produced in flowers protected from insects for 2 hr period was measured using disposable micropipettes. Sugar concentrations were determined with a pocket refractometer and sugar composition by paper chromatography and spectrophotometry (Harborne 1973). Proteins and amino acids were identified by the method of Baker & Baker (1973). The insect visitors of the flowers were collected periodically at different localities and were identified thorough the courtesy of CAB identification

services, Commonwealth Institute of Entomology, London, and Zoological Survey of India, Calcutta. Butterflies caught over the flowers were identified with the help of Wynter-Blyth (1957) and the nomenclature used is after Varshney (1983).

Flower visitors' behaviour, the length of a visit and flower visits in a unit time were studied carefully with the help of a stopwatch. Pollen removed from anthers and pollen depositions on stigmas under foragers' activity were studied at 2 hr intervals. The ability to pick up pollen by each visitor was assessed through body washings by catching the visitor species on the flowers and subsequently washing the pollen off their bodies using alcohol drops on a slide. The pollen grains obtained in the body washings were counted under the microscope after adding aniline blue in lactophenol. Pollen loads on a stigma after the first visit of a particular insect was also observed.

The operation of a particular breeding system was revealed by hand-pollination of 50 flowers and observing them for fruit formation. The extent of fruit set, seed set and fecundity were assessed through observing 500 flowers for fruit development after their pollination.

Results and Discussion

Blooming Phenology

One or the other plant of the species was seen in flower all through the year. However, two flowering seasons: July-November and March-May, could be delineated. The former is associated with the monsoon and the latter with the dry period of the year.

The cymose inflorescence varied in length from 10 to 20 cm and the total number of flowers from 69 to 179 with a life span of 17-32 days. The number of flowers produced for inflorescence in a given day varied from 2-14 ($\bar{x} = 4.7$) as indicated by observations on 10 inflorescences. The distribution of opened flowers over the inflorescence lifetime was left skewed with the curve showing an increase up to day 10 and thereafter a gradual decrease.

Floral Phenology

On fine weather days, flowers opened between 0830-1300 hr with a relatively high frequency at 1100 hr. The temperatures ranged between 28.5 and 31.5°C and RH between 85 and 62%.

Pollen grains are presented after a longitudinal dehiscence of the anthers. They are small (12-17 μm in diam), spheroidal to ellipsoidal, exine smooth, tricolporate, cytoplasm granular. The number of pollen grains per anther ranged from 2280-2900 and averaged 2570. Pollen ovule-ratio came to 2570:1. The grains remained viable for 10 hr after anther dehiscence as determined by fruit formation after hand-pollinations with stored pollen. Fruit set decreased from 80% with 2 hr old pollen to 6% with 10 hr old pollen. Stigma attained receptivity with anthesis and remained receptive up to the 20th hour of anthesis. Fruit set rate decreased from 88% with 2 hr old stigmas to 10% with 20 hr old ones.

Flowers lasted for 24 hr after anthesis as indicated by the corolla drop. Thus they are also available on the second day morning along with the stamens. Sometimes the corolla dropped off even 4-5 hr early due to the disturbances caused by flower visitors, particularly *Graphium agamemnon* and *Xylocopa* sp.

Nectar Dynamics

It was observed that nectar is secreted around the ovary base from the mature bud stage to the time the corolla drops off. The amounts of nectar accumulated every 2 hr from 1000-1800 hr in protected flowers ranged from 0.03 to 1.0 μl. Nectar sugar concentration ranged from 20-25%. It contained glucose, fructose and sucrose, the

former being dominant. Proteins and aminoacids were present, the histidine scale being 3.0.

Flower Visitor Activity Dynamics

Composition and abundance: Altogether 22 species of insects were captured while they were foraging at the flower (table 1). Ten of these fed on pollen and nectar as well, 2 on pollen, and the other 10 on nectar only.

Ten of these 22 species (*Apis cerana indica, Ceratina* sp., *Amegilla* sp., *Rhynchium metallicum, Delta conedus, Sphex* sp., *Ropalidia saptulata, Ropalidia* sp. *Graphium agamemnon* and *Catopsiliea pyranthe*) were encountered at all the 4 study sites. The total number of species caught at each of the study sites was - Chinawaltair site = 20, Research *Scholars'* Hostel site = 13, and Harbour Park and Siripuram site = 11 each. The number of visits made by different insect species at the four study sites are provided in table 2. On the wole the wasps frequented more and shared 43% of the total visits, followed by bees 39.3% and butterflies 17.7%. Among each group of insects, the visits of individual species varied numerically from site to site (figure 1).

