Pollination ecology of the annual herb *Hedyotis corymbosa* (*Rubiaceae*)

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Abstract. *Hedyotis corymbosa* is an annual herb that grows in open, moist and highly disturbed habitats during the wet and winter seasons. It is isostylous, weakly protandrous, self-compatible, auto-selfing and entomophilous. The natural fruit set is >90 % but only half the ovules produce seeds, which could be due to unfertilized ovules, selective abortion of low-quality seeds arising from self-pollination, and state of nutrient environment in the soil. The fruits are non-fleshy, erect, cup-like capsules, which dehisce loculicidally. Seed dispersal is anemo-, baro-, ombro-, hydro-, and anthropochorous. The seeds are dormant, germinate and produce new plants during the wet season. Therefore, the study suggests that this plant is evolved to complete its entire life cycle seasonally.

Key words: *Hedyotis corymbosa,* auto-selfing, entomophily, anemochory, barochory, ombrochory, hydrochory, and anthropochory.

Introduction

Rubiaceae is one of the largest angiosperm families distributed mainly in the tropics and sub-tropics of the world (Eriksson & Bremer 1991). The species of this family have remarkable ecological, economic and taxonomic importance (Perveen & Qaiser 2007). The genus *Hedyotis* with 515 species belongs to this family and is distributed in the tropical and sub-tropical regions worldwide, mainly in Australia, Africa, East and Southeast Asia, and the Americas. It is very variable and includes annual or perennial herbs, sub-shrubs, weak stragglers, weak climbers, shrubs or small trees (Vaes & al. 2006; Viswanathan & Manikandan 2008; Tao & Taylor 2011; Wikstrom & al. 2013). In India, there are about 75 species, many of them restricted to the hill areas of South India, especially the Western Ghats (Dutta & Deb 2004). These species are important constituents of the herbaceous layer in open habitats, at roadsides and in agricultural fields.

Hedyotis has a wide range of breeding systems, including distyly, dioecy and herkogamy (Robbrecht 1988; Wagner & Lorence 1998; Ko 1999). Pollen dimorphism occurs with respect to size, shape and exine characteristics in some distylous species of *Hedyotis* (Naiki & Nagamasu 2004; Castro & al. 2004). *H. nigricans* is distylous and displays heteromorphic incompatibility, which precludes self- and intra-morph cross-fertilization and allows only inter-morph cross-fertilization (Ornelas & al. 2004). *H. salzmannii* is distylous, self-compatible and pollinated by bees and flies (Riveros & al. 1995). *H. acutangula* is dimorphic, distylous, cryptically self-incompatible and dominated by legitimate (inter-morph) mating (Wu & al. 2010). *H. pulcherrima* is isoplethic and contains pin and thrum flowers at 1:1 ratio. The two flower morphs exhibit a precise reciprocal herkogamy (Liu & al. 2012). *H. corymbosa* has supposedly originated in Africa and subsequently spread across the tropics (Burger & Taylor 1993). Characteristically, it has variable morphological characters and loculicidal capsules with very small-

sized oldenlandoid seeds. It grows in sandy soils, even in areas with pebbles and is often subjected to humidity of the water courses. During monsoon, it is also found frequently in agricultural fields across India, Sri Lanka, tropical East Asia, and up to Java and the Philippines (Mammen & Daniel 2011; Juan Antonio & al. 2013). This species is represented by two varieties. On the basis of the morphological characters of leaves, flowers, fruits and seeds, Pramod Kumar (2008) has classified *H. corymbosa* into two varieties: *corymbosa* and *linearis*. However, Sivarajan & Biju (1990) maintained that *H. corymbosa* represents only var. *corymbosa,* while *H. erecta* represents var. *linearis*. Lewis (1964) described three races in *H. corymbosa:* diploid, tetraploid and hexaploid. There is no information on any aspect of reproduction or regeneration in *H. brachiata*, even not on its taxonomy. With this backdrop, the present study is expected to provide information on the pollination ecology of *H. corymbosa,* so as to understand its invasiveness as a weed.

