Reproductive ecology of *Tephrosia purpurea* and *T. villosa* (*Fabaceae*)

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Abstract. *Tephrosia purpurea* and *T. villosa* display vegetative growth and sexual reproduction throughout the year. *T. purpurea* produces resupinate and non-resupinate flowers with an explosive pollination mechanism, while *T. villosa* produces exclusively non-resupinate flowers with a valvular pollination mechanism. Flower-tripping is essential for the function of pollination mechanism and occurrence of self- or cross-pollination; tripping is effected by wind, rain, temperature, thrips, bees and wasps. Both species are facultatively xenogamous. Empty pods, ill-formed seeds and variation in the number of produced seeds has been attributed to the internal regulation by plants, in order to allow cross-pollinated flowers to produce more seeds and abort selectively the genetically inferior pollen resulting from self-pollination. The low fruit and seed set rates recorded in open-pollinated flowers of both *Tephrosia* species have been attributed to the presence of untripped flowers, extent of cross-pollination rate, short tenure of pollen viability, number of foraging visits made by pollinating insects, flower closure by the end of the day of anthesis, and availability of energy resources. Both species are autochorous, with short-range seed dispersal. Seeds germinate if the soil has moisture and nutrients but their continued growth and development is largely related to their efficiency in utilizing the available nutrients over other co-occurring seedlings or plants growing simultaneously with them. The plants also sprout from the perennial root stock, usually during rainy season. Therefore, the dual modes of propagation ensure these species' recolonizing of the same habitat and invading new habitats.

Key words: autochory, entomophily, explosive pollination mechanism, facultative xenogamy, resupinate flowers, *Tephrosia*, valvular pollination

Introduction

In the subfamily *Faboideae* of the *Fabaceae* family, the most distinctive morphological feature is the highly specialized papilionate flower. This flower, with clearly differentiated one standard, two wing and two keel petals, and partially or entirely fused staminal tube enveloping the ovary, is typically distinct from the mostly radially symmetrical mimosoid flower and the generally bilateral but non-papilionate caesalpinioid flower. Furthermore, this floral organization involves a strong bilateral symmetry and three-dimensional depth that often limit access to the nectar and pollen; such specialized petal and sex organs arrangement is closely associated with bee pollination (Hutchinson 1926; Arroyo 1981; Westerkamp & Claβen-Bockhoff 2007). In this subfamily, Lavin & Delgado (1990) described a ciliate style, ciliate and penicillate stigma, and some pollen-brush types of styles. Brummitt (1980) reported that the genus *Tephrosia* is the only member of the Millettieae tribe in this subfamily that includes species with a pollen brush designated as "barbistyled Tephrosias". Faegri & van der Pijl (1979) mentioned that the pollen brush is not just a physical aggregation of erect trichomes emanating from the style, but also a functional aggregation involved in secondary pollen presentation.