Diurnal activity: All the insect species recorded at the flowers are diurnal in their activity. They appeared at the flowers during 0600-1800 hr. Most of the dominant visitors exhibited a period of greater activity: *R. metallicum* 0730-1300 hr, *Ropalidia* sp. 0800-1300hr. *Delta* sp. 0900-1400hr, *Bembix* sp. 0900-1400hr, *Sphex* sp. 0900-1400 hr. *Amegilla* sp. 0800-1500 hr, *A. cerana indica* 0900-1400hr, *Xylocopa* sp., 0900-1300hr and *G. agamemnon* 1000-1400hr.

Insect Behaviour at the Flowers

Rhynchium metallicum and *Ropalidia* sp. were the first to visit the flowers. They appeared at the unopened flowers and punctured the corolla to get at the concealed nectar. The holes were single and ovoid. By the time the other foragers appeard, these species completed boring more than 50% of flower buds. Even the right foragers (foragers having free access to nectar through flower entrance) which included the bees *Amegilla* sp., *A.c. indica, Ceratina* sp., *Campsomeris* sp., *Nomia* sp., *T. histrio* and the wasps *D. conedus, R. spatulata, Bembix* sp. and *Sphex* sp., were seen searching holes at the flower

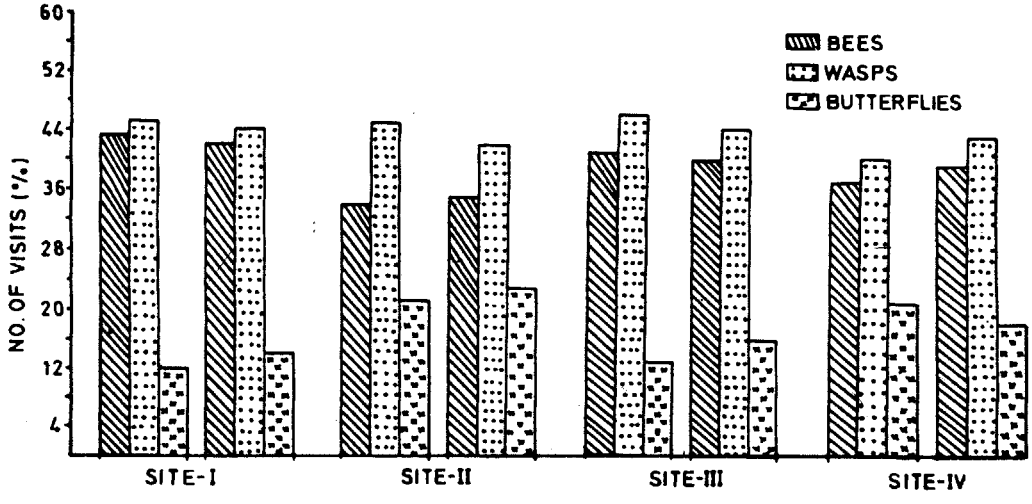

Figure 1 Insect group abundance on *V. negundo* flowers at different study sites

Table 1 *List of flower-visitors of V. negundo, their forage type and body region of pollen deposition*

Visitor species	Forage type		Body region of pollen deposition
	Pollen	Nectar	
HYMENOPTERA			
Apidae			
Apis cerana indica	+	+	*Head, ventral side, Dorsal side*
A. florea	+	+	"
Trigona sp.	+	–	Head, ventral side
Halictidae			
Nomia strigata	+	+	Head, ventral side Dorsal side
Anthophoridae			
Ceratina sp.	+	+	Head, ventral side
Amegilla sp.	+	+	Head, ventral side Dorsal side
Pithitis binghami	+	–	Head, ventral side
Thyreus histrio	+	+	Head, ventral side, Dorsal side
Xylocopidae			
Xylocopa latipes	+	+	"
X. pubescens	+	+	"
Scolidae			
Campsomeris sp.	+	+	"
Megachilidae			
Megachile sp.	+	+	"
Eumenidae			
Rhynchium	–	+	Dorsal side
Sphecidae			
Delta conedus	–	+	"
Bembix sp.	–	+	"
Vespidae			
Sphex sp.	–	+	Dorsal side
Ropalidia spatulata	–	+	"
Ropalidia sp.	–	+	"
LEPIDOPTERA			
Nymphalidae			
Phalanta phalantha	–	+	Proboscis, legs
Papilionidae			
Graphium agamemnon	–	+	"
Pieridae			
Catopsilia c. pomona	–	+	"
Catopsilia pyranthe	–	+	"

