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The Reproductive Ecology of *Asystatia gangetica* (L.) T. Anderson (Acanthaceae)

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Abstract: *Asystasia gangetica* is represented by two subspecies in the study area *alba* and *micrantha* of which the former is the most common, and the latter is very rare. It is hermaphroditic, prominently protandrous, self-compatible, self-pollinating, facultatively xenogamous, melittophilous but also psychophilous. Floral rewards for pollinators include nectar and pollen, both with several essential and non-essential amino acids and proteins. The fruit and seed characteristics enable the plant to produce several batches of plants in the same growing season. The dual modes of reproduction and the flexible facultative xenogamous mating system ensure the plant's formation of huge populations within a short period of time even in the absence of its pollinators and sustains it as a successful invasive weed.

Keywords - *Asystasia gangetica*, Facultative xenogamy, Melittophily, Psychophily.

Introduction

The *Asystasia* genus belongs to the Justicieae tribe, the Acanthoideae subfamily, and the Acanthaceae family. Jackson (1990) described the name *Asystasia* by inconsistency which relates to the fact that the corolla is more or less regular, which is unusual in Acanthaceae. Acevedo-Rodriquez (2005) confirmed that the genus *Asystasia* includes about forty species of a paleotropical origin. Tsai-Wen *et al.* (2005) reported that *Asystasia* consists of of seventy species, distributed across Africa, India, mainland China, and Australia, but there is almost no information on any aspect of the reproductive ecology of the different species of this genus throughout their distribution. Mardan and Kiew (1985) reported that *A. intrusa* is used as a nectar plant by *Apis cerana* in Malayasia. Edwards and Norris (1987) reported that *A. pinguijolia* in Natal, South Africa, is visited by domestic bees. Aliakbarpour and Rawi (2012) reported that *A. coromandeliana* is the breeding site for *Thrips hawaiiensis* and *Megalurothrips usitatus* in the Mango Orchards of Pulau Pinang, Malaysia. Jongjitvimol and Poolprasert (2014) noted that *A. salicifolia* is an important pollen source for different species of stingless bees in the Nam Nao National Park, Thailand.

Asystasia gangetica is indigenous to the tropics of the Old World from Malaya to Africa (Kamemoto and Storey, 1955). However, later, *A. gangetica* was believed to be originated in India and South Africa, even though it is widely distributed in tropical regions such as the Pacific Islands, Central America, and Australia (Josekutty *et al.,* 2002; Burg *et al.,* 2012). The species epithet "*gangetica*" was named after the Ganges River in India where it was presumed to be occurring (Jackson, 1990). It is introduced into some countries as an ornamental plant or as a cover crop (Burg *et al.,* 2012). It grows rapidly, reproduces excessively, and damages native flora in the new areas, where it prevails as an invasive weed (Josekutty *et al.,* 2002). In the Hawaiian Islands, the flowers are purple, white, and yellow with the purple flowers being the most dominant (Kamemoto and Storey, 1955, Elliot *et al.,* 2004) attracting bees, black ants, and butterflies regularly (Murali *et al.,* 2013). The pollen of this plant species was found to be dominant in the colonies of the meliponine bee, *Heterotrigona itama* throughout the year in Malaysia (Lob *et al.,* 2017).

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Oecophylla smaragdina acts as a predator by capturing *Apis cerana* when the latter is engaged in probing the flowers of *Asystasia gangetica* for pollen and/or nectar (Rodriquez-Girones *et al.,* 2013). The plant is used as a folklore medicine in India, Kenya, and Nigeria (Akah *et al.,* 2003; Reddy *et al.,* 2010).

Ensermu (1994) recognized two subspecies in *A. gangetica*: *gangetica* and *micrantha*; the former is the large-flowered type distributed in areas from India, throughout Asia to Indonesia and the Pacific Islands, while the latter is the smaller-flowered type and is confined to Africa. Westaway *et al.* (2016) stated that the subsp. *gangetica* is cultivated more widely in tropical zones. Kiew & Vollesen (1997) and Daniel and Figueiredo (2009) documented the subsp. *micrantha* as native to the African continent but it is also cultivated and is now widely naturalized in Asia, the Pacific, and central and southern America. The National Herbarium of New South Wales (PlantNet) and the Australian Plant Census (APC) have relegated the two subspecies as synonyms (Westaway *et al.,* 2016). The *A. gangetica,* subsp. *Gangetica* was named *A. gangetica.* subsp. *alba* (Grubben, 2004) which differs from the second subspecies; *A. gangetica* subsp. *micrantha*; on the basis of the petal colour. The *A. gangetica* subsp. *micrantha* is intricately designed for bee-pollination and butterflies use it as a nectar source. Furthermore, it is the larval host for the nymphalid butterflies *Doleschallia bisaltide pratipa, Hypolimnas bolina bolina, H. bolina jacintha* and *Junonia orithya wallacei* in Singapore (Tan and Khew, 2012), *Hypolimnas misippus* in Sri Lanka (Jayasinghe *et al.,* 2014), *Precis almana* and *P. hierta* (Harinath and Venkata Ramana, 2014) and the hesperiid butterfly *Celaenorrhinus leucocera* in the Western Ghats of India (Churi *et al.,* 2020). *A. gangetica* is a nectar host plant for papilionid *Papilio polytes, Princeps demoleus*, the pierid *Pareronia valeria* and the hersperiid *Borbo cinnara* in Visakhapatnam, India (Deepika *et al.,* 2014). This work is aimed at investigating the reproductive ecology involving phenology, flowering, floral biology, sexual system, breeding system, pollinators, fruiting ecology, seed dispersal and the regeneration of *Asystasia gangetica* (L.) T. Anderson.