The genus *Tephrosia* with over 400 species of annual and perennial woody herbs is distributed in tropical, subtropical and arid regions of the world, with more species distributed in Africa and Australia. It is valued for its ability to increase nitrogen content in soil from root nodules in symbiotic association with the *Rhizobium* bacteria (Bosman & De Haas 1983; Zhi & Pedley 2010). This genus is a source of a number of phytoconstituents, such as flavonoids, terpenoids, sterols, and rotenoids. These chemicals have diverse pharmacological impact, such as hepatoprotective, antidiabetic, antioxidant, antihyperlipidemic, antiulcer, antibacterial, antifungal, larvicidal, anti-inflammatory, wound healing, anti-cancer, and antifeedant effect (Samuel & al. 2019). In spite of the ecological, commercial and medicinal values of the genus, there is very little information available on the reproductive ecology of its species; this information is important for understanding their propagation, expansion and distribution across the globe. *T. vogelii* is self-pollinated, without external agents, but some large bees, such as *Xylocopa brasilianorum,* increase the amount of self-pollination (USDA Technical Bulletin 1970). *T. purpurea* is native to tropical Asia and found from India and Sri Lanka to South China, and from Southeast Asia to tropical Australia and the Polynesian Islands, but now it is naturalized and cultivated pantropically (Bhatnagar 1986). It is distributed across India (Dalwadi & al. 2014) and is used as an important constituent in several herbal medicines for treatment of different diseases related to stomach, heart, liver, lungs, skin, blood, diabetes, and cancer (Soni & al. 2006). The Lycaenid butterfly, *Catochrysops crabo* uses *T. purpurea* as its larval host plant and also acts as a pollinator for this plant in the Maruthamali Hills of Southwestern Ghats of India (Jothimani & al. 2014). The butterflies *Papilio demoleus* (Papilionidae), *Eurema hecabe, Catopsilia pomona* (Pieridae), *Junonia hierta* (Nymphalidae), *Leptotes plinius, Jamides celeno, Pseudozizeeria maha, Acytolepis puspa* (Lycaenidae), and *Pelopidas conjuncta* (Hesperiidae) use *T. purpurea* as a nectar host plant in the Pune region of Maharashtra, India (Nimbalkar & al. 2011). In this species, the papilionaceous flowers with a special type of flower structure offer a suitable micro environment for foraging, feeding, breeding, and an oviposition site for the thrips species, *Megalurothrips distalis* and *Frankliniella schultzei*. Their movements inside the keel petals enable pollen transfer to the receptive surface of the capitate stigma and, hence, they act as important pollinators (Annadurai & Velayudhan 1986). Rao (1977) reported that *T. purpurea* is a predominantly self-pollinating species. Kumari & Sharma (2018) reported that *T. purpurea* flowers are chasmogamous, with high percentage of viable pollen, pod and seed production. *T. villosa* is a widespread species distributed in tropical and subtropical Africa, West Pakistan, India, Sri Lanka, Indo-China, Java, Flores, and possibly is native to India (Bosman & De Haas 1983). Its populations are severely fragmented and mature individuals are declining continually, but this species is included in the Least Concern category of IUCN Red List (Groom 2012). There is a total lack of information on any aspect of the reproductive ecology of this species. Considering the widespread distribution, ecological and medicinal importance of *T. purpurea* and *T. villosa*, the present study has been aimed at elucidating their flowering phenology, floral biology, sexual system, breeding systems, pollination, pollination mechanisms, fruiting behavior, seed dispersal, and seedling ecology. These species are ecologically important in various dry and semi-dry habitats and in carbon sequestration and, finally, they have their say as glue of biodiversity.

Material and methods

Tephrosia purpurea and *T. villosa* growing in wild patches still available in the Andhra University Campus (17°42'N and 82°18'E) were selected for study during the period April 2018 –September 2019. Regular visits to the populations of these species were made to record flowering and fruiting seasons. Ten inflorescences, which were about to initiate flowering on five plants, were tagged and followed 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 floral morphological aspects, 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 the probing and forage collection activities of insects. Ten mature buds, two each on five plants, were bagged and tagged to measure nectar volume and sugar concentration using the protocols provided by Dafni & al. (2005). A micropipette was inserted into the flower