Material and methods

The seasonal annual herb, *Hedyotis corymbosa* was selected for study during 2014–2016, in Visakhapatnam and its surroundings, Andhra Pradesh, India (17°42'N and 82°18'E). The inflorescence type and the number of flowers per inflorescence were noted down. Ten inflorescences were tagged prior to commencement of their flowering and studied daily for recording the flowering duration of each inflorescence. Twenty-five fresh flowers were used to record the floral details such as flower shape, colour, odour, sex, symmetry, floral mechanism, calyx, corolla, stamens and style, and stigma and ovule number. Ten inflorescences, which have not initiated flowering, were tagged and observed daily to record the duration of flowering, anthesis schedule and timing of anther dehiscence. Twenty-five fresh flowers were used to record their morphological details. Nectar cannot be measured and analyzed due to its secretion in minute quantities, which are further depleted by thrips during mature bud and flower life. Twenty mature, but undehisced anthers were collected from different plants and examined for pollen output, according to the protocol described by Dafni & al. (2005). Pollen output per flower and pollen-ovule ratio were calculated according to the formulas described by Cruden (1977). Ten flowers, eachfrom five individuals, were used to test the stigma receptivity. It was tested with hydrogen peroxide from the mature bud stage to flower closure/drop, according to Dafni & al. (2005). Furthermore, the receptivity was also traced out visually: whether the stigma was shiny, wet or changing colours, or withering. Insects foraging at the flowers were observed from morning to evening on four different days for their mode of approach, landing, probing behavior, and contact with the floral sexual organs. Bees, wasps and flies were identified with the representative specimens available at the Department of Environmental Sciences, Andhra University, Visakhapatnam. Butterflies were identified by consulting the books of Kunte (2007). The foraging visits of insects were recorded on a 2 × 2 m area of a flowering patch, for 10 min at each hour of the entire day, on four different days, and the data was tabulated to record the foraging pattern and the percentage of visits made by them. The pollen/nectar collection behaviour of insects was carefully observed, so as to assess their role in effecting pollination. Ten specimens of each insect species were captured during their peak foraging and brought to the laboratory. Each specimen was washed in ethyl alcohol, stained with aniline-blue on a glass slide and observed under microscope to count the number of pollen grains carried by it. Thus, the average number of pollen grains carried by each insect species was calculated to find out the pollen carryover efficiency. Plants that have not initiated flowering were tagged and observed to record fruit and seed set rate in open pollinations. Fruit maturation period and fruit and seed morphological characteristics were recorded to evaluate the adaptations for dispersal by different means. Furthermore, the seed dispersal modes were examined in the field. The aspects of seed germination and establishment of populations were observed briefly in the field.

Results

Flowering phenology: The plant is a spreading, suffuruticose, low, annual, erect, glabrous, herbaceous weed, with sessile, less than 5 mm broad linear leaves. It grows in open, moist and highly disturbed habitats during the rainy and winter seasons; it also grows in summer, in areas where the soil is sufficiently wet. Generally, it is found in agricultural fields during the

rainy season. The plant is multi-stemmed and branches out in prostrate form, carpeting the sandy soils. Seeds germinate during July-February. Flowering intensity is confined to September-November (Fig. 1a). The inflorescence is a 2–5 flowered pedunculate cyme, anthesing in 3-4 days and axillary in position.

Flower morphology: The flowers are pedicellate (1 cm long), funnel-shaped, and stand out prominently against the foliage. They are small (3.15 ± 0.5 mm long and 2.45 ± 0.4 mm wide), white, odourless, actinomorphic, and bisexual. The calyx consists of four 1 mm long and 1 mm wide green, triangular-lanceolate and glabrous sepals, which are fused at the base. The corolla is white with blue tinge, funnel-shaped (2 mm long), tubular with a ring of pubescent hairs inside and 4-partite at the top, with spathulate lobes. The stamens are four, white, inserted, adnate to the throat of the corolla tube; the filaments are short (0.8 to 1 mm long), pubescent, the anthers are 0.6 to 1 mm long, dithecous and stand in close proximity to the stigma. The ovary is bicarpellary, bilocular, syncarpous, with 56.1 ± 10.99 ovules (Fig. 1g) arranged on axile placentation. The style and stigma are white, with yellow tinge and spring up from the center of the flower; the style is 1.5-2.5 mm long and terminates in 0.3 to 0.5 mm long capitate-like papillose bifid stigma (Fig. 1f).

Floral biology: The mature buds slowly bulge (Fig. 1b,d) and open at 07.00–09.00 h and the anthers dehisce in mature bud stage by longitudinal slits. The pollen grains are white, powdery, oblate-spheroidal, tricolporate, 22.23 × 21.6 µm in size, the ornamented and thick sexine is composed of bacula and reticulations (Fig. 1e). The pollen output is 871.4 ± 68.77 per anther and 3485.6 ± 275.09 per flower. The pollen-ovule ratio is 62:1. The close proximity between the stigma and anthers facilitates autonomous selfing during mature bud and flower life (Fig. 1c). The stigma is semi-wet, shiny and receptive after anthesis and ceases by about 15.00 h of the same day. The nectary disc present around the ovary secretes nectar in traces only. The flowers close back partially at 16.00 h. The petals, stamens, style and stigma fall off on the next day, while the calyx gradually bulges into a fruiting calyx.

