

Reproductive ecology of nutritionally important perennial climber *Canavalia gladiata* (Fabaceae: Faboideae)

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Abstract. *Canavalia gladiata* is a fast-growing perennial climber, which blooms throughout the year, with prominent flowering and fruiting during the rainy season. At the base of leaf petiole and flower pedicel, it produces button-shaped extra-floral nectaries, with minute volume of nectar with low sugar concentration. Ants feeding on these nectaries protect the plant from herbivores during leaf flushing, flowering and fruiting. The flowers' resupinate monomorphic, is an explosive pollination mechanism adapted for tripping and nototribic pollination by the large-bodied Carpenter Bees of genus *Xylocopa*. The plant is protandrous but unfunctional, because the stigmatic surface is ruptured by the nectar-seeking Carpenter Bees to enable pollen germination and, hence, pollination is obligately pollinator-dependent. The plant is facultatively xenogamous but in open pollinations it is able to set a low fruit-set rate compensated by a high seed set. Its year-long prolific flowering ensures constant production of seeds. Seed dispersal occurs by explosive pod dehiscence and gravity and the dry season is ideal for that event, while in the rainy season seed germination and new plant production take place.

Key words: ant activity, Carpenter Bees, extra-floral nectaries, pollination, protandry, seed dispersal, xenogamy

Introduction

In the *Faboideae* subfamily of the *Fabaceae* family, *Canavalia* is one of the genera with about 50 species in tropical and subtropical parts of the Old World and New World and the Islands of the West Indies and South Pacific. These species inhabit sandy and rocky coastal seashores, woodlands, and thickets (Sauer 1964; Allen & Allen 1981). Their name is derived from the Malabar word *kavavali*, which means *forest climber* (Austin 2004). In most countries where the *Canavalia* species are distributed, local people are unaware of their importance as nutritious crops and the species are gradually becoming extinct in many regions (Sauer & Kaplan 1969). In this genus, such species like *C. gladiata*, *C. virosa* and *C. ensiformis* are reported as useful sources of human food, forage, cover crop, green compost,

soil erosion controllers, and phytochemical and pharmaceutical products (Arun & al. 2003; Eknayake & al. 1999; Molina & Bressani 1974; Nanda & al. 1993). Furthermore, all species of this genus are useful producers of pest and disease-resistant and extremely environment-tolerant varieties (Sahai 2009).

Sauer (1964) claimed that available information on the pollination ecology of *Canavalia* was inadequate, despite its importance in the restoration and expansion of populations of its species. He maintained that *Canavalia* species, with monotonous corolla and broad flowering schedules, had a slim possibility for adaptation to specific pollinators and that pollination was similar in most species. Watmough (1974) noted that the floral mechanism in genus *Canavalia* is adapted to operation by Carpenter Bees, with pollination as end result. Verdcourt (1971) and Westphal (1974)

provided a general account of the floral aspects of *C. maritima* and *C. ensiformis*. Different authors reported that *C. maritima* is pollinated by Carpenter Bees (*Xylocopa violaceae* and *X. aestuans*), Digger Bees (*Anthophora* sp.) and butterflies (unlisted) (Scott-Elliott 1891; Piper & al. 1922; Vogel 1954). In South Africa, *C. virosa* was reported as pollinated by Carpenter Bees (*Xylocopa flavorufa*) and Leafcutter Bees (*Megachile combusta*).

Carpenter Bees act as natural pollinators, while Leafcutter Bees as opportunistic pollinators. Furthermore, the honey bee, *Apis mellifera* and other bees also visit the flowers but they are not pollinators (Stirton 1977). *C. reflexiflora* is probably indicative of ornithophily (Snak & al. 2015).

Canavalia gladiata is believed to have originated in the Asian continent and spread throughout the tropics. It is now cultivated on a limited scale across Asia, the West Indies, Africa, and South America. Recently, it has been introduced for cultivation as an important pulse crop into the tropical parts of Australia (Herklots 1972; Kay 1979). In ancient India, people used the seeds of this plant for food. Now the seeds are consumed by the urbanized populations for their high protein content (Vishnu Mittra 1981) and, potentially, it is an important food source (Ekanayake & al. 2000).

Sahai (2009) reported *C. gladiata* as largely outcrossing or self-incompatible species. The flowers are visited by a wide range of insects, mostly moths, butterflies, ants, and honeybees. He also mentioned that pollination rate is high in the absence of foraging ants for extra-floral and floral nectar. Ma et al. (2017) reported that the nectar of *C. gladiata* manifests a scant defense mechanism. Although many insects visit the flowers, only the long-tongued butterflies and moths reach the nectar, due to its deep seating at the base of the corolla. Ants feeding on the floral and extra-floral nectar may probably benefit the plant by driving away herbivores. Visiting bees apparently cannot access the floral nectar and gather pollen instead. In view of these conflicting reports and incomplete details regarding the reproductive ecology of *C. gladiata* despite its value as a potential source of food and fodder, the present study is intended to provide information on all aspects of its reproductive ecology. Such information can be used in the restoration of its populations in the natural areas and for cultivation of the species in suitable spots, where cultivation of traditional crops is impossible.

Material and methods

Canavalia gladiata growing in wild patches still existing in the Andhra University Campus (17°42'N Latitude and 82°18'E Longitude) was selected for study in the period of April 2018 – September 2019. Regular visits to the plant site were made to record the phenological events: leaf flushing, flowering and fruiting. Parts of the leaves and inflorescence were closely observed for the presence of extra-floral nectaries. After confirmation of their presence at the base of leaf petiole and flower pedicel, their function, nectar volume and sugar concentration was recorded. After reporting ants as resident foragers, their role in the protection of leaf, flowers and fruits against herbivores was noted down during their foraging activity at these extra-floral nectaries.