base to extract nectar for measurement. The average nectar of ten flowers was taken as the total volume of nectar/flower and expressed in µl. Hand Sugar Refractometer (Erma, Japan) was used for this purpose. Nectar analysis for sugar types was done according to the Paper Chromatography Method described in Dafni & al. (2005). Ten mature but undehisced anthers were collected from five plants and placed in a Petri dish. Subsequently, the anthers were taken out one by one and placed on a clean microscope 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 under a compound microscope (40× objective, 10× eye piece). This procedure was followed for counting the number of pollen grains in each collected anther. Based on these counts, the mean number of pollen grains produced per anther was determined. The mean pollen grain 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 microscope for recording the pollen grain features. Twenty ovaries, five each from four plants were examined under microscope for recording the range and average number of ovules per flower. The pollen-ovule ratio was determined by dividing the average of the number of pollen grains per flower by the average number of ovules per flower. The obtained value was taken as pollen-ovule ratio. *In vitro* pollen viability tests were made at selected intervals on the day of anthesis, using 10 % sucrose solution because it showed maximum pollen germination. The stigma receptivity was observed visually and by a H_2O_2 test. In the visual method, the physical state of the stigma (wet or dry) was considered to record the commencement of receptivity; its colour change and withering were taken as loss of receptivity. A H_2O_2 test, as given in Dafni & al. (2005), was applied for confirmation of the stigma receptivity period. Breeding systems were assessed based on the results from hand-pollination tests. The number of flowers used for each mode of pollination and open pollination for the fruit and seed set was recorded in Table 1. The flowers were bagged without hand pollination for spontaneous autogamy. The stigmas were pollinated with pollen of the same flower manually for manipulated autogamy, with the pollen of different flowers of the same plant after emasculation for geitonogamy, and with pollen from the flowers of other plants after emasculation for xenogamy. All these modes of pollination were followed for one month to record the percentage of fruit and seed set rates in each mode. Five inflorescences, each from twenty plants, were tagged prior to the initiation of flowering and followed to record fruit and seed set rate in open pollinations. The fruit maturation period and fruit and seed characteristics were recorded. Field observations were made regularly to record the fruit and seed dispersal mode. Casual observations were also made to record whether the seeds after their dispersal germinate immediately or not. Seventy-five 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 during February was kept under observation for their germination in late February and March 2019. Based on the number of germinated seeds, seed germination

Table 1. Results of the breeding systems in *Tephrosia purpurea* and *Tephrosia villosa*.

Treatment	Number of flowers sampled	Number of flowers with fruit	Fruit set (%)	Seed set (%)
Tephrosia purpurea				
Autonomous autogamy	25	8	32	25*
Manipulated autogamy	25	15	60	27*
Geitonogamy	25	17	68	34*
Xenogamy	25	22	88	75
Open pollination (Inflorescences tagged before initiation of flowering)	70	30	43	44
Tephrosia villosa				
Autogamy (Mature buds bagged)	25	10	40	40*
Autogamy (manipulated)	25	18	72	47*
Geitonogamy	25	20	80	60*
Xenogamy	25	24	96	84
Open pollination (Inflorescences tagged before initiation of flowering)	70	57	82	94

*Most pods are either empty or with ill-formed seeds, indicating that initiation of seed production is preceded by a completely developed pod.

percentage was calculated to indicate the ideal season for the production of new plants.

Floral mechanism of the papilionaceous flower was examined in terms of petal configuration, placement and position of sex organs during bud and flower life, and also the movement of petals and release of sex organs during flower probing by nectar- and pollen-seeking insect foragers, in order to define the functional pollination mechanism in the plant. Insects foraging on the flowers were observed throughout the day on four different days for their mode of approach, landing, probing behaviour, and contact with the floral sexual organs. Thrips and insects were identified from the representative specimens available at the Department of Environmental Sciences, Andhra University, Visakhapatnam. The foraging visits of insects were recorded on a 1 × 1 m area of the flowering patch for 10 min each hour for the entire day on four different days 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 insects was carefully observed to assess their role in effecting pollination. Ten specimens of each insect species were captured during 08:00–14:00 h 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 on it. Then, the average number of pollen grains carried by each bee species was calculated to find out the pollen carryover efficiency.