Materials and Methods

The Study Area and the Identification of Subspecies:

A large population of *Asystasia gangetica* growing in an area of about 500 sq. m at the outskirts of Visakhapatnam City (17°42'N Longitude and 82°18'E Latitude, altitude 45 m amsl), Andhra Pradesh, India was selected for the study over the period from March 2018 to December 2019. Regular field visits were made to this population site for the sake of studying the vegetative growth, flowering and fruiting aspects. Petal color variations were noted and−two subsp. *alba* and *micrantha* were identified; the former is the most common, while the latter is very rare, and both occur in the same population. All aspects investigated and described in the results section relate to the subsp. *alba* only unless otherwise specified.

Anthesis and anther dehiscence:

Twenty-five mature buds nearly open were tagged and observed to record the timing of anthesis. The same buds were also used to record the timing and mode of anther dehiscence.

Flower morphology:

The flower aspects such as flower sex, shape, size, colour, odour, sepals, petals, stamens, and ovary were recorded. The stamens were described regarding their position, that is whetherthey were exposed or hidden during the open state of the flower. The stamen attachment and detachment in the proximal and distal portion of the corolla were examined and the details were described in relation to their role in nectar concealment in order to give access to appropriate foragers which are involved in effecting pollination.

Pollen production:

Ten mature but un-dehisced anthers were collected from different plants and kept in a Petri dish. A single anther was taken out each time and placed on a clean microscope slide (75 x 25 mm). It was crushed with a glass rod and a small drop of lactophenol-aniline-blue was added to disperse the pollen grains equally to the fixed area on the slide and the pollen grains were counted under a compound microscope (40x objective, 10x eye piece). This procedure was followed for the sake of counting the number of pollen grains in all ten anthers. Based on the pollen counts of each anther, 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 to obtain the mean number of pollen grains per flower. At the same time, the pollen grain characteristics were recorded. Also, the pollen-ovule ratio was calculated using the formula noted by Cruden (1977).

Mondal *et al.* (2009) described the protocols for the analysis of amino acids in the pollen. These protocols were followed for identifying amino acid types present in the pollen. The pollen was collected from mature anthers and was sieved using meshes of different sizes (100, 200 and 300 µm) to remove the debris. After that, the pollen was rapidly dried over silica gel at 30°C and stored. Free amino acids were extracted from the pollen using the method of Bieleski and Turner described in Mondal *et al.* (2009).

Later, the extract thus obtained was used for the qualitative analysis of the free amino acids of the pollen using thin-layer chromatography. The protocol described in Sadasivam & Manickam (1997) was followed for the extraction of protein from the pollen samples using phosphate buffer of pH 7.4 and then the protocol described by Lowry *et al.* (1951) was followed for estimating the protein content in the sample.

Nectar production and analysis:

The presence of nectar was determined by observing the mature buds and open flowers. The micropipette was inserted into the flower to extract the nectar for measurement. The average of the nectar of ten flowers was taken as the total volume of nectar/flower and was expressed in µl. The same sample size was used for measuring the nectar's sugar concentration; the Hand Sugar Refractometer (Erma, Japan) was used in recording the sugar concentration. Nectar analysis for sugar types was done according to the paper chromatography method described in Dafni *et al.* (2005). The sugar content/flower is expressed as the product of nectar volume and sugar concentration per unit volume, mg/µl. This was done by first noting the conversion value for the recorded sugar concentration on the Refractometer scale and then by multiplying it with the volume of nectar/flower. The procedure in Table 5.6 given in Dafni *et al.* (2005) was followed to record the value converted to mg of sugars present in one µl of nectar. The protocols provided by Sadasivam *and* Manickam (1997) were followed for the quantitative estimation of sucrose, glucose, and fructose in mg/flower. The caloric reward of nectar/flower/day was measured according to the formula given by Heinrich (1975). He assumed that 1 mg of sugar yields 16.74 joules or four calories of energy, and accordingly he used the formula for calculating the caloric reward of the nectar.

$$\frac{\text{Nectar volume (µl)} \times \text{Concentration of nectar (\%)}}{100} \times 16.74$$

The paper chromatography method described in Dafni *et al.* (2005) was followed to identify the amino acid types in the nectar. Lowry *et al*. (1951) described the method for measuring the protein content.