bases. When they came across these holes, mostly they ignored the right path and took the nectar through these holes. Out of the total number of visits performed in a period of 6 hr, 459 were to unperforated flowers and 428 to perforated ones. Out of the 428 visits paid to perforated flowers, 379 (ca. 89%) were made through the holes and remaining (ca. 11%) through the legitimate way. Besides foraging on nectar, the bees *Amegilla* sp., *Apis* sp., *Trigona* sp., *Ceratina* sp. occasionally foraged on pollen of perforated flowers.

The large bodied bees *Apis, Xylocopa* and wasps *Bembix, Delta,* and *Sphex* utilised the lower corolla lip as a landing platform and foraged in the legitimate way, then the back of the their head/thorax contacted the essential flower parts and received pollen nototribically. But the bee *Amegilla* never alighted on the flower, it always directly plunged into the corolla tube to get at the nectar. Pollen deposition was nototribic. But while collecting pollen it touched the anthers by its underside. *Apis* sp. also while collecting pollen received the pollen sternotribically. The small bodied bees as *Trigona* and *Ceratina* concentrated on pollen collection. They alighted on the upperside of anther and collected pollen, then their abdominal surfaces get smeared with pollen.

The butterfly *G. agamemnon* fluttered before the flower and inserted its proboscis. The other butterflies settled on the inflorescence and then inserted their proboscides into the flower. As the stamens and style are placed towards the upper lip, contact between butterfly proboscides and the essential organs are unlikely.

Flower visits per unit time and length of a visit: Table 3 gives the data relating to the length of a visit and total number of flowers visited in a minute. *G. agamemnon, Amegilla* sp., *Bembix* sp. *Sphex* sp., *Trigona* sp., and *Ceratina* sp. visited a larger number of

Table 2 *Census of flower visitors on* ***V. negundo*** *in two blooming seasons*

Insect species	Chinawaltair site I				Harbour park site II			Siripuram site III				R S Hostel site IV		
	1986		1987		1986		1987	1986		1987		1986	1987	
	7/10	28/10	25/3	28/4	19/8	3/9	30/4	1/10	24/10	27/3	26/4	22/8	20/8	13/9
BEES														
Apis cerana indica	389	477	437	439	316	346	357	349	391	240	391	205	278	236
A. florea	108	235	160	231	142	88	91	0	0	0	0	0	0	0
Trigona sp.	0	0	0	0	0	108	102	0	110	118	127	0	0	0
Nomia strigata	115	0	0	0	0	128	0	0	0	0	0	0	0	0
Ceratina sp.	124	191	119	175	96	109	103	92	94	92	115	104	105	104
Amegilla sp.	695	793	617	758	770	686	709	542	681	488	675	773	643	788
Pithitis binghami	0	0	0	0	0	0	0	0	0	0	0	97	0	150
Thyreus histrio	0	0	162	130	0	0	0	0	0	0	0	0	0	0
Xylocopa latipes	207	325	0	369	0	0	0	0	150	132	0	0	0	0
X. pubenscens	272	201	0	227	0	0	0	197	171	78	0	0	0	0
Campsomeris sp.	153	324	131	0	0	0	0	0	0	0	0	0	0	0
Megachile sp.	83	143	121	0	0	0	0	0	0	0	0	0	0	0
WASPS														
Rhynchium metallicum	463	656	223	277	603	380	391	289	376	211	396	576	470	584
Delta conedus	424	420	275	343	412	372	324	363	352	283	371	218	238	203
Bembix sp.	347	397	147	0	0	0	0	0	0	0	0	0	0	0
Sphex sp.	495	641	523	563	566	514	471	401	428	279	422	129	226	180
Ropalidia spatulata	234	241	145	216	132	176	186	133	185	210	218	149	232	153
Ropalidia sp.	293	344	240	325	272	262	278	245	271	262	251	193	142	138
BUTTERFLIES														
Phalanta phalantha	0	46	0	0	60	0	0	0	47	0	48	0	0	0
Graphium agamemnon	616	686	602	695	665	692	694	365	406	431	449	577	467	457
Catopsilia C. pomona	0	0	0	0	0	49	83	0	0	0	43	62	0	56
Catopsilia pyranthe	94	0	0	0	118	121	123	0	47	73	70	82	67	65
Total visits	5,112	6,120	3,902	4,748	4,152	4,031	3,912	2,976	3,709	2,788	3,576	3,065	2,868	3,314