Results

The plant and flowering aspects. *Tephrosia purpurea* and *T. villosa* are small, erect, self-regenerating and profusely branched perennial herbs or subshrubs (Fig. 1a, 5a). They grow together intermingled with each other, and also form distinct populations in open and waste places, along roadsides, on shallow sandy, rocky and disturbed soils; they also tolerate long dry season and heavy rains. *T. villosa* is widespread and occupies vast areas, when compared to the occurrence levels of *T. purpurea*. Still, they have a symbiotic relationship with rhizobial bacteria forming nodules on the roots and fixing atmospheric nitrogen. The stem is densely clothed with white and silky hairs in *T. purpurea* and with densely matted woolly hairs in *T. villosa*. In both species, the branches produce imparipinnate compound leaves; the leaflets are of the same size, measuring 19–20 mm in length and 4–5 mm in width; they are oblanceolate in *T. purpurea* and obovate to elliptical in *T. villosa*. Both species flower throughout the year but profuse flowering occurs during August-November (Fig. 5a). The inflorescence is a pseudo-raceme borne terminally or opposite to leaf axil (Fig. 5b). In *T. purpurea*, the flower number is 11.93 ± 0.75 in terminal racemes and 6.7 ± 0.5 in racemes borne opposite to leaf axils. In *T. villosa*, the flower number is 12.73 ± 0.35 in terminal racemes and 9.4 ± 0.65 in racemes borne opposite to leaf axils. The flower stalk is densely covered with hairs in both species. In *T. purpurea*, the flowers are of two types: resupinate and non-resupinate. Resupinate flowers are characterized by inverted position of the standard petal, a wing-keel petal complex, with the former taking the lower position and upper position of the latter. Non-resupinate flowers are characterized by upright position of the standard petal, a wing-keel petal complex, with the former taking the upper position and lower position of the latter. These two types of flowers are produced in the same and in different inflorescences of the same plant (Fig. 2); the resupinate flowers account for 26 %, while the non-resupinate flowers for 74 % at plant level (Fig. 7). In *T. villosa*, there are only non-resupinate flowers.

Flower morphology. The flowers are dark pink, 9.1± 0.4 mm long and 7.2 ± 0.5 mm wide in *T. purpurea,* and light pink, 10. 5 ± 0.8 mm 10.8 ± 1.0 mm in *T. villosa*. In both the species, the flowers are odourless, hermaphroditic and zygomorphic. The calyx is light green to light yellow, velutinous and slightly tubate at base; the vexillary lobe is triangular, deltoid, while the lateral lobes are narrowly triangular. In both species, the corolla is typically papilionaceous, with one standard petal, two wing petals and two keel petals (Fig. 1e); it is 7. 2 ± 0.5 mm long in *T. purpurea* and 11.3 ± 0.8 mm long in *T. villosa*. In both species, the standard petal is broadly ovate, with slightly emarginate apex covered with dense hairs on the dorsal side. The wing petals are about as large as the keel petals. The wings are glabrous and auricled on the standard petal, slightly pubescent on the carinal lobe formed by the keel petals. The keel petals are also glabrous, auricled at base on the standard petal and the connected part of both petals is slightly pubescent; they are

adherent to each other along the carinal margins from about halfway up to the apex. They are interlocked by the wing petals by means of lateral bulgy pockets. In *T. purpurea*, the standard petal is 6.4 ± 0.6 mm long and 7.5 ± 0.6 mm wide; the wing petals are 6.7 ± 0.4 mm long and 4.4 ± 0.6mm wide; and the keel petals are 4.7 ± 0.6 mm long and 3.8 ± 0.6 mm wide. In *T. villosa*, the standard petal is 8.7 ± 0.4 mm long and 10.9 ± 0.7 mm wide; the wing petals are 10.5 ± 0.5 mm long and 5.1 ± 0.7 mm wide; and the keel petals are 6.5 ± 0.5 mm long and 3.5 ± 0.5 mm wide. Stamens are 10, monadelphous and of different heights due to variation in the length of filaments in both species (Fig. 1f). The staminal tube is 4.6 ± 0.4 mm long in *T. purpurea* and glabrous, 5.6 ± 0.6 mm long in *T. villosa,* and hairy at the auricles in both species. The filaments are tipped with creamy-white to yellow 1–2 mm long fertile dithecous anthers in both species. The ovary is monocarpellary and monolocular, with 5.8 ± 0.5 (Range 5–7) ovules in *T. purpurea* (Fig. 1i-k) and 6.2 ± 0.5 (range 5–8) ovules in *T. villosa*; the ovules in both species are arranged in a linear row and attached to the marginal placenta. In *T. purpurea*, the ovary is 4.5 ± 0.5 mm, and style and stigma together are 3.6 ± 0.5 mm long. In *T. villosa*, the ovary is 5.5 ± 0.5 mm, and style and stigma together are 3.5 ± 0.5 mm long. In both species, the style is laminar, tipped with simple creamy-white stigma and bent horizontally at an angle to the ovary in the mature bud, but in *T. villosa*, the stigma shows a slight curvature at its distal end adjoining the light creamy-white stigma. The stigma is simple with a few ciliate hairs in *T. purpurea,* and capitate with penicillate hairs extending beyond its surface in *T. villosa*.