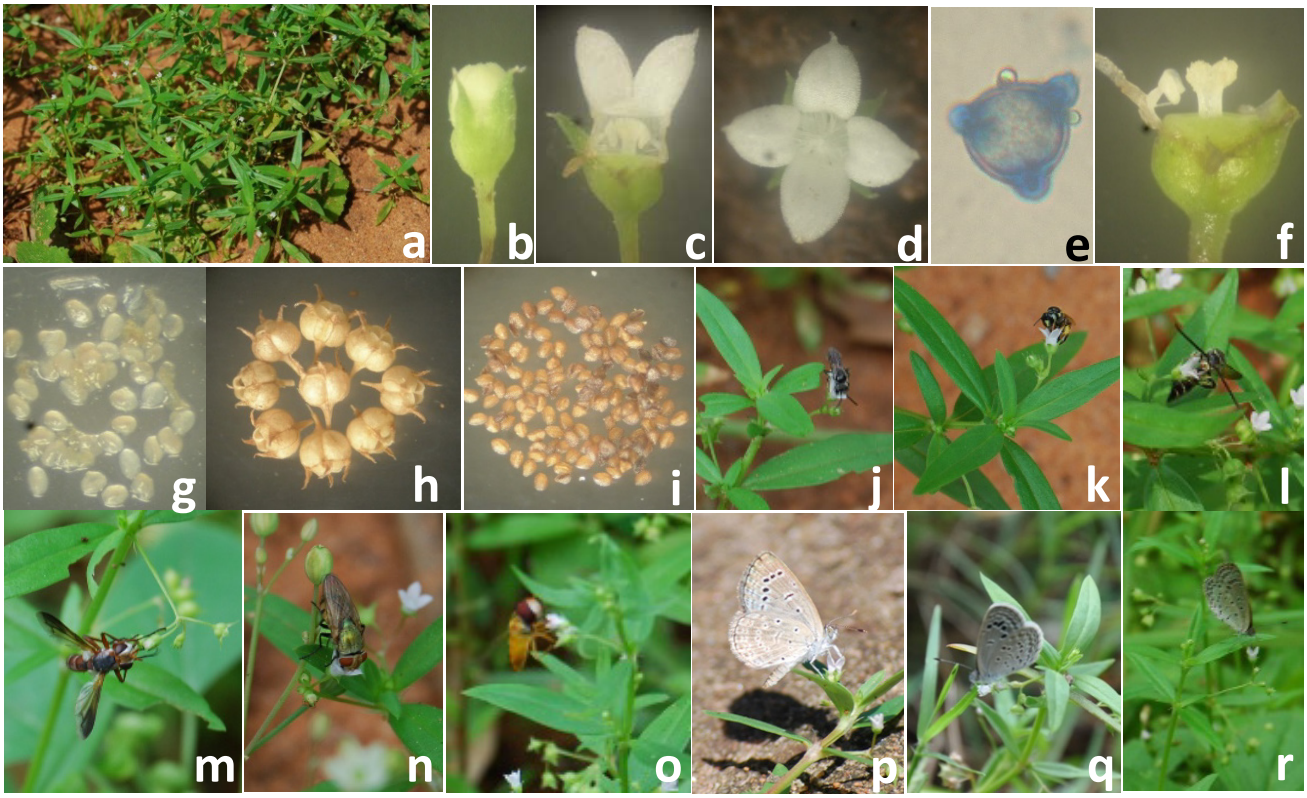

Fig. 1. *Hedyotis corymbosa*: **a.** Flowering phase, **b.** Mature Bud, **c.** Position of stamens and stigma in flower, **d.** Upper view of flower, **e.** Pollen grain, **f.** Bifid stigma, **g.** Ovules, **h.** Mature and dry fruits, **i.** Seeds, **j.** *Trigona iridipennis*, **k.** *Ceratina* sp. **l.** *Campsomeris* sp., **m.** Wasp (unidentified), **n.** *Musca* sp. , **o.** *Eristalinus* sp., **p.** *Zizeeria karasandra*, **q.** *Zizina otis*, **r.** *Zizula hylax*.