flowers and spent comparatively less time on a flower.

Pollen transfer in the first visit of various visitors: Insects with a considerable body size removed a sizeable number of pollen in their first visit. These include *Xylocopa* sp., *Sphex* sp., *Bembix* sp., *Amegilla* sp., and *A.c. indica* (table 4).

Table 3 *Numbers of flowers visited per unit time and length of a visit of foragers on* ***V. negundo***

Name of the visitor	Sample size	No. of flower visits/min		Length of a visit/sec	
		Range	Mean	Range	Mean
Apis cerana indica	10	7-15	9.5	4-9	6.5
Apis florea	10	6-11	8.0	6-10	7.0
Trigona sp.	10	12-35	26.0	2-5	3.0
Amegilla sp.	10	25-32	41.0	1-3	2.0
Ceratina sp.	10	12-32	25.0	3-5	4.0
Xylocopa latipes	10	10-24	19.0	3-6	4.5
X. pubescens	10	12-22	18.0	3-5	4.5
Rhynchium metallicum	10	5-16	11.0	4-12	8.0
Delta conedus	10	16-22	21.0	3-5	3.5
Bembix sp.	10	20-39	30.0	2-3	3.0
Sphex sp.	10	15-33	28.0	2-4	3.0
Ropalidia spatulata	10	10-36	24.0	2-6	4.0
Ropalidia sp.	10	5-12	8.5	5-12	9.0
Graphium agamemnon	10	34-56	54.0	1-2	1.5

Table 4 *Pollen-depletion from anthers vs. pollen deposition on stigmas in first visit of some foragers on* ***V. negundo***

Visitor species	Mean no. of pollen/flower after 1st visit	Pollen depletion (%)	Mean no. of pollen/stigma after 1st visit	Pollen deposition (%)
Apis cerana indica	7120	31	22	8
A. florea	7350	28	15	5
Trigona sp.	8470	17	13	4
Amegilla sp.	6980	32	32	11
Ceratina sp.	7450	27	19	7
Xylocopa latipes	6520	36	54	18
X. pubescens	5780	44	44	16
Delta conedus	8750	15	8	2
Bembix sp.	6840	33	36	12
Sphex sp.	6630	35	43	15
Ropalidia spatulata	8940	13	5	2

Average number of pollen produced per flower = 10,280
No. of flowers sampled = 10

Pollen in body washings of different visitors: The number of grains found on the visitors body also depended on the body size. Thus *Xylocopa* sp. *Sphex* sp., *Bembix* sp., *Amegilla* sp. and *A.c. indica* carried more pollen (table 5).

Pollen depletion from anthers vs. pollen deposition on stigma under foragers activity: Pollen depletion from the anthers and deposition on the stigma corresponded with the visitors activity. These events started at 0900 hr with a low rate and gradually reached a peak at 1100-1300 hr when the flower visitors activity was brisk and then declined (table 6).

Breeding system: Controlled tests revealed the absence of apomixis. Autogamy gave 40% fruit set, geitonogamy 80% and xenogamy 90%. Seed set and fecundity each was 100%.

Effect of corolla perforation: In order to ascertain whether corolla perforation detract the success of pollination, stigmas of 400 perforated flowers were observed for pollen. It was found that 36 of them bore pollen loads which 28 set fruit amounting to 7%.

Natural fruit set: In nature, 58% fruit set was observed. Seed set and fecundity each was 100%.