Floral biology. In both species, the flower-opening process is initiated by appearance of a prominent slit in the mature bud, which gradually unfolds the standard petal but not the keel and wing petals (Fig. 1b-d, 5c). The process of flower opening from initiation to unfolding of the standard petal occurs within a time span of about 30–40 minutes. The mature buds open daily during 07:00–10:00 h, with pronounced anthesis during 08:00–09:00 h in *T. purpurea;* and during 09:00–13:00 h, with pronounced anthesis during 11:00–12:00 h in *T. villosa*. Anther dehiscence occurs by longitudinal slits and all anthers dehisce synchronously in the mature bud stage in both species. The pollen output per anther is 612.4 ± 63.1 and the pollen-ovule ratio is 1021:1 in *T. purpurea,* with corresponding values for *T. villosa* of 946.7 ± 93.5 and 1578:1, respectively. In both species, the pollen grains are creamy white, powdery, tricolporate, surface reticulate, and with significantly varying grain size: 27.3 ± 4.0 µm in *T. purpurea* (Fig. 1g) and 32.3 ± 6.1 µm in *T. villosa.* In both species, the pollen grains are viable and showed maximum germination in 10 % sucrose concentration, but the pollination germination rate varied with each species. In *T. purpurea*, the pollen germination rate is 92 % at anthesis, 76 % two hours after anthesis, 62 % four hours after anthesis, 34 % six hours after anthesis, and 4 % at the time of flower closure. The corresponding percentages of pollen germination rate for *T. villosa* are 98 %, 81 %, 59 %, 32 %, and 5 %, respectively. In *T. purpurea*, the style is curved horizontally, almost half-way to its entire length, but seated 3–4 mm above the anthers during bud stage, while the stigma is straight in horizontal position during bud stage and curved downwards during and after anthesis. In *T. villosa*, similar situation exists but the degree of style bending is less, it is seated 1–2 mm above the anthers and the stigma is very slightly curved inwards during bud and flower stages. In these species, spatial separation of stamens and stigma during flower life indicates the functionality of herkogamy. In both species, the stigma attains receptivity, indicated by its wet state almost in synchrony with anther dehiscence, which designates the functionality of homogamy, and this receptivity ceases by the end of the day of anthesis. In both species, the flowers begin nectar secretion after anther dehiscence and complete the secretion process one hour after anthesis. The nectar can be seen through nectar windows, which are enclosed by the standard petal (Fig. 1h). The total volume of nectar produced by individual flowers is 2.2–2.5 µl, with 28–31 % sugar concentration in *T. purpurea,* and 3.3–3.5 µl, with 27–34 % sugar concentration in *T. villosa*. The nectar in both species produces sucrose, glucose and fructose, with the first sugar as the most dominant. In both species, the standard petal folds back inwardly enclosing the wing and keel petals by the end of the day, around 18:00 h (Fig. 5d, e); the flowers fall off on the 3rd day, if not pollinated. In pollinated flowers, the corolla together with stamens fall off after 6–7 days, while the style and stigma remain as vestigial parts at the tip of the fruits even after their maturation.