Stigma receptivity:

The stigma receptivity was tested with hydrogen peroxide from the mature bud stage to the late evening of the secondday of anthesis. When applied to bifid stigma, Hydrogen peroxide did not stain but produced

bubbles as a result of catalase (peroxidase) presence. This test is widely followed although it does not indicate the exact location of the receptive area (Dafni *et al.* 2005). The period of the release of bubbles from the stigma surface following the application of hydrogen peroxide was taken as the total duration of stigma receptivity during the flower life.

Breeding systems:

Breeding systems were tested for apomixis, self-pollination and cross-pollination. The number of mature buds selected was thirty for each mode of pollination; they were observed for four weeks for fruit set. Based on the flowers that produced fruits, the percentage of fruit set was calculated. Mature buds were emasculated and bagged to test apomoxis. Mature buds were bagged without emasculation and pollination to test the spontaneous self-pollination (autogamy). Mature buds were bagged on the evening of the previous day, and were opened in the afternoon of the next day by which time anthesis, anther dehiscence, and stigma receptivity occurred; the stigma was then pollinated with the pollen of the same flower using a brush and was bagged to test hand self-pollination (autogamy).

The mature buds were bagged after emasculation, and were opened in the afternoon of the next day by which time anthesis and stigma receptivity occurred; the stigma was then pollinated with the fresh pollen of a different flower of the same plant using a brush and was bagged to test hand self-pollination (geitonogamy).

The mature buds were bagged after emasculation, and were opened in the afternoon of the next day by which time anthesis and stigma receptivity occurred. The stigma was then pollinated with the fresh pollen from the flower of a different plant using a brush and was bagged to test hand cross-pollination (xenogamy).

Fruit set in open pollinations:

Eighty flowers taken at random from thirty plants were tagged prior to anthesis and were observed for fruit set. Based on the flowers that produced fruits, the percentage of fruit set was calculated.

Flower-visitors and pollination:

Flowers were observed from morning to evening for four days to record flower-visitors. The flower visitors included bees and butterflies only; the beeswere identified by the Zoological Survey of India, Calcutta, India, while the butterflies were identified with the help of the Field Guide of Butterflies book by Gunathilagaraj *et al.* (1998).

The approach of the flower visitors to the flowers, flower-probing, the forage collected by them, and the contact between the body parts of the flower visitors with the stigma and stamens were carefully observed by standing close to the flowering patch and also by using a field binocular to record their pollination role. The number of foraging visits made by the flower visitors was recorded at each hour for ten minutes from morning to evening for four days at thirty profusely flowering plants. The data collected on the foraging visits of these insects were tabulated, and the mean number of foraging visits at each hour was calculated to know the foraging pattern of insects through time. The same data were also used to calculate the percentage of foraging visits made by bees and butterflies separately.

Fruit and seed aspects:

Forty mature buds were tagged and observed over a period of three months to record fruit growth, development, and the maturation period. The fruit dehiscence mode and seed dispersal aspects were observed carefully. Fruit and seed morphological characteristics were described to understand fruit/seed dispersal modes. Seventy-five mature and dry fruits were collected from twenty-five plants, and were classified according to their seed number per fruit, and the seed set rates were calculated. Field observations were made of the seed germination and the production of new plants during the rainy season and the winter season.

Results

Plant and flowering phenology:

It is a fast-growing low-ground perennial prostrate glabrous weedy herb with a mat-forming habit in open, dry, and sandy soils especially during the rainy season (Figure 1a). It spreads by seeds as well as by stems which turn into roots aftercontacting moist soils. The stem is quadrangular and slightly covered with hairs. The leaves are opposite, petiolate with simple, ovate blade—and entire margin. Even with its mat-forming habit, this herb is intermingled with several weedy species such as *Sida cordifolia, S. acuta* (Malvaceae), *Triumfetta rhomboidea* (Tiliaceae), *Pedalium murex* (Pedaliaceae), *Merremia tridentata* (Convolvulaceae), and *Antigonon leptopus* (Polygonaceae). All these weedy species flower simultaneously along with *A. gangetica*. The flowering occurs during the rainy season from July to November. The inflorescence is an axillary and terminal secund raceme with 13.2 ± 1.40 buds/flowers arranged on one side only (Figure 1b, c). The flowers are pedicellate and horizontally-oriented, but are projected above the leaves making them quite distinct against the foliage. Based on the flower color, two subspecies have been identified, the *A. gangetica* subsp. *alba* with a complete creamy white corolla with a yellow throat and the *A. gangetica* subsp. *micrantha* with a white corolla consisting of bluish-purple blotches in two parallel lines on the inside of the bottom petal lobe, of which the former is the most common, while the latter is very rare occuring in combination with the subsp. *alba*. The description of observations and results relate to the *A. gangetica* subsp. *alba* only unless otherwise stated.