Ten inflorescences about to start flowering on five plants were tagged and the processes followed, in order to record the flower-opening schedule and the timing and mode of anther dehiscence. Anther dehiscence timing was confirmed by observing the anthers under a 10× hand lens. Twenty fresh flowers were used to record the morphological aspects of the flower, flower type, sex, shape, colour, odour, symmetry, calyx, corolla, stamens, ovary, style, and stigma. Floral configuration and reward presentation aspects were observed in relation to probing and forage collection by the insects. In order to measure the nectar volume and sugar concentration according to Dafni & al. (2005), fifty mature buds, ten on each of five plants, were bagged and tagged. The nectar volume and sugar concentration were measured in the period after anthesis for two consecutive days, in order to note the nectar removal rates and to record the changes in nectar sugar concentration. A micropipette was inserted into the flower base to extract nectar for measurement. The average amount of nectar of five flowers was taken as total volume of nectar/flower and expressed in µl. Hand Sugar Refractometer (Erma, Japan) was used for the purpose. Ten mature undehisced anthers were collected from five plants and placed in a Petri dish. Subsequently, one at a time, a single anther was taken out and placed on a clean slide (75×25 mm) and dabbed with a needle in a drop of lactophenol-aniline blue. The anther tissue was then observed under the microscope for pollen. The pollen mass was drawn into a band, and the total number of pollen grains was counted un-

der a compound microscope (40× objective, 10× eye piece). This procedure was followed for counting the number of pollen grains in each collected anther. On the basis of these counts, the mean number of pollen grains produced per anther was determined. The mean pollen output per anther was multiplied by the number of anthers in the flower for obtaining the mean number of pollen grains per flower. The same pollen grains were examined under the microscope for recording the pollen grain features. Twenty ovaries, five from four plants each, were examined under the microscope in order to record the range and average number of ovules per flower. The pollen-ovule ratio was determined by dividing the average number of pollen grains per flower by the average number of ovules per flower. The obtained value was regarded as the pollen-ovule ratio (Cruden 1977). *In vitro* pollen viability tests were made on selected intervals at the day of anthesis, using a 15% sucrose solution showing the maximum pollen germination. The stigma receptivity was observed visually and by H₂O₂ test. In the visual method, the physical state of the stigma (wet or dry) was considered in order to record the commencement of receptivity; its colour change and withering was regarded as loss of receptivity. The H₂O₂ test, as given in Dafni & al., (2005) was followed for confirmation of the stigma receptivity period. Observations of pollen germination on the stigmatic surface were made during the period of mature bud and after flower-tripping by foraging insects to record the functionality of protandry and demand of insects for flower-tripping for stigma receptivity by way of scratches and rupture on its surface. Breeding systems were assessed on the basis of results from hand-pollination tests. The number of flowers used for each mode of pollination and for open pollination for the fruit and seed sets was recorded in Table 3. The flowers were bagged without hand pollination for spontaneous autogamy. For manipulated autogamy, the stigmas were slightly ruptured and pollinated with the pollen of the same flower manually: with the pollen of different flowers of the same plant after emasculation for geitonogamy, and with the pollen from flowers of other plants after emasculation for xenogamy. All these modes of pollination were followed for two months to record the percentage of fruit and seed set rates in each mode. One inflorescence each from ten plants was tagged prior to the initiation of flowering and followed to record

fruit and seed set rate in open pollinations. Fifty inflorescences on ten plants were tagged and followed for recording the flowers that produce fruits, according to their position on the inflorescence, and the maximum number of fruits produced per inflorescence. Fruit maturation period, fruit and seed characteristics were also recorded. Field observations were made regularly to record the fruit and seed dispersal mode. Casual observations were also made to record whether the seeds germinated immediately after they were dispersed or not. Seventy seeds fallen in the vicinity of the parental plants were kept under observation for their germination in July and August 2018 and another set of forty seeds fallen in February were kept under observation for their germination in late February and March 2019. On the basis of the number of germinated seeds, seed germination percentage was calculated to indicate the ideal season for production of new plants.

The floral mechanism in papilionaceous flowers was examined with reference to petal configuration, placement and position of sex organs during bud and flower life, and the movement of petals and release of sex organs during flower probing for nectar. Examined were also the pollen-seeking insect foragers, in order to define the functioning pollination mechanism in the plant. On four different days, insects foraging on the flowers were observed throughout the day for their mode of approach, landing, probing behavior, and contact with the floral sexual organs. Thrips and bees were identified from the available representative specimens at the Department of Environmental Sciences, Andhra University, Visakhapatnam. On four different days, the foraging visits of insects were recorded on a 1×1 m flowering patch for 10 min each hour for the entire day, and the data were tabulated to record the foraging pattern and the percentage of visits made by different insect categories. The pollen/nectar collection behaviour of thrips and bees was closely observed to assess their role in effecting pollination. Ten specimens of each bee species were captured during the time period 08:00–14:00 h and brought to the laboratory. Each specimen was washed out in ethyl alcohol, stained with aniline-blue on a glass slide and observed under microscope to count the number of present pollen grains. Thus, the average number of pollen grains carried by each bee species was calculated to determine the pollen carryover efficiency.

Results

The Plant and Flowering aspects: *Canavalia gladiata* is a fast-growing, vigorous, deep-rooted perennial climber, with glabrous, slightly woody stems and branchlets (Fig. 1a). It scrambles over the ground in areas, where supporting plants are unavailable, and twines, if supporting plants are available. Leaves are pinnately trifoliate, with large, pubescent, apically short-pointed acuminate leaflets. Flowering occurs throughout the year in wet to semi-wet soils, and during the rainy season from July to November in semi-wet to semi-dry soils. Inflorescence is a pedunculate (12–27 cm long) stalked terminal raceme, with 20.92 ± 4.3 flowers, borne singly and arranged laxly in succession (Fig. 1b–d). The flowers open in acropetal manner from base to apex of the raceme over a period of 8–10 days, but only 60% of the total buds produce flowers, while the remaining fall off as immature buds. The flowers stand out prominently above and against the foliage.