Foraging activity: Thrips used flowers for pollen and nectar; while collecting the forage, they effected pollination. The flowers were indiscriminately foraged by bees, wasps (Hymenoptera), flies (Diptera), and butterflies (Lepidoptera) at 08.00–14.00 h, with highest activity at 10.00–12.00 h (Fig. 2). The bees were *Trigona iridipennis* (Fig. 1j) and *Ceratina* sp. (Fig. 1k). The wasps were *Campsomeris* sp. (Fig. 1l) and one unidentified sp. (Fig. 1m). The flies were *Musca* sp. (Fig.1n) and *Eristalinus* sp. (Fig. 1o). The butterflies were exclusively Lycaenids, *Zizeeria karsandra* (Fig. 1p), *Zizina otis* (Fig. 1q), *Zizula hylax* (Fig. 1r), *Freyeria trochylus,* and *Chilades pandava*. Of these, bees foraged for both pollen and nectar, while all other insects foraged for nectar only. Bees and lycaenid butterflies were regular foragers, while all other insects were occasional foragers, although all effected pollination. Bee visits accounted for 21 %, wasps for 18 %, flies for 17 %, and butterflies for 44 % of all insect visits (Fig. 3). While probing and collecting the forage from the flowers, they contacted the stigma and stamens effecting pollination. The body washings of these insects revealed that both regular and occasional foragers carry pollen to a different extents, with *Ceratina* sp. (bee) carrying the highest number of pollen grains (Table 1).