Breeding systems. Results of hand-pollination tests in both *T. purpurea* and *T. villosa* indicated that the

plants reproduce by autogamy (autonomous and manipulated), geitonogamy and xenogamy, but the fruit and seed sets varied greatly in each mode of pollination. Fruit set and seed set rates are the lowest in the first mode (autonomous autogamy) and the highest in the last mode. Moreover, most of the fruited flowers in both modes of autogamy and geitonogamy only formed pods without seeds, or with ill-formed seeds. In open pollinations, the fruit set rate stood at 43 % and the seed set rate at 44 % in *T. purpurea,* and at 82 % and 94 % in *T. villosa,* respectively, indicating facultative xenogamy, which enables sexual reproduction through self- and cross-pollination, which occurs when the flowers are tripped by visiting foragers and also by intra- and inter-flower movements of thrips (Table 1).

Pollination mechanism. In both *T. purpurea* and *T. villosa*, the staminal column and pistil are held under tension within the pouch-like keel petals, enclosed on both sides by wing petals. Upon the release of tension, the staminal column and stigma snap forward against the standard petal, causing violent release of pollen from the dehisced anthers. This process makes up the tripping mechanism and is accomplished when the keel petals are pressed down by a foraging insect. High wind speed, heavy rain and high temperatures, which weaken the turgidity of the restraining keel tissues, may cause tripping of the wing and keel petal complex, which spontaneously release the staminal column and stigma. If the flowers are untouched, the stamens and pistil remain within the keel and subsequently fall off. Insects approaching in upright position and landing on the front side of the flower, especially on the wing petals, ride the keel petals with their legs by pushing down the wing petals, which in turn pull back the keel petals by the notched folds. In *T. purpurea*, once the tripping occurs, the staminal column and the pistil do not return back to the keel petals, nor do the keel petals in both resupinate and non-resupinate flowers. This wing-keel complex tripping mechanism characterizes an explosive pollination mechanism (Fig. 3a, b). However, in *T. villosa*, the upper rim of the keel petals is not sealed and stays in an open state along its entire length. Once the tripping occurs by the insect, which visits the flower from the front side, the staminal column and the pistil are violently released and snap forward against the standard petal, but they return to the keel petals, as the keel petals do with departure of the insect. This wing-keel complex tripping mechanism makes up a valvular mechanism, which enables the flower to release pollen with every subsequent visit made by the insect that causes tripping. Since the stigma stands above the anthers, the foraging insect during probing first brushes against the ciliate (*T. purpurea*)/penicillate (*T. villosa*) stigma and then against the anthers, effecting with its ventral side either cross- or self-pollination in non-resupinate flowers in both species. In resupinate flowers of *T. purpurea*, the foraging insect during probing brushes the stigma and anthers with its dorsal side effecting cross- or self-pollination. In case abiotic factors, namely wind, rain and temperatures, affect the tripping of the keel complex, only self-pollination occurs. The explosive and valvular pollination mechanisms functional in these species are adapted for manipulation by both biotic and abiotic factors.

Insect visitors and pollination. The thrips, *Megalurothrips distalis* Karny and *Frankliniella schultzei* Trybom (sub-order: Terebrantia, family: Thripidae, subfamily: Thripinae), were found to use the buds of both *T. purpurea* and *T. villosa* for their breeding. They moved out with the gradual unfolding of the standard petal resulting in exposure of the wing-keel petal complex. Since the flower base is not completely closed, these minute thrips came out with great ease from slight gaps between petals and through the staminal column, and kept moving in and out of the flower base to feed on nectar and pollen and also crawled all over the plant. Bud infestation with thrips was 25 % in *T. purpurea* and 28 % in *T. villosa*. The crawling of the thrips within and between flowers was considered to effect self-pollination to some extent within the plant, despite the position of stigma above the anthers. Ciliate stigmas in *T. purpurea* and penicillate stigmas in *T. villosa* capture the pollen with great ease from the pollen-laden thrips, when the latter crawl on the stigmas.