Flower morphology: The flowers are short-stalked, bluish-purple, odourless, papilionaceous, 36.5 ± 2.4 mm long, 30.1 ± 1.6 mm wide, bisexual, and zygomorphic. Calyx is green, bi-lipped, campanulate, 5-lobed, 14.9 ± 2.2 mm long; upper lip is 2-lobed, while lower lip is 3-lobed and the lobes of both lips are ovate and acute. Corolla is bluish-purple, 3.4 to 4.1 cm long, and displays standard petals at the bottom and wing, and keel petals at the top; this form of petal position outlines the resupinate flow-

er (Fig. 1e). The standard petal is orbicular, emarginated, with two small-lunar processes at the base. The wing petals are oblong-spatulate, slightly curved, longitudinally bulged along the upper surfaces, and auricled at the base. Two pin-like callosities situated at the base of the standard petal rest on the portion of wing tissue between bulges and auricles of the wings. The wing auricles articulated with the standard petal allow the wing and keel petals to return to their original position, when the standard petal is depressed by the visiting insect. Wing petals enclose keel petals, which in turn enclose loosely the stigma and stamens. The stamens are 10 (6 long and 4 short), almost monadelphous, with nine totally connate from the base and one vexillar stamen free at the base and connate with all other stamens from the middle point, all slightly curved terminally. The anthers are uniform in size and shape, 2–3 mm long, dithecous and dorsifixed. The ovary is sessile, green, hairy, 19.2 ± 1.1 mm long, monocarpellary, monolocular, with 8–12 anatropous ovules arranged on marginal placentation. The style and stigma are creamy-white and 13.5 ± 1.1 mm long; the stigma is capitate, incurved within keel and situated at the height between the long and short stamens.

Floral biology: Mature buds opened from 06:00 to 08:00 h. Individual buds opened slowly by unfolding the standard petal, a process, which took about 40 minutes. The stigma and stamens remained enclosed in the keel petals, which, in turn, were covered by wing petals. In mature buds, all anthers dehiscenced synchronously by

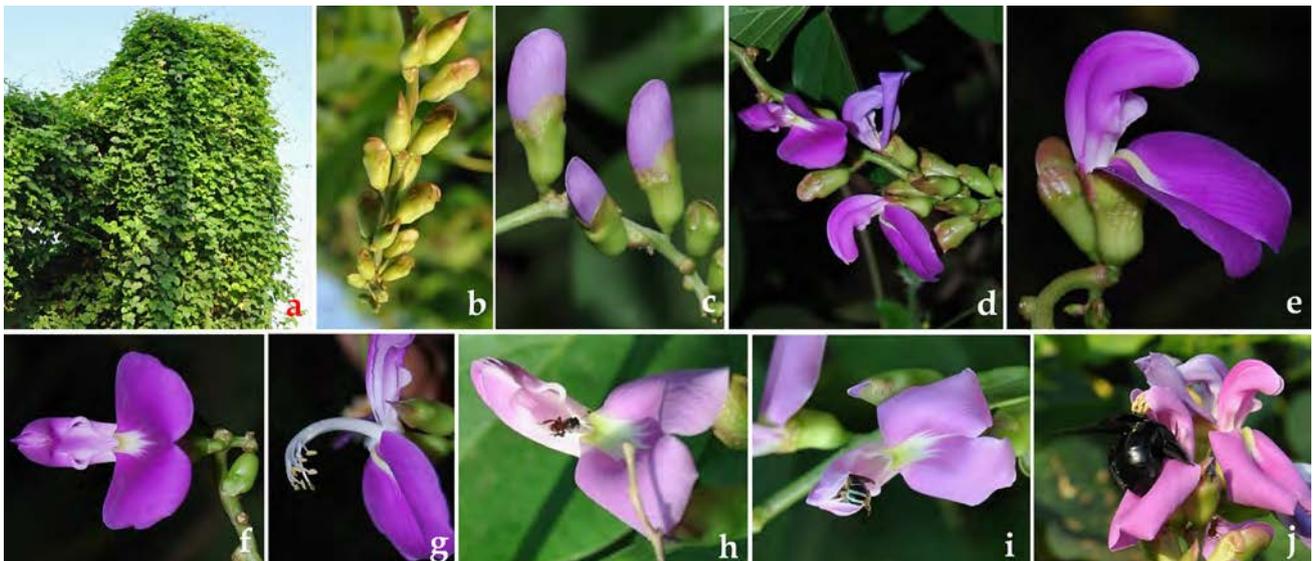


Fig. 1. *Canavalia gladiata*: a. Habit, b. Inflorescence with immature buds, c. Inflorescence with mature buds, d. Flowering inflorescence, e. Resupinate flower (corolla), f. Flower with un-tripped keel and wing petals, g. Flower with tripped keel and wing petals and staminal column with stamens and stigma, h. *Trigona iridipennis* making unsuccessful attempt to probe un-tripped keel petals for nectar, i. *Nomia* sp. collecting pollen from un-tripped keel petals, j. *Xylocopa latipes* collecting nectar from un-tripped flowers during which tripped keel petals violently release the stamens and stigma which in turn are brushing against the thorax of the bee.

longitudinal slits, about 40 min prior to their opening. The pollen grains were creamy-white, powdery, $63.53 \pm 5.81 \mu\text{m}$ in size, endocolpium $8\text{--}9 \mu\text{m}$, and exine $3\text{--}5 \mu\text{m}$ thick. They were spherical prior to anther dehiscence, and triangular and tricolporate after anther dehiscence. The pollen output was 1592.2 ± 98.82 and $15\ 922$ per flower. The pollen-ovule ratio was 1592:1. The pollen grains were viable for five hours from the time of anther dehiscence and shriveled gradually thereafter. Pollen viability was the highest during/after anthesis, gradually decreased and reached its lowest by 15:30 h, after which the pollen grains did not show any germination (Table 1). The stigmas collected after anther dehiscence, before and after flower opening from bagged buds/flowers showed that the pollen of the same flower did not germinate on the surface of the stigmas. That indicated that protandry was not functional for autogamy. In flowers that were visited by Carpenter Bees, the stigma surface had either scratches or rupture and was also dusted with pollen. When these stigmas were observed under the microscope, the pollen was found germinating, which indicated that self-incompatibility was functional. The stigma attained receptivity shortly before flower opening and remained receptive until noon of the following day. Gradually, the stigma turned light-to dark-brown and withered. Slowly, from the morning of the next day, the corolla and stamens showed signs of loss of vigour and colour and fell off by the evening of that day from the pollinated flowers. The entire flower, along with the pedicel, fell off by the evening of the 2nd day, if not pollinated.