Flower morphology:

The flowers are large (3.45 ± 0.07 cm long), funnel-shaped, creamy white, slightly pungent, and bisexual. The calyx is green, 5-7 mm long, lanceolate, hairy, fused basally and five-lobed terminally. The corolla is tubate and hairy with five 1.1 ± 0.04 cm wide rounded lobes apically forming a yellow-colored throat. The stamens and pistil (3 cm long) are inserted on the corolla tube. The stamens are four, epipetalous and free terminally; the filaments are green while the anthers are creamy white, dithecous, and dorsifixed (Figure 1d). The stamen and petal portions display two types of regions, synstapetal and apostapetal. In the synstapetal region, the proximal portion of the corolla and the stamens are fused while in the apostapetal region, the stamens are free from the distal portion of the corolla wall. In the synstapetal region, the filament ridges form channels that lead to the nectar. The corolla lobe traces are geniculate and positioned at the border of synstapetal and apostapetal corolla regions. Along this border, the corolla tube expands into a wider corolla throat. The filaments are decurrent and are situated along the synstapetal corolla region and fused in two-by-two mode proximally in the apostapetal region. The ovary is ellipsoid, densely pubescent, bicarpellary syncarpous with two locules, and each locule consists of two ovules on the axile placentation (Figure 1g- j). The style is subulate and velutinous, while the stigma is slightly capitate and bi-lobed (Figure 1h).

Floral biology:

The flowers are open during 0600-0800 h. Anthers dehisce by longitudinal slits an hour after anthesis. The subulate style runs from the center of the corolla tube and extends beyond the height of the stamens but remains inside the corolla throat (Figure 1e). The anthers are positioned laterally inside the corolla throat but below the level of the stigma. The pollen output is 787.8± 29.19 per anther and 3, 151.2 per flower. The pollen ovule ratio is 787.8. The upright style bends at the stigmatic end, and the stigmatic surface exudes a sticky substance which enables the capture and germination of the pollen deposited by foraging insects. The stigma is receptive from the afternoon of the day of anthesis and remains so until

Figure 1. *Asystasia gangetica*: a. Habit, b. Mature bud, c. Flower, d. Stamens, e. Relative positions of stamens and stigma, f. Pollen grain, g. Ovary, h. Style with bifid stigma, i. Placement of ovules in ovary, j. Ovules, k. Growing fruit, l. Mature and dry seeds.

the evening of the next day. Nectar secretion begins by nectary situated at the base of the corolla tube during the mature-bud stage and ceases soon after anthesis, it is 3.35 ± 1.35 µl with a sugar concentration of 31.7 ± 4.07% per flower. The total sugar content is 106.20 mg with 17.8 joules of energy per flower. The sugar types in the nectar include sucrose, glucose, and fructose; their quantity per flower varies with sugar type. The sucrose is 0.2 mg, glucose 0.078 mg, and fructose 0.069 mg. The nectar contains five essential amino acids and ten non-essential amino acids. The essential amino acids include threonine, valine, isoleucine, histidine, and arginine. The non-essential amino acids include alanine, amino butyric acid, aspartic acid, cysteine, cystine, glutamic acid, glycine, hydroxyproline, proline, and serine (Table 1). The protein content in the nectar is 0.012 mg/flower. The pollen contains six essential amino acids and seven non-essential amino acids. The essential amino acids are threonine, valine, isoleucine, lysine, histidine, and arginine. The non-essential amino acids include alanine, amino-butyric acid, cysteine, cystine, glutamic acid, glycine and hydroxyproline (Table 1). The total protein content per 1 mg of pollen is 204.05 µg. The corolla together with the stamens fall off on the third day any time, while the calyx remains intact and turns into a fruiting calyx. The style

Table 1. Essential and non-essential amino acids present in the nectar and pollen of *Asystasia gangetica*.

Essential amino acids			Non-essential amino acids		
Amino acid type	Nectar	Pollen	Amino acid type	Nectar	Pollen
Threonine	+	+	Alanine	+	+
Valine	+	+	Amino butyric acid	+	+
Methionine	-	-	Aspartic acid	+	-
Leucine	-	-	Cysteine	+	+
Iso leucine	+	+	Cystine	+	+
Lysine	-	+	Glutamic acid	+	+
Phenyl alanine	-	-	Glycine	+	+
Histidine	+	+	Hydroxy proline	+	+
Arginine	+	+	Proline	+	-
Tryptophan	-	-	Serine	+	-
			Tyrosine	-	-

(+) = Present; (-) = Absent

and stigma remain as a vestige at the tip of the fruit.

Breeding systems:

The hand-pollination tests showed that the flowers do not set fruit through apomixis. Fruit set occurs through self- and cross-pollination. Spontaneous self-pollination is not functional. Fruit set is 27% in hand self-pollination, 47% in geitonogamy, 87% in xenogamy and 82% in the open-pollination mode (Table 2).
The results of hand-pollinations indicated that the plant is facultatively xenogamous.