Table 5 *Pollen amounts in body-washings of some foragers on* ***V. negundo***

Visitor species	No. of pollen	
	Range	Mean
Apis cerana indica	45-82	58.0
A. florea	34-58	43.0
Trigona sp.	19-31	24.0
Amegilla sp.	66-102	77.0
Ceratina sp.	26-51	35.0
Xylocopa latipes	143-207	173.0
X. pubescens	169-226	187.0
Rhynchium metallicum	17-32	21.0
Delta conedus	15-29	19.0
Bembix sp.	84-139	115.0
Sphex sp.	75-118	93.0
Ropalidia spatulata	16-29	19.0
Ropalidia sp.	13-24	16.0
Graphium agamemnon	5-11	7.0

No. of visitor species sampled = 10

Table 6 *Pollen-depletion from anthers vs. pollen-deposition on stigmas of* ***V. negundo*** *under foragers activity*

Time (hr)	No. of pollen grains depleted/ flower	Rate of pollen depletion (%)	No. of pollen deposited/ stigma	Pollen deposition (%)
0900	440	7	14	5
1110	1400	21	62	23
1300	2220	34	89	34
1500	1350	71	64	24
1700	1090	17	37	14

Pollination

The flowers are zygomorphic and oblique, but not perfectly horizontal. The stamens and style are located adjacent to the upper lip but their ends are curved facing the lower lip. The dehiscent side of one lobe faces the upper corolla lip and the other the lower one. The two lobes of the stigma are divergent and both face the lower lip. In effect, the flower is a 'gullet blossom' (Faegri & Pijl 1979) and is primarily adapted for nototribic pollination a device known for its precision and economy in pollen transfer. It is considered that the zygomorphy and the associated nototriby are evolved under selection pressure to increasing adaptation to higher bees (Proctor & Yeo 1972, Fageri & Pijl 1979).

Although the flowers are hermaphrodite and homogamous, spontaneous autogamy is precluded because the stigma is located a little above the anthers and the lobes face the lower corolla lip. When bees such as *Apis* sp., *Amegilla* sp., *Xylocopa* sp. and the wasps

Bembix sp., *Sphex* sp., *Delta* sp. and *Ropalidia spatulata* forage on nectar, they land on the expanded lower corolla lip and insert their probocides slightly pushing their heads in. Then depending on the size of the forager, pollen deposited nototribically on the back of head or thorax; small sized insects do not touch the anthers and stigma. As the flowers are compatible to auto-, geitono- and xeno- gamy, it is likely that any or all of these modes of pollination may takes place under the insect activity at the flowers. It may be noted that out of the three modes of pollination, xenogamy is rather more successful. As already indicated in the results part, *Amegilla* sp., *Apis* sp., *Trigona* sp. *Ceratina* sp., and *Pithitis* sp., foraged on pollen, then they effect sternotribic pollination. Thus *Apis* and *Amegilla* exhibited dual behaviour-pollen deposition is 'nototribic' when they probe for nectar, it is 'sternotribic' when they collect pollen. A pollination mechanism that operates in these two ways was also reported in *Pedicularis sudentica* by Macior (1973). Also a situation where the same pollinator exhibits two distinctly different behaviour patterns on the same floral mechanism has also been reported in *Pedicularis grayii* by Macior (1973), in *Jatropha gossypiifolia* by Reddi & Subba Reddi (1983) and in *Anisomeles malabarica* by Solomon Raju (1986).

Among the 22 flower visitors, the bees such as *A.c. indica, Ceratina* sp., *Amegilla* sp. and the wasps *Sphex* sp., *Delta conedus* and *Ropalidia spatulata* are the major pollinators (Baker et al. 1971). The others are minor pollinators except butterflies. These lepidopterans are unlikely to make contact with the essential parts because of the wide spacing between the position of the anthers and stigma from the lower corolla lip, hence they are to be treated as flower-visitors in this species. However, their nectar harvesting may indirectly cause the bees and wasps to make repeated visits to more number of flowers to get satiated quantity of nectar and/or pollen (Cruden 1976).

Of the minor pollinators, the wasp, *Bembix* sp. and carpenter bees *Xylocopa latipes* and *X. pubescens*, because of their body size, are highly effective in bringing about nototribic pollination. Because of their limited number of visits, they are considered as minor pollinators. In habitats where these insects abound, they assume a major role in the pollination of *V. negundo*.

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