Nectar secretion was initiated during the mature bud stage and continued one hour after anthesis. On the day of anthesis, the total mean volume of nectar registered was $32.5 \mu\text{l}$, with 31.6% sugar concentration at 08:00 h, $16.4 \mu\text{l}$ with 39.2% sugar concentration at 18:00 h, and on the 2nd day of anthesis, $15.3 \mu\text{l}$ with 28.2% sugar concentration at 08:00 h and $6.9 \mu\text{l}$ with 27.2% sugar concentration at 18:00 h (Table 2). The nectar was totally concealed at the flower base by holding the standard, wing and keel petals in tension. Gradual decrease in the nectar volume during the two days of flower life was recorded as extent of the foraging activity of nectar-feeding Carpenter Bees. Furthermore, on the day of anthesis, a gradual increase in nectar sugar concentration from morning to evening was considered to result from partial exposure of nectar to sunlight during visits by Carpenter Bees and gradual release of tension between standard versus wing and keel petals. On the 2nd

Table 1. *In vitro* pollen viability in *Canavalia gladiata*.

Time (h)	Pollen sample	No. of germinated pollen grains	Germination (%)
0730	50	45	90
0930	50	42	84
1130	50	31	62
1330	50	9	18
1530	50	2	4
1730	50	0	0

Pollen germination was recorded in 15% sucrose solution

Table 2. Nectar volume and sugar concentration per flower at Day 1 and Day 2 flowers of *Canavalia gladiata*.

Time (h)	Day 1 flower		Day 2 flower	
	Mean nectar volume/flower (μl)	Mean sugar concentration (%)	Mean nectar volume/flower (μl)	Mean sugar concentration (%)
0800	32.5	31.6	15.3	28.2
1000	27.5	35.7	8.5	28.5
1200	23.3	38.2	7.7	29.4
1400	21.4	40.6	7.6	28.8
1600	17.7	40.1	7.4	28.6
1800	16.4	39.2	6.9	27.2

day of anthesis, the low sugar concentration levels recorded throughout the day were considered as partial and gradual withdrawal of sugars from the nectar.

Breeding systems: Hand-pollination tests indicated that the plant reproduces by autogamy (manipulated), geitonogamy and xenogamy, but the fruit set and seed set varied greatly at each mode of pollination. Fruit set and seed set rates were the lowest at the first mode and highest at the last mode. In open pollinations, fruit set rate reached 11%, while seed set 90%, indicating facultative xenogamy, which enabled sexual reproduction through self- and cross-pollination, occurring only when the stigma was scraped or ruptured by visiting foragers (Table 3). Furthermore, fruits developed only from basal flowers of the inflorescence at all modes of pollination. In the open-pollination mode, fruits developed from any inflorescence and varied from 1 to 4 in number, irrespective of the number of buds/flowers produced.

Pollination mechanism: The staminal column and pistil remained tensed within the keel petals after unfolding of the standard petal, and when the tension was released by tripping, the stigma and staminal column snapped forward against the standard petal situated below, thus causing instant pollen release (Fig. 1f,g). Untripped flowers eventually dropped off without pollination. Insects seeking nectar landed on the standard petal and pushed up the wing and keel petals to access the nectar at the ovary base. Then, the tensed keel pet-

Table 3. Results of the breeding systems in *Canavalia gladiata*.

Treatment	Number of flowers sampled	Number of flower set fruit	Fruit set (%)	Seed set (%)
Autogamy (mature buds bagged)	20	0	0	0
Autogamy (stigma ruptured and bagged)	20	2	10	30
Geitonogamy (stigma ruptured, hand-pollinated and bagged)	20	3	15	50
Xenogamy (stigma ruptured, hand-pollinated and bagged)	20	12	60	83
Open pollination (inflorescences tagged before initiation of flowering)	75	8	11	90

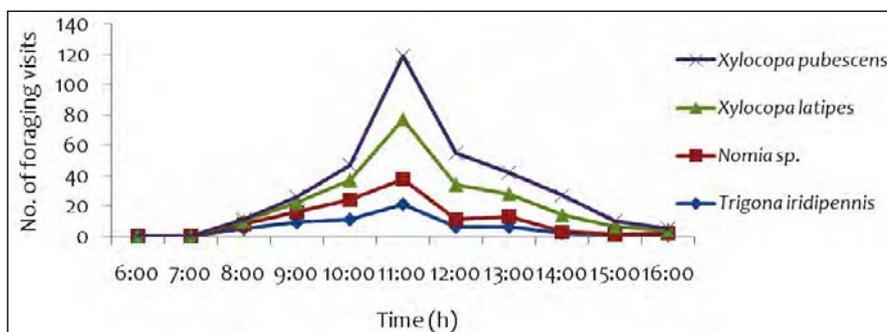
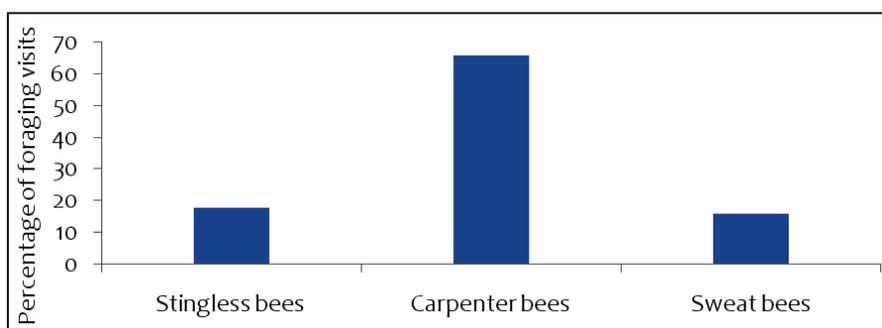
* In all treatments, only basal flowers of the inflorescences were bagged/tagged, as there was no fruit set from the flowers borne from the middle to apical part in open pollinations.

als released the stigma and stamens, which spontaneously brushed against the dorsal side of the thorax and part of the pollinator's abdomen. In the process of this, the stigma surface was scraped or slightly ruptured, due to its rubbing against the insect's body. If the visiting forager already had pollen from the earlier visited flowers on its dorsal surface, that would entail either in self- or in cross-pollination, depending on the pollen source. Simultaneously, the stamens ejected pollen explosively from the anthers onto the dorsal surface of the foragers.