Foraging activity and pollination:

The flowers were foraged by bees and butterflies during day-light hours. The bees were *Apis dorsata* (Figure 2a-d), *A.cerana*, *A. florea* (Figure 2e,f), *Xylocopa pubescens* (Apidae) (Figure 2h), *Anthophora cingulata* (Anthophoridae) (Figure 2g), and *Megachile* sp. (Megachilidae) (Figure 2h) from 0700 to1800 h.
The butterflies included *Papilio polytes* (Papilionidae) (Figure 2i), *Pareronia valeria* (Pieridae) (Figure 2j), *Iambrix salsala* (Figure 2k), *Pelopidas mathias* (Figure 2l),

Table 2. Results of breeding systems in *Asystasia gangetica*.

Treatment	Number of flowers sampled	Number of flowers' fruit set	Fruit set (%)
Apomixis	30	0	0
Spontaneous self-pollination (Mature buds just bagged)	30	0	0
Hand self-pollination (Flowers hand-pollinated and bagged)	30	8	27
Geitonogamy (Flowers hand-pollinated and bagged)	30	14	47
Xenogamy (Flowers hand-pollinated and bagged)	30	26	87
Open pollination (Flowers tagged)	80	66	82

Figure 2. *Asystasia gangetica*: a-d. subsp. *alba*: Different stages in flowering probing for nectar collection by *Apis dorsata,* e. *Apis florea* collecting pollen, f. *Apis florea* probing for nectar collection, g. subsp. *micrantha*: *Anthophora cingulata* collecting pollen from subsp. *micrantha*, h-m. subsp. *alba*: left side – h. *Megachile* sp. approaching the flower and right side *Xylocopa pubescens* puncturing the base of corolla tube for nectar collection, i. Papilionid butterfly, *Papilio polytes*, j. Pierid butterfly, *Pareronia valeria*, k-m. Hesperiid butterflies – k. *Iambrix salsala,* l. *Pelopidas mathias*, m. *Borbo cinnara*.

and *Borbo cinnara* (Hesperiidae) (Figure 2m) (Table 3). Among bees, *X. pubescens* probed the flowers regularly for nectar illegitimately by making puncture at the base of the corolla tube, and it never probed legitimately from the front side of the flowers. This foraging behavior characterized this bee as an exclusive nectar robber. All other bees probed the flowers legitimately from the front side of the flower. For nectar collection, they first landed on one of the petal lobes, usually on the lower lobe and then inserted their tongues into the corolla tube through the central space of the stamens to access the nectar. In the subsp. *micrantha,* the lower petal lobe with the bluish-purple was always used as a landing platform for probing the flowers for nectar and/or pollen by the bees and butterflies. The horizontal orientation of the flowers enabled the nectar to leak and flow slightly towards half-way of the proximal portion of the corolla tube. Such a placement of nectar enabled the bees to collect the nectar with great ease. During the bees' probing for nectar collection from the side of the lower petal lobe , the backside of their head and thorax brushed against the stigma first and then their dorsal and ventral lateral side against the anthers receives pollen onto those areas and subsequently,

Table 3. List of insect foragers on Asystasia gangetica.

Order	Family	Genus	Species	Common name	Forage sought
Hymenoptera	Apidae	*Apis*	*dorsata* F.	Rock Honey Bee	Pollen and Nectar
		Apis	*cerana* F.	Indian Honey Bee	Pollen and Nectar
		Apis	*florea* F.	Dwarf Honey Bee	Pollen and Nectar
		Xylocopa	*pubescens* Spinola	Carpenter Bee	Pollen and Nectar
	Anthophoridae	*Anthophora*	*cingulata* F.	Blue Banded Bee	Pollen and Nectar
	Megachilidae	*Megachile*	sp.	Leafcutter bee	Pollen and Nectar
Lepidoptera	Papilionidae	*Papilio*	*polytes* L.	Common Mormon	Nectar
	Pieridae	*Pareronia*	*valeria* Cr.	Common Wanderer	Nectar
	Hesperiidae	*Iambrix*	*salsala* Moore	Chestnut Bob	Nectar
		Pelopidas	*mathias* F.	Dark Small Branded Swift	Nectar
		Borbo	*cinnara* Wallace	Rice Swift	Nectar