Field observations showed that *T. purpurea* with dark-pink flowers were foraged by a few individuals of insect foragers, while *T. villosa* with light-pink flowers were foraged by many individuals of insect foragers, and many insect forager species were common to both species. The flowers of *T. purpurea* and *T. villosa* were foraged by bees and lycaenid butterflies; *T. villosa* flowers were also foraged by wasps and hesperiid butterflies. In *T. purpurea*, the bees visited the flowers from 08:00 to 17:00 h, with a greater number of

visits during 10:00–12:00 h, while butterflies manifested foraging activity from 08:00 to 16:00 h, with more foraging visits during 10:00–11:00 h (Fig. 8, 9). In *T. villosa*, the bees and wasps visited the flowers from 08:00 to 15:00 h, with more visits during 11:00–13:00 h, while butterflies made visits from 08:00 to 14:00 h, with maximum visits during 12:00–13:00 h (Fig. 10, 11). Honey bees (*Apis cerana* – Fig. 3c, 5f and *A. florea* –Fig. 3d), small carpenter bees (*Ceratina smaragdula* –Fig. 3e, f, 5j) and sweat bees (*Nomia* sp. – Fig. 3g, 5g, h) visited the flowers of both the plant species. Larger carpenter bees (*Xylocopa latipes* -Fig. 5i and *X. pubescens*), leaf cutter bees (*Megachile* sp. –Fig. 5k), and the potter wasps (*Eumenes petiolata* -Fig. 5l) visited the flowers of *T. villosa* only. Among these, the large carpenter bees foraged for nectar only, while the rest foraged for both nectar and pollen. The foraging butterflies were lycaenids with 10 species and hesperiids with one species; the hesperiid butterflies visited only *T. villosa* (Fig. 5s). Among lycaenids, *Chilades pandava* is the only species which visited the flowers of both *T. purpurea* and *T. villosa*. *Azanus jesous* (Fig. 3h), *Chilades laius* (Fig. 3i), *Chilades pandava* (Fig. 3j, 5m), *Euchrysops cnejus* (Fig. 3k), *Spindasis vulcanus* (Fig. 3l, 5p), and *Zizina otis* (Fig. 3m) visited the flowers of *T. purpurea* only, while *Freyeria trochylus* (Fig. 5n), *Lampides boeticus* (Fig. 5o), *Tarucus nara* (Fig. 5q), and *Zizeeria karsandra* (Fig. 5r) visited the flowers of *T. villosa* only (Table 2). Bees accounted for 57 % and butterflies for 43 % of all foraging visits made to the flowers of *T. purpurea* (Fig. 12). In case of *T. villosa*, bees accounted for 59 %, wasps for 8 % and butterflies for 33 % of all foraging visits (Fig. 12).

In *T. purpurea*, the foraging insects visited both non-resupinate and resupinate flowers indiscriminately. Bees approached the non-resupinate flowers in upright position, landed on the wing-keel petal complex and proceeded towards the flower base to collect nectar, due to which keel petals were tripped exposing the stamens and stigma and effecting sternotribic pollination spontaneously. For pollen collection, they approached the flowers of both non-resupinate and resupinate flowers in upright position, landed on the standard petal, with head facing towards the wing-keel petal complex, and proceeded to probe the keel petals for pollen, during which tripping and sternotribic pollination occurred spontaneously. These bees never attempted to collect nectar from the resupinate flowers, which indicates that they did not distinguish between non-resupinate and resupinate flowers in terms of the change in position of the standard petal versus other petals. Furthermore, the bees seldom visited tripped flowers of the non-resupinate flowers, due to the central position of the staminal complex, along with the extended style and stigma seated very closely to the standard petal; such placement of the sex organs complex was not conducive to subsequent visits by bees to collect nectar. In *T. villosa*, the bees and wasps exhibited the foraging behaviour employed by bees for collecting nectar and pollen from the flowers of *T. purpurea* and tripped the flowers effecting sternotribic pollination. They visited repeatedly the tripped flowers, because the staminal complex along with style and stigma returned back into the keel petals and the flowers looked untripped, enabling bees to make subsequent visits with great ease and effect pollination. Occasionally, honey bees approached the flowers laterally to probe for nectar but they were unsuccessful in accessing it and flower tripping did not occur. Sweat bees occasionally attempted to collect pollen

Table 2. Insect foragers recorded on *Tephrosia purpurea* and *Tephrosia villosa*.