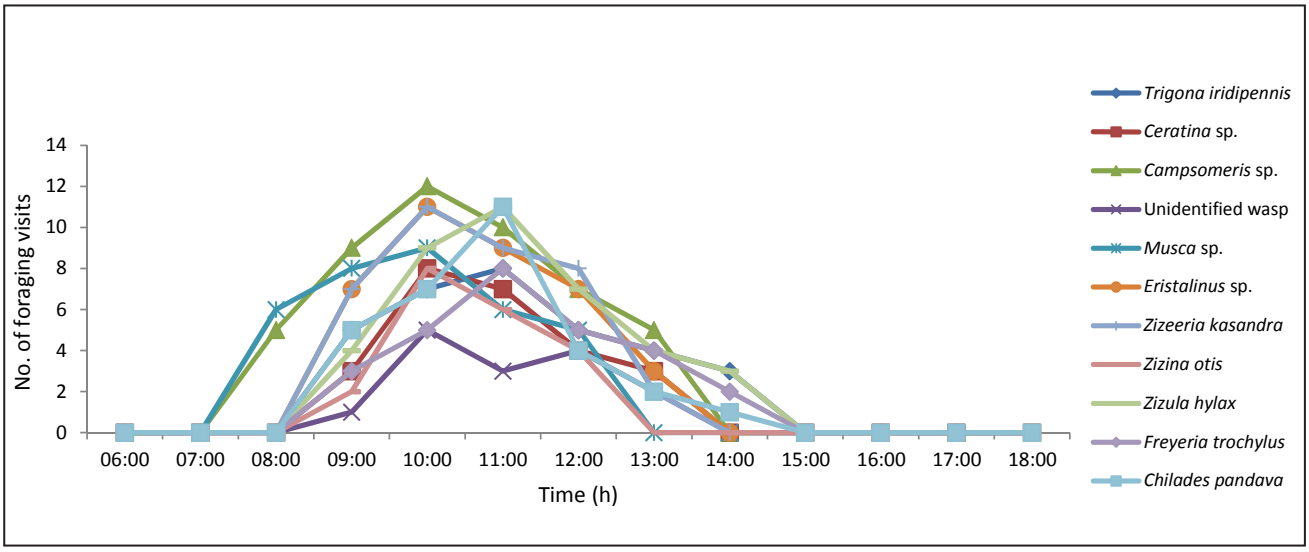

Fig. 2. Hourly foraging activity of insects on *Hedyotis corymbosa.*

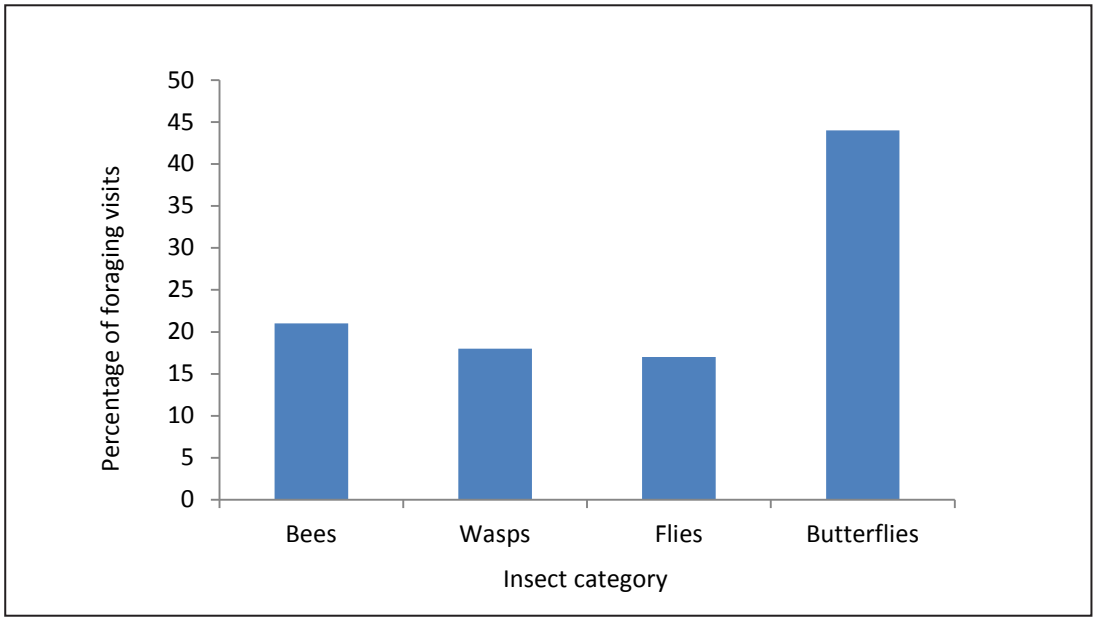

Fig. 3. Percentage of foraging visits of different categories of insects on *Hedyotis corymbosa.*

Table 1. Pollen recorded in the body washings of insects on *Hedyotis corymbosa*.

Insect species	Sample size (N)	Number of pollen grains		
		Range	Mean	S.D
Trigona iridipennis	10	37–83	54.6	14.28
Ceratina sp.	10	52–102	71.2	16.71
Campsomeris sp.	10	21–64	43.4	12.62
Wasp (Unidentified)	10	26–57	35.9	9.94
Musca sp.	10	11–45	27.6	10.30
Eristalinus sp.	10	15– 61	38.4	13.32
Zizula hylax	10	14–53	30.9	11.31
Zizeeria karsandra	10	25–66	42.9	13.03
Zizina otis	10	16–49	30.4	9.99
Freyeria trochylus	10	21–54	38.9	10.23
Chilades pandava	10	11–61	35.6	15.48

Fruiting behaviour: Fruits were produced within three weeks. The peduncle of the inflorescence and pedicels of flowers elongated rapidly and markedly as fruits develop. The natural fruit set rate was 93 to 98 %. The fruit was a non-fleshy ovoid or globose, bilocular, membranous, glabrous, 2.5 × 2.8 mm capsule (Fig. 1h). The seed set rate per fruit was 50.25 ± 7.92 %. Mature and dry fruits dehisced loculicidally into two valves releasing seeds into the air. The seeds were then carried away by the prevailing wind and also by gravity. Rain water drops falling into the fruit capsule caused ejection of seeds, which were subsequently carried away by flowing water. Furthermore, seed dispersal was prompted by human activities. The seeds were pale-brown, tiny, 3-ridged shining, smooth, exotesta reticulate, with a straight wall, and 0.5 × 0.6 mm in size (Fig. 1i). They were dormant and germinated only during the rainy season to produce new plants.

Discussion

Pramod Kumar (2008) classified *Hedyotis corymbosa* into two varieties, *corymbosa* and *linearis,* on the basis of morphological characters of the leaves, flowers, fruits, and seeds. However, Sivarajan & Biju (1990) maintained that *H. corymbosa* represented only var. *corymbosa,* while *Hedyotis erecta* represented var. *linearis* in India. In line with that report, the present study treated *H. corymbosa* as belonging to var. *corymbosa*, and hence, it is mentioned as *H. corymbosa* throughout the text. Burger & Taylor (1993) reported that this species supposedly originated in Africa and subsequently has spread across the tropics. Lewis (1964) noted that the pollen of *H. corymbosa* varies greatly in size, aperture number and sexine; on the grounds of these criteria, he distinguished three different races based on the pollen characters: diploid, tetraploid and hexaploid. On the basis of polyploidy and pollen morphology, this author stated that East Africa was the center of origin of the diploid race, which is now absent here. It spread in two directions before decay: westwards to West Africa, where it is very common, and subsequently to the Americas; and eastwards to South Asia and the western Pacific Ocean region, with a remaining relict in the Seychelles. In the center of origin, currently the tetraploid race is dominant because of the absence of a diploid race. According to his explanations, in that ancient area of distribution, there was ample time for the diploid race to mutate into a tetraploid race. The process was followed by a successful competition of the tetraploid mutant with the diploid race and subsequent elimination of the diploid and dominance of the tetraploid race. Furthermore, the author found another hexaploid mutant, just a few miles away from the known diploid and tetraploid races in West Africa. He suggested that the hexaploid mutant probably