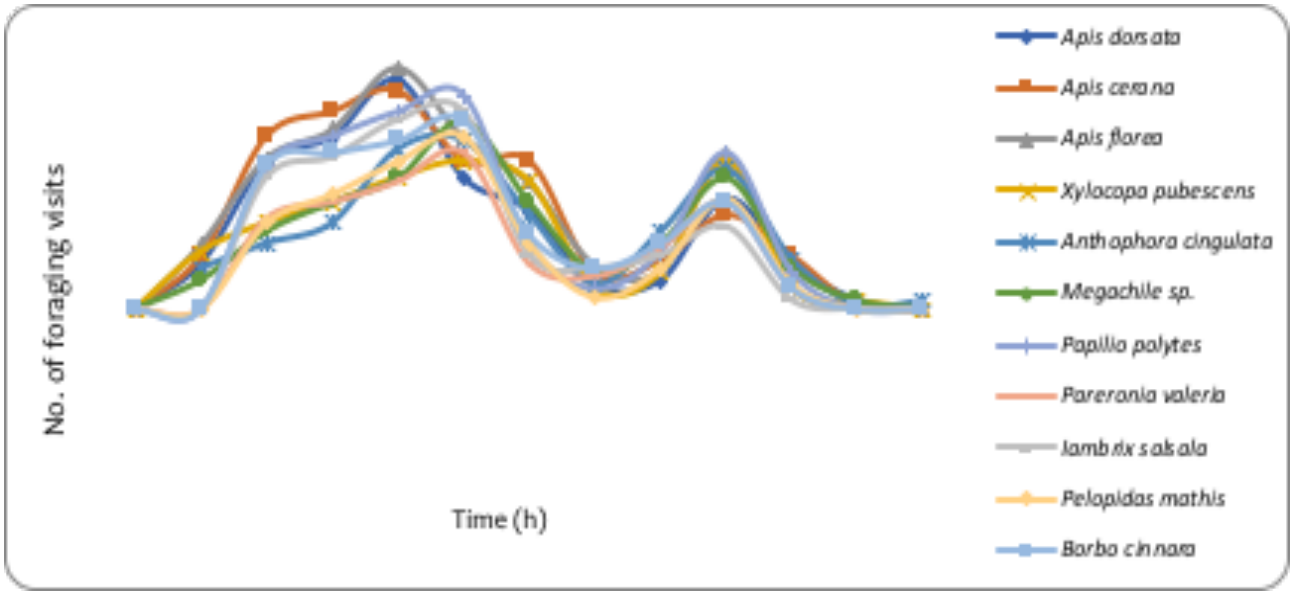

Figure 3. Hourly foraging activity of bees and butterflies on *Asystasia gangetica*.

the pollen is spread on the dorsal surface of the rear part of the head, thorax, and even the abdomen. Such a temporal difference in the contact between the nectar probing bee and the stigma and the anthers was viewed as an adaptation for the promotion of cross-pollination. In this foraging mode, if the bee is already pollen-laden, it affects cross-pollination, and if not, it is loaded with the pollen, carries it along, and pollinates another flower while collecting the nectar. In the same visit, the bees also collected pollen by rotating themselves around the throat to access the anthers. All bee species recorded were regular and consistent foragers to the flowers. Since *A. gangetica* has a mat-type population with numerous flowers on any given day, the bees mainly concentrated on this floral source. The bees foraged daily from 0700 to 1800 h with a peak activity occurring from 0800 to1300 h and again between 1500 and 1600 h (Figure 3); they made 56% of the total foraging visits. The butterflies recorded were not as regular and consistent as bees. They foraged exclusively for nectar legitimately from the front side of the flowers from 0800 to 1700 h with a peak activity between 0800 and 1300 h and again between 1500 and 1600 h (Figure 3); they made 44% of the total foraging visits.

As for the butterflies, *P. polytes* and *P. valeria*, theyinserted their long proboscis into the corolla tube and accessed the nectar with great ease when compared to hesperiid butterflies which have relatively short proboscis.

However, the hesperiids also accessed the nectar easily due to the wide nature of the corolla tube. During nectar collection, all these butterflies contacted the stigma first and the stamens later with their proboscis, antennae, and the forehead affecting cross- or self-pollination.

The depletion of nectar due to the nectar robbery practiced by *X. pubescens* appeared to be a driving force for the bees to make multiple visits to the standing crop of flowers, and such repeated visits to the same flowers did increase the pollination rate, especially the out-crossing. Therefore, the plant is principally melittophilous and supplemented by psychophily.

Fruiting and seed ecology:

Fruits mature rapidly within two to three

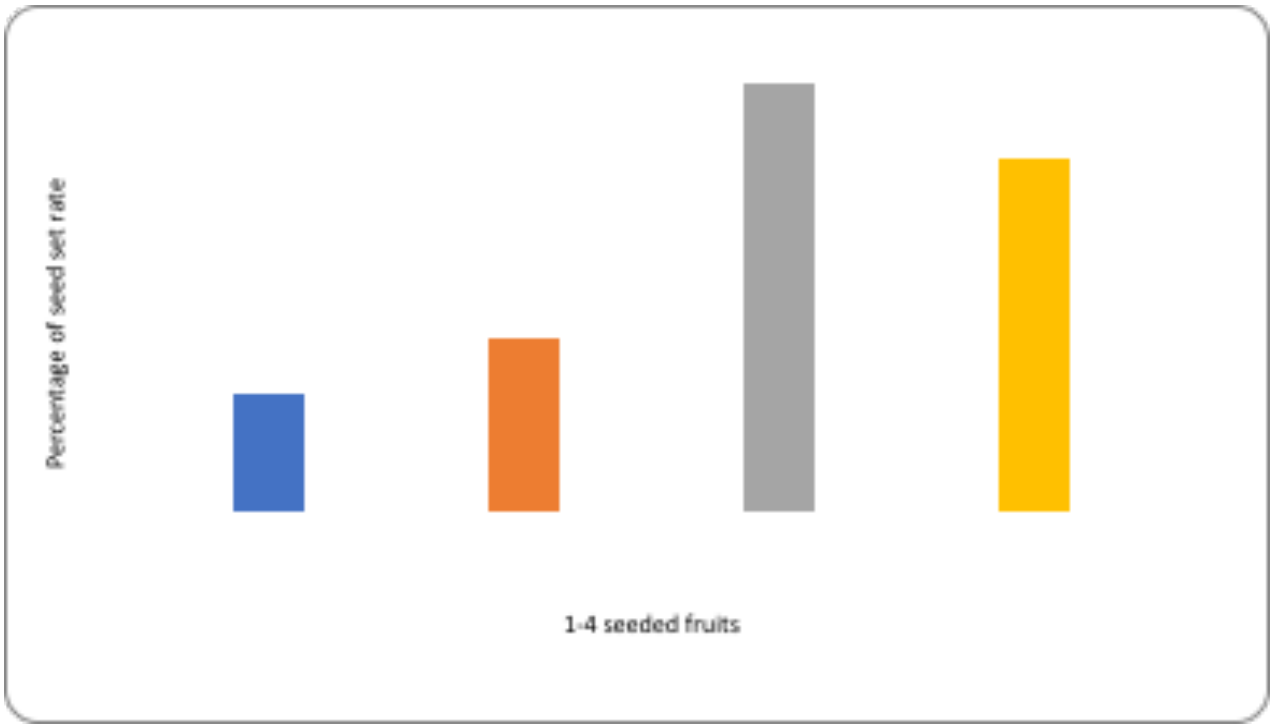

Figure 4. Percentage of seed set rate in *Asystasia gangetica.*

weeks. They are initially green (Figure 1k), brown and turn to black dry fruits at maturity. The fruit is an elongated 26-33 mm long capsule with two compartments, each compartment with zero to two seeds. The seeds are whitish to brownish black, circular, flattened, beaked, 5 mm long and 1 mm wide (Figure 1l). The seed set rate is 11% in one-seeded fruits, 16% in two-seeded fruits, 40% in three-seeded fruits and 33% in four-seeded fruits (Figure 4).

The dry capsules dehisce loculicidally with an explosive force around midday when sunlight is very intense, and the seeds released disperse to short distances only. Fruit dehiscence did not occur on rainy days due to the low ambient temperature and the high relative humidity. The seeds falling to the ground were subsequently dispersed by the rainwater. They are non-dormant, germinate immediately and form new plants if the soil is moderately wet. In addition to the seed propagation, the plant propagates by a vegetative mode through trailing stems that produce roots when nodes establish a contact with moist soils.

Therefore, the plant, with its ability to propagate through seeds and rhizomes, spreads easily and forms a huge mat-type population in open areas either in natural areas or in agricultural ecosystems.

Discussion

The color of the petal in *Asystasia gangetica* has been used by many authors to distinguish between the subspecies under this taxon (Kiew and Vollesen, 1997; Daniel and Figueiredo, 2009; Grubben, 2004; Danthanawanit *et al*, 2015). In this work, the A. gangetica populations showed the subspecies, alba and micrantha of which the former is the most common, while the latter is very rare. The color of the petal of the subspecies *alba* matches the description of the petal color provided by Grubben (2004), while that of the subspecies *micrantha* matches the description of the petal color provided by Danthanawanit *et al.*, (2015). In Acanthaceae, most of the species distributed in Mexico are self-compatible and are capable of self-pollination when pollinators are scarce (Daniel, 2004; 2008). In the present study, *Asystasia gangetica* subsp. *alba* is prominently protandrous, self-compatible, and self-pollinating as substantiated by the hand-pollination tests. The placement of stigma ahead of the anthers at the corolla throat is designed for promoting cross-pollination and the legitimately probing bees and butterflies also contact the stigma first and then the anthers which suggests that the plant is principally adapted for out-crossing, but the function of self-compatibility does not preclude the occurrence of autogamy and geitonogamy. However, the self-pollination modes are strictly vector-dependent indicating that the plant has evolved its breeding system destined for maximizing the cross-pollination rate and minimizing the self-pollination rate. Therefore, the *A. gangetica* subsp. *alba* is strictly facultatively xenogamous.

Olmstead *et al.*, (1993) stated that the characteristic of the fusion of petals into the sympetalous corolla is most prevalent and is key to the Acanthaceae family. Ritterbusch (1991) used the terminology of the “synstapetal” and “apostapetal” regions in the corolla; the former refers to the fusion of the proximal portion of the corolla and the stamens, while the latter refers to the condition of the free state of the stamens from the wall of the distal portion of the corolla. Manktelow (2000) identified four types of filament curtains, namely the phaulopsoid type, the corolla fold, the reduced type and the strobilanthoid type in Acanthaceae. In the present study, the *A. gangetica* subsp. *alba* as well as the subsp. *micrantha* represent the phaulopsoid filament curtain type in which the decurrent filaments form a barrier to the nectar. Manktelow (2000) also noted that most species representing the phaulopsoid type of filament curtain produce flowers characterizing the melittophilous syndrome. The present study found that both subspecies of *A. gangetica* have floral traits adapted to melittophily. The flowers open during early morning indicating that they are destined for