In tripped flowers, the keel and wing petals returned back, enclosing the stamens and stigma, and concealing nectar, but multiple visits to the same flowers resulted in partial exposure of the sex organs and in freeing slightly the petals that enclosed them. That wing-keel complex tripping action characterized the explosive pollination mechanism triggered out only by nectar-seeking foragers.

Insect visitors and pollination: The thrips, *Frankliniella schultzei* Trybom (Sub-order: Terebrantia, Family: Thripidae, Sub-family: Thripinae), used buds for breeding. They moved out when mature buds gradually unfolded the standard petal. Since the flower base was not completely closed, the minute thrips came out easily from the slight gaps between petals and through the staminal column, and kept moving in and out of the flower base feeding on nectar as well as on pollen, and crawling all over the plant. Bud infestation with thrips was only 20%. The flowers visited by thrips did not show any scratches or rupture on the stigmas and, hence, the thrips were considered mere foragers, without any thrips role.

At daytime, from 08:00 to 16:00 h, the flowers were foraged by Stingless Bees, Carpenter Bees and Sweat Bees, with intensive foraging activity from 10:00 to 11:00 h (Fig. 4). Stingless Bees included only one species, *Trigona iridipennis*; Carpenter Bees two species, *Xylocopa latipes* and *X. pubescens*; and Sweat Bees only one species, *Nomia* sp. Of all foraging visits recorded during the observation period, Stingless Bees claimed 18%, Carpenter Bees 66% and Sweat Bees 16% (Fig. 5). Carpenter Bees always approached the flower from the front, with head facing the

**Fig. 4.** Hourly foraging activities of bees on *Canavalia gladiata*.**Fig. 5.** Percentage of foraging visits of different bees on *Canavalia gladiata*

wing-keel petal complex. They landed on the standard petal, pushed up the wing-keel petal complex and inserted the glossa into the flower base to collect nectar. At that probing behavior, the keel petals were tripped, stigma brushed and pollen ejected violently and spontaneously against the dorsal side of thorax and abdomen of the bees, which entailed cross and/or self-pollination (Fig. 1j). These bees never made attempts at collecting pollen. Stingless Bees (Fig. 1h) and Sweat Bees attempted to reach the flower base for nectar, but failed. However, they collected pollen from untripped and tripped flowers by landing on the wing-keel petal complex in upright position and facing towards the base of that complex (Fig. 1i). After their visits, the stigmas collected from first-time visited untripped flowers were without any scratches or rupture, indicating that they were only pollen robbers, without any role in pollination. However, they were considered pollinators in the flowers tripped by Carpenter Bees. The ventral side both of Stingless and Sweat Bees rubbed against the stigmas in tripped flowers during their pollen collection activity. Of these bees, Carpenter Bees were regular and consistent foragers, while the others were irregular and inconsistent foragers. However, only two or four Carpenter Bees visited the flowers of a plant. The pollen-carrying efficiency evaluated by body washings of the captured bees indicated that Carpenter Bees carried more pollen than other bees. The average number of recorded pollen grains varied from 94 to 242 in case of Carpenter Bees, from 61 to 156 in case of Sweat Bees and from 44 to 135 in case of Stingless Bees (Table 4). Carpenter Bees foraged the flowers in quick succession from one flower to another and from one inflorescence to another on the same and/or different plants, in order to collect as much nectar as possible; this inter-inflorescence/plant foraging activity was regarded as promoting cross-pollination, while at the same time effecting self-pollination. Pollen-collecting bees gathered pollen very slowly from each flower they visited and, hence, were considered minor pollinators only in flowers tripped by Carpenter Bees.

Table 4. Pollen recorded in the body washings of bees on *Canvalia gladiata*.

Insect species	Sample size (N)	Number of pollen grains		
		Range	Mean	SD
<i>Trigona iridipennis</i>	10	44–135	97.5	24.2
<i>Xylocopa latipes</i>	10	113–242	173.7	44.9
<i>Xylocopa pubescens</i>	10	94–206	137.8	35.1
<i>Nomia</i> sp.	10	61–156	114.7	27.5

Extrafloral nectaries and ant activity: Extrafloral nectaries are produced at the base of leaf petiole (Fig. 2a) and flower pedicel (Fig. 2b). Nectaries of the leaf petiole occur as a pair of large green round cushion forms, with small invaginations of accumulated nectar and tumescent surrounding tissue. They are visible externally and active mainly during the leaf-flushing phase, which indicates that their function is not in synchrony with the inflorescence production. Furthermore, these nectaries did not secrete nectar after completion of leaf flushing, by which time the initiation of inflorescences occurs. The nectar volume in these nectaries ranged from 0.4 to 0.6 μl , with sugar concentration varying from 3 % to 9 %. Nectaries of the flower pedicel occur as a pair of swollen cushion-like mounds, with large invaginations of accumulated nectar, which varied in volume from 1.8 to 3.5 μl , with sugar concentration ranging from 14 % to 23 %. When buds are aborted and flowers fallen, the pedicel base shows a depression or scar and the tissue around it becomes swollen and glandular, depositing its sugar content in the scar throughout the lifespan of the inflorescence. The continued secretion in the nectaries at the pedicel base appears to have a protective role for the existing and to be yet produced flowers and growing fruits from herbivores.