dominated in that region, particularly along the coast. All this indicates that the diploid form has migrated to South Asia and the Western Pacific region, to West Africa and, eventually, to the Americas, and decayed in the area of origin. Furthermore, the tetraploid race has also migrated to South Asia, where it has become the dominant race, and subsequently to South and West Africa, but the diploid race is still dominant in West Africa. Studied presently on the Indian territory of South Asia, *H. corymbosa* exhibits the pollen characters defined for the diploid race, *sensu* Lewis (1964) and, hence, the diploid race is dominant in the study region. However, detailed studies of the pollen characters of *H. corymbosa* from different regions of India are required, so as to find out the extent of prevalence of the diploid race and the other two races.

Anderson (1973) reported that in hermaphroditic isostylous flowers, protandry is predominant; the pollen matures early and is shed during or soon after anthesis. Before anthesis and in some cases in a period after anthesis, the elongation of the style is arrested and the immature stigmas are temporarily retained within the tube of the corolla, below the level of the anthers. During and/or after the release of pollen, the style elongates, eventually equaling or surpassing the anthers, and the stigmas mature belatedly. In *H. corymbosa*, the flowers are isostylous, weakly protandrous and self-compatible. A close proximity of dehisced anthers with the receptive stigma facilitates occurrence of autonomous selfing but it is not absolute. In this context, mention deserves the fact that thrips, which use the flower buds for breeding, also serve as foragers of both pollen and nectar during flower life and their foraging activity ensures occurrence of selfing within and between flowers of the same (geitonogamy) or different individuals nearby (xenogamy). The position of both stamens and stigma at the mouth or rim of the corolla tube facilitates the occurrence of self- or cross-pollination during flower probing by the visitors for pollen and/or nectar. The flowers attract small bees, wasps, flies and small lycaenid butterflies, of which only bees and butterflies are regular pollinators. Their pollination role is supported by the record of considerable number of pollen grains on their bodies. The plant characters, such as patchy growth pattern, production of a few flowers each day by individual plants, and nectar and pollen depletion by thrips, compel the regular and occasional foragers to visit the same flowers repeatedly in the same and different plants, promoting both self- and cross-pollination rate to produce enhanced levels of natural fruit and seed set rates. The seed set rate recorded in this species indicates that not all ovules produce seeds, despite autonomous selfing and insect activity**.** This situation could be due to unfertilized ovules, selective abortion of low-quality seeds arising from self-pollination, and state of the nutrient environment in the soil.

Neupane & al. (2009) noted that in *H. scandens*, the capsule has a beak protruding beyond the calyx lobes and it is loculidically dehiscent from the top and then divides septicidally into two valves. These authors mentioned that the capsules are indehiscent in *H. corymbosa*. Randall (2002) reported that in *H. corymbosa*, the seed is very small and is likely to be dispersed by gravity or wind. Human-mediated dispersal is also possible as a result of the transport of seeds in contaminated machinery, produce and soil from the agricultural fields. In the present study, it was found that *H. corymbosa* dehisces fruits loculicidally into two valves and releases seeds into the air. The seeds disperse by four different means: anemochory, barochory, ombrochory, and hydrochory. Furthermore, human-mediated seed dispersal is also possible due to the plant's occurrence in agricultural fields. These modes collectively enable *H. corymbosa* to be a colonizer species, especially in open, moist and highly disturbed habitats, and also in agricultural fields. This study indicates that the species apparently has developed adaptations to grow in agricultural and non-agricultural areas, maximize seed production by means of local bees and lycaenid butterflies every year, and subsequently colonize the favourable areas during the growth season. Individual plants flower for a few weeks but at population level they appear in flowering for two seasons: the wet and the winter seasons. This situation appears to be a function of the fixed period of seed dormancy, which germinates during the wet season as an invasive weed.

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