pollination during daytime. The floral traits of the subsp. *alba* such as the tubate corolla with the anthers at the corolla throat and the stigma situated beyond the height of anthers, the moderate volume of the nectar with a high sugar concentration, and the presence of an energetically rewarding sucrose-rich nectar with several essential and non-essential amino acids are adaptations for bee-pollination (Baker and Baker, 1983; Opler, 1983). The production of sucrose-rich nectar by *A. gangetica* has also been reported by Freeman *et al.*, (1991). Furthermore, the pollen of the subsp. *alba* is an important source of several essential and non-essential amino acids and proteins. The field study showed that bees exhibit fidelity to this floral source to collect both nectar and pollen and in this process, they affect both self- and cross-pollination. The carpenter bee, *Xylocopa pubescens*, collects only nectar illegitimately by making a puncture at the base of the corolla tube, and hence it is a mere nectar robber. However, its nectar-robbing behavior compels other bees, which collect the forage legitimately by probing the flowers from the front side of the corolla throat, to make multiple visits and contribute to the promotion of pollination rate, mostly cross-pollination. Additionally, while collecting nectar legitimately from the flowers of *A. gangetica* subsp. *alba* as well as the subsp. *Micrantha,* butterfliesaffect self- and/or cross-pollination, but they are not as regular and consistent as bees, and hence they are supplementary pollinators. Therefore, the *A. gangetica* subspecies are primarily melittophilous but psychophily is supplementary.

Murali *et al*. (2013) reported that the *A. gangetica* flowers attract bees, black ants, and butterflies regularly in South India. Lob *et al.* (2017) reported that the *A. gangetica* pollen is the most dominant pollen stored in the colonies of the meliponine bee, *Heterotrigona itama*, in Malaysia. Tan and Khew (2012) reported that the *A. gangetica* subsp. *micrantha* is intricately designed for bee pollination and butterflies use it as a source of nectar . Deepika *et al.* (2014) reported that *A. gangetica* is a nectar host plant for papilionid *Papilio polytes, Princeps demoleus*, the pierid *Pareronia valeria*, and the hersperiid *Borbo cinnara* in Visakhapatnam, India. The present study also found that the *A. gangetica* subspecies attract bees and butterflies which while collecting nectar and/or pollen affect pollination, and hence this characterizes a mutualistic relationship between the plant and the bees/ butterflies; the former for pollination and the latter for food.

Josekutty *et al.* (2002) reported that *Asystasia gangetica* grows rapidly, reproduces excessively, and prevails as an invasive weed in the areas they were introduced to. Kamemoto and Storey (1955) stated *A. gangetica* produces seeds profusely under a wide range of environmental conditions, ranging from wet to dry and from sunny to shady conditions. It propagates vegetatively very easily by means of cutting. The present study found that the *A. gangetica* subsp. *alba* is an invasive species when occurring in both agricultural and non-agricultural open areas. The invasiveness functions through both the sexual and asexual system, the former through facultative xenogamy andthe latter through rhizomes. The characteristics of explosive fruit dehiscence and non-dormant seeds enable the plant to produce several batches of plants in the same growing season. Furthermore, the seeds dispersed through fruit explosion migrate to distant areas via rainwater. Therefore, the dual mode of reproduction and the flexible facultative xenogamous mating system ensure the plant'sformation of huge populations within a short period of time even in the absence of its pollinators and susutains it as a successful invasive weed.

Elliot *et al*. (2004) recommended *Asystasia gangetica* for cultivation as a cover crop in mature oil palm plantations because it fulfills several conditions such as its quick covering of the land, and the ability to increase the N, P, K available in the soil. Different authors reported that *A. gangetica* is an important forage source for bees and butterflies (Tan and Khew, 2012; Murali *et al.,* 2013; Rodriquez-Girones *et al.,* 2013; Deepika *et*

al., 2014; Lob *et al.,* 2017). This plant is also the larval host for the nymphalid butterflies *Doleschallia bisaltide pratipa, Hypolimnas bolina bolina, H. bolina jacintha* and *Junonia orithya wallacei* (Tan and Khew, 2012), *Hypolimnas misippus* (Jayasinghe *et al.,* 2014), *Precis almana* and *P. hierta* (Harinath and Venkata Ramana, 2014), and the hesperiid butterfly *Celaenorrhinus leucocera* (Churi *et al.,* 2020). These reports indicate that *A. gangetica* is important in increasing soil fertility, providing pollen and/ or nectar for local insects and is useful as a larval host plant for several butterflies. The present study proves that this plant, being a prolific weed, covers the soil and prevents soil erosion, and hence is useful for consideration in the restoration of ecologically destroyed, degraded, and damaged ecosystems and natural areas. Therefore, *A. gangetica* as a C3 plant with a less efficient carbon fixation system, as mentioned by Hnatiuk (1980), is ecologically valuable and useful in carbon sequestration in the context of global warming and climate change problems regradles of being a menace, especially in agricultural areas.

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