Field observations indicated that the ant *Camponotus compressus* (Family: Formicidae, Sub-family: Formicinae) was resident forager, crawling all over the plant and involved in nectar collection from extrafloral nectaries at the base of leaf petiole and flower pedicel (Fig. 2c). That ant species made unsuccessful attempts to collect nectar from the flowers, because the nectar was concealed and the ant body weight was insufficient to push up the wing-keel petal complex for access to it. Therefore, the ant activity was regarded as providing protection against herbivores during the leaf flushing, flowering and fruiting periods.

Fruiting and seed ecology: Fruits are characteristically pods that grow to full size in 6–7 weeks and mature in 5–6 months (Fig. 3a,b). In the growing stage of fruits, the endocarp is very thick and juicy, but thin, white and papery at maturity. Fruit pods are linear, flattened, compressed between the seeds, with a prominent ridge along the upper suture, slightly curved, minutely beaked, 17–19 cm long, 2.2–3.4 cm wide, and 8–12 seeded (Fig. 3c,d). The seeds are large, of various shades of reddish-brown, ellipsoid, partially compressed and 2.5–3.5 cm long. The hilum is creamy-



Fig. 2. *Canavalia gladiata*: Extrafloral nectaries at the base of leaf petiole (a) and flower pedicel (b), and Ant, *Camponotus compressus* feeding on nectar at flower pedicel – Nectar secretion is continuous until fruit development.



Fig. 3. *Canavalia gladiata*: a. & b. Fruiting phase, c. Mature and dry pods, d. Dry pod with seeds, e. Seeds, f. New growth from seeds.

white, extending almost along the entire length of the seed coat (Fig. 3e).

When mature, pods twist and dehisce explosively to disperse the seeds. Most of dispersed seeds fall around the parental plants. Most of the pods dehisce in March–May, although pod dehiscence occurs across the year due to year-long flowering and fruiting. Dry ambient conditions have proved ideal for dehiscence of mature pods by explosion. Seeds fallen to the ground are non-dormant and germinate within 3–4 days, if the soil is saturated with water, and within 8–12 days if the soil is semi-wet. However, only the seeds that germinate during the rainy season usually form seedlings and subsequently produce new plants (Fig. 3f). Field studies indicated that 93 % of the seeds, which germinated during the rainy season, have produced seedlings, in contrast to 15 % of the seeds, which germinated outside the rainy season. Production of new plants from these seedlings is apparently related largely to moisture levels in the soil.

Discussion

With its vigorous climbing habit, *Canavalia gladiata* uses the nearby available woody shrubs and trees. It covers them entirely, up to the top part, but the inflorescences remain oriented horizontally and in hanging position, thus displaying the flowers outside the foliage. This climbing habit is important for the species, in order to survive and populate itself through sexual reproduction. The plant is a perennial species with a deep-root system. It grows throughout the year, with leaf flushing, flowering and fruiting depending on the soil moisture levels, although most of the flowering occurs during the rainy season and accordingly fruit set occurs. Sahai (2009) reported that *C. virosa* is also a fast-growing climbing perennial, with a phenology similar to *C. gladiata*. He also noted that *C. virosa* has a peduncled raceme, which flowers from its middle part, while *C. gladiata* produces flowers in axillary racemes. The present study has shown that *C. gladiata* produces mainly terminal racemes, although occasionally axillary racemes are also produced, and flowering occurs acropetally from the base to the apex of the raceme. However, terminal buds usually do not reach maturity and eventually fall off. Purselove (1968) mentioned that in *C. ensiformis* the flowers from any part of the inflorescence take part in the

fruit set. In *C. gladiata*, only flowers from the basal part of the inflorescence take part in the fruit set. Furthermore, the fruit number varies from 1 to 4 only, although the mean number of flowers produced per inflorescence reaches 20.92, which indicates the plant's ability to use only basal flowers for the fruit set.

Sahai (2009) reported that in *C. gladiata* anther dehiscence and stigma receptivity occur during the mature bud stage, and the stigma surface is covered by pollen load, but pollen germination occurs only when the stigma is ruptured by foragers, which indicates that autogamy is not functional. He used such terms as xenogamy, largely out-crossing and self-incompatibility, to explain the functional breeding system. Ma & al. (2017) noted that *C. gladiata* is an obligate out-crosser. With this in view, the present study has carefully examined the breeding system of that species. The investigations have shown that autogamy is unfunctional, despite the functionality of protandry, as pollen did not germinate on the stigma surface during bud stage and after flower opening, prior to any visits by pollinator insects seeking nectar. This indicates that stigmatic surface should be ruptured by the nectar-seeking pollinators for the pollen to germinate and, irrespective whether it is self- or cross-, pollination is obligately pollinator-dependent. Hand-pollination tests have also indicated that only the surface-ruptured receptive stigmas set fruit, depending on the compatibility levels for self- and/or cross-pollen. Furthermore, the highest fruit and seed set rates evidenced in xenogamy indicate that it is largely out-crossing, although it is also self-compatible and self-pollinating. That is further supported by the short period of pollen viability and long period of stigma receptivity. This mixed breeding system in *C. gladiata* represents facultative xenogamy, for which pollinator interaction is required to rupture the stigmatic cuticle and to transport pollen (Krietner & Sorensen 1985), so that finally pollen germination occurs (Rowlands 1958). Facultative xenogamy promotes cross-pollination, facilitating the genetic variability, while the capacity for self-pollination with the aid of pollinators is probably adaptive to a changing environment, where pollinators are not available or reliable (Bock & al. 2019).

Harley & al. (2017) reported resupinate dimorphism (dimorphism) in some *Eplingiella* species of Hyptidinae of the family *Lamiaceae*. Individual plants produced either resupinate or non-resupinate flower morphs. Half of the population produced resupinate

flowers, with stamens above the style facilitating the nototribic pollination, while the other half of the population produced non-resupinate flowers, with stamens below the style facilitating the sternotribic pollination. In both resupinate and non-resupinate flowers, the boat-shaped median lobe of the anterior corolla lip held the stamens inside under tension and released pollen similarly by explosion. Resupinate dimorphic is an adaptation for promoting cross-pollination, while keeping the option for self-pollination. Stirton (1977) reported similar resupinate dimorphic in *Canavalia virosa*, but in that species both upright flowers with standard petal above and inverted flowers with standard petal below are borne on the same individuals. When visited by appropriate foragers for nectar collection, the wing and keel petals in both, upright and inverted flowers are depressed, facilitating the way for the insects to insert their mouthparts for nectar collection, during which pollination occurs sternotribically in the upright flowers and nototribically in the inverted flowers and, after departure of the forager, these petals return to their original position.

In this study, *C. gladiata* produced only inverted flowers with wing and keel petals above, consisting of stamens and stigma, and standard petal below. The wing petals were longitudinally bulged along the upper surface and auricled at the base. Two pin-like callosities rested on the wing petal portion between the bulges and auricles at the base of the standard petal. That petal configuration represented a specialized floral mechanism adapted for tripping by the specialized pollinating insects. It was referred to as explosive pollination mechanism, because of the spontaneous and violent release of stamens and stigma, and their violent rubbing against the dorsal side of pollinator entailing nototribic pollination. A similar pollination mechanism was reported in *C. virosa* by Stirton (1977).

Faegri & van der Pijl (1979) noted that papilionaceous flowers are usually bee-pollinated. Watmough (1974) stated that Carpenter Bees alone have the necessary power and weight to operate the floral mechanisms ending in pollination in the *Papilionaceae* members in South Africa. *Canavalia* is a genus adapted to the Carpenter Bees for the function of its floral mechanism, in order to achieve successful pollination. Gross (1993) stated that bees are commonly reported as pollen vectors for the *Canavalia* species. Snak & al. (2015) suggested that *C. reflexiflora* with red flowers, reflexes the standard petal and wing and keel pet-

als displays of the ornithophilous pollination system. Different authors reported that *C. maritima* is pollinated by Carpenter Bees, such as *Xylocopa violacea* (Scott-Elliot 1891) and *X. aestuans* (Piper & al. 1922), Digger Bees, such as *Anthophora* sp. (Vogel 1954) and butterflies (Scott-Elliot 1891). In South Africa, *C. virosa* is pollinated by *Xylocopa flavorufa* and *Megachile combusta*. The former is a natural pollinator, while the latter is an opportunistic forager, which effects pollination while probing the flower for nectar (Stirton 1977). In China, *C. gladiata* is visited by many insects, namely, butterflies, moths and bees. The mouthparts of butterflies and moths facilitate the probing and access to the nectar, but bees can access the floral nectar and, hence, gather only pollen (Ma & al. 2017). In India, *C. gladiata* and *C. virosa* are visited by a wide range of insects, mostly moths, butterflies, ants, and honeybees, but the latter are occasional visitors (Sahai 2009; Ma & al. 2017).

In the present study, Carpenter Bees, representing *Xylocopa latipes* and *X. pubescens*, were regarded as natural pollinators of *C. gladiata*. These bees were large-bodied and had the required weight and energy to thrust their mouthpieces and head into the flower base by pushing up and tripping the keel-wing petal complex to access nectar. Consequently, the stamens and stigma were released violently and nototribic pollination occurred. Nototriby entailed precision and economy in pollen transfer, as the pollen deposited on the dorsal side of the bee was inaccessible for grooming and ensured successful pollination. That mode of pollination was related to the position of sex organs on the upper side of the flowers.

Cruden & al. (1983) reported that Carpenter Bees with their short-tongue can get sucrose-rich nectar. Heinrich & Raven (1972) stated that the large in size Carpenter Bees require high energy and reach the reward only if the energetic reward is proportional to the energy expended. The concealed nectar of *C. gladiata* is expected to be sucrose-rich, with high sugar concentration so as to fulfil the energy levels required by the Carpenter Bees. Stingless and Sweat Bees with their small bodies are inappropriate for tripping the keel-wing petal complex and, hence, their attempts to access the nectar are futile. However, they effect pollination after visiting the flowers tripped by Carpenter Bees, because the stigma in tripped flowers is ruptured and pollen collection by non-Carpenter Bees results in pollination. Since those bees visit the *C. gladiata*

flowers irregularly and inconsistently, they act either as accidental or opportunistic pollinators. Therefore, *C. gladiata* is obligately dependent on the Carpenter Bees for pollination.

Different authors have reported the presence of extra-floral nectaries and foraging activity of ants in many legumes and other plant species protected by the ants from harmful herbivores and seed predation (Kost & Heil 2005; Raine & al. 2002; Martin & Mckey 2003). The *Fabaceae* members, *Canavalia gladiata*, *C. lineata*, *C. maritima*, *Dunbaria fusca*, *D. podocarpa*, *D. rotundifolia*, *Erythrina caffra*, *E. speciosa* and *E. variegata* possess two button-shaped extra-floral nectaries at the leaf base and the three latter species possess two more such nectaries below the terminal leaflet (May Ling 2004). In *Canavalia lineata* and *C. cathartica*, the presence of extra-floral nectaries and the nectar feeding activity of ants on these nectaries indicates a beneficial relationship for both sides (Yamashiro & Yamashiro 2008). Nieuwenhuis-Von Uxkull-Goldenbrandt (1907) noted that *C. gladiata* possesses extra-floral nectaries in each leaf axil, consisting of a large green cushion representing nearly ten dot-like surface depressions. Sahai (2009) and Ma & al. (2017) reported that in *C. gladiata* the extra-floral nectaries are situated at the base of the pedicel and initiate nectar secretion during the mature bud stage, with continuing secretion up to the full bloom. Sahai (2009) reported some confusing and contradictory findings regarding the association between ants and extra-floral nectaries of *C. gladiata*. He documented that ants, such as *Monomorium minimum* and *Campylomma verbasci*, feed on extra-floral and floral nectars regularly and act as nectar-robbers. Their feeding on floral nectar results in accidental pollination and on extra-floral nectaries in flower abscission and low fruit set. The ants surround the flowers and the tissues forming a turgid joint of the pedicel loosen causing flower drop without being involved in pollination. He also stated that ants are probably responsible for most successful self-pollen transfer and tripping of the stigmatic cuticle in the absence of potential bee pollinators. Furthermore, the larvae of the Bean Aphid, *Aphis fabae*, feed on the stigmas and anthers of almost half of the buds produced by the plant and their feeding activity largely contributes to non-availability of flowers for fruiting. Ma & al. (2017) noted that the ant foraging activity on extra-floral nectaries of *C. gladiata* may benefit the latter by driving away its predators.

In the present study, it was found that *C. gladiata* produced extra-floral nectaries from the base of the leaf petiole, as well as from the base of the pedicel. The nectar-secreting structures used the same vascular tissue that ran towards and extended into the inflorescence internally (Vogel 1998). The extra-floral nectaries of the leaf petiole were largely functional during the leaf-flushing phase and, accordingly, involved in the protection of leaves against herbivores. Likewise, the extra-floral nectaries of the flower pedicel initiated nectar secretion shortly before anthesis and continued secretion even after the abscission of pedicel, cessation of flowering and until fruit development, in order to protect the flowers and the growing fruits against herbivores. The nectar of the extra-floral nectaries was harvested continuously by the resident ants, *Camponotus compressus*, with high foraging activity during the flowering and fruiting phase. That ant species made attempts at collecting the concealed floral nectar but failed to access it as the ants weight was insufficient either to push down the standard petal, or to push up the wing-keel petal complex and, hence, the ant activity in pollination was ruled out.

Pascal & al. (2000) suggested that extra-floral nectaries are structurally very simple in *Caesalpinioideae*, which are considered a primitive sub-family within the *Fabaceae* family. On the contrary, the extra-floral nectaries are derived and structurally more complex in the sub-family *Papilionoideae* (Diaz-Castelazo & al. 2005). *Canavalia* belongs to that sub-family and, hence, the extra-floral nectaries in *C. gladiata* are complex and exhibit protective roles: in leaf protection and in flower and fruit protection against herbivores.

In the present study, Bean Aphids have not been observed to cause damage either in the buds or in the flowers and, hence, the plant was absolutely free from bud and flower predation by Aphids.

Sahai (2009) reported that a natural fruit set in *C. gladiata* varies from 8% to 10% and 90–95% of the ovules produce mature seeds in the fruited flowers. The mature seed set is highest in the middle ovule position and declines towards the stylar end. It is completely absent in the terminal ovule position.

The present study recorded the highest fruit and seed set rates in *C. gladiata* through xenogamy, followed by geitonogamy and manual autogamy, which indicated that the plant was predominantly out-crossing, although self-pollination occurred. Low seed set

rates in self-pollination modes indicated that the plant presumably had internal regulation to allow cross-pollinated flowers to produce more seeds and abort selectively the genetically inferior seeds resulting from self-pollination. That finding was in agreement with Stephenson & Bertin (1983) who reported that self-pollinated flowers always produced fewer seeds and were more likely to abort than the fruits of cross-pollinated flowers. The present study also showed that the seed set rate was not related to the position of the ovule in the ovary, as stated by Sahai (2009). Furthermore, the low natural fruit and seed set rates could be attributed to the production of fruits only from basal flowers, bud drop from the terminal part of the inflorescence, timely tripping of flowers by the pollinating Carpenter Bees, extent of the cross-pollination rate, short duration of pollen viability, number of the foraging visits made by bees, and energy resources available to the plant during flowering and fruiting phases. However, the low natural fruit/seed set to flower ratios did not affect the colonizing success rate of *C. gladiata*, because individual plants flower prolifically across the year, ensuring constant production of seeds. Similar situation was reported for *C. rosea* by Gross (1993).

Takayama & al. (2008) pointed out that *Canavalia rosea*, *C. cathartica*, *C. lineata*, *C. sericea*, and *C. bonariensis* use sea dispersal for spreading their seeds. Sauer (1964) reported that *Canavalia* species, other than the species mentioned by Takayama & al. (2008), exhibit mechanical seed dispersal by throwing out seeds by dehiscence of pods or gravity.

In this study, *C. gladiata* has been found to have a mechanical mode of seed dispersal, in which the seeds were ejected by the explosive dehiscence of pods, as well as by gravity. However, seed dispersal was effective only during dry ambient conditions, which occurred largely during the summer season. Despite the ability of seeds to germinate as soon as they fall onto the ground, their germination and subsequent production of new plants was greatly influenced by the soil moisture levels. The ideal soil conditions occurred during the rainy season and it was then that most of the germinated seeds produced new plants.

Canavalia gladiata is traditionally consumed as an important source of food and cultivated as an important pulse crop in different parts of the world (Herklots 1972; Kay 1979; Ekanayake & al. 2000). Its pods and seeds were even consumed by the urbanized populations in ancient India for their high protein content

(Vishnu Mittra 1981). Rodrigues (1990) stated that *C. gladiata* is able to grow under extremely difficult conditions and offers a means for extending protein production to marginal areas, particularly to tropical lowlands with depleted soils and to areas with unpredictable climate or varying soil types, high altitudes, and pest infestations. Therefore, *C. gladiata* can be promoted for cultivation in different ecological areas, with different soil conditions, in order to use its pods and seeds as a potential source of high-protein food. Furthermore, that plant can be also cultivated in butterfly parks and gardens as a larval host plant for the nymphalid butterfly, *Neptis hylas*, and lycaenid butterflies *Eychrysops cnejus*, *Chilades pandava* and *Jamides celeno* (Soman & Churi 2019; Kunte 2000), in order to help them breed and multiply, thus enhancing through ecotourism the awareness how important butterfly diversity is in tropical ecosystems.

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