Order / Family	Insect species	*Tephrosia purpurea*	*Tephrosia villosa*	Forage sought
Hymenoptera				
Apidae	*Apis cerana* F.	+	+	N + P
	Apis florea F.	+	+	N + P
	Xylocopa latipes Drury	-	+	N
	Xylocopa pubescens Spinola	-	+	N
	Ceratina smaragdula Cockerell	+	+	N + P
Halictidae	*Nomia* sp.	+	+	N + P
Megachilidae	*Megachile* sp.	-	+	N + P
Vespidae	*Eumenes petiolata* F.	-	+	N + P
Lepidoptera				
Lycaenidae	*Azanus jesous* Guerin	+	-	N
	Chilades laius Cr.	+	-	N
	Chilades pandava Horsefield	+	+	N
	Euchrysops cnejus F.	+	-	N
	Freyeria trochylus Freyer	-	+	N
	Lampides boeticus L.	-	+	N
	Spindasis vulcanus F.	+	-	N
	Tarucus nara Kollar	-	+	N
	Zizeeria karsandra Moore	-	+	N
	Zizina otis F.	+	-	N
Hesperiidae	*Borbo cinnara* Wallace	-	+	N

N = Nectar; P = Pollen

from the bottom part of the keel petals of mature buds but they were unsuccessful because the anthers are not situated in the place of their probing. Still, the carpenter bees are sufficiently large to accomplish pollination. The flowers bent downwards and touched the inflorescence rachis upon landing by carpenter bees and returned back to their original position with the departure of bees. These bees were very efficient and effective pollinators. Butterflies approached the flowers from the front and laterally and landed on the standard petal or on other petals for inserting their proboscis into the narrow space between them to access nectar and were successful in collecting it. They never caused flower-tripping in both plant species. However, they touched the stamens and stigma with their proboscis/head/underside of the abdomen thus effecting pollination in the tripped flowers of *T. purpurea* but not in the tripped flowers of *T. villosa,* which indicates that butterflies have a minor role in the pollination of *T. purpurea* and no role whatsoever in the pollination of *T. villosa*. All insect species were regular and consistent foragers on both *T. purpurea* and *T. villosa*. The pollen-carrying efficiency evaluated by body washings of captured bees, wasps and butterflies indicated that bees carried more pollen than wasps and butterflies in both plant species. In *T. purpurea*, the average number of pollen grains carried by bees varied from 71.5 to 184.5 and by butterflies from 23.7 to 35.7. In *T. villosa*, the average number of pollen grains carried by bees and wasps varied from 32.4 to 178.5 and by butterflies from 22.2 to 29.6 (Table 3). In both plant species, bees and wasps collected nectar quickly from individual flowers and moved from flower to flower on the same and/or different conspecific plants, in order to collect as much nectar as possible; this intraplant foraging activity was considered to be resulting in self-pollination and the interplant foraging activity in cross-pollination. Bees and wasps collected pollen slowly from each flower they visited and this pollen foraging activity was considered to effect mostly self-pollination within the plant. In *T. purpurea*, butterflies as nectar foragers spent more time on each visited flower; they acted as nectar robbers in their visits to untripped flowers and as minor pollinators in their visits to tripped flowers, effecting mostly self-pollination. In *T. villosa*, butterflies acted exclusively as nectar robbers in their visits to untripped and tripped flowers.

Table 3. Pollen recorded in the body washings of insects on *Tephrosia purpurea* and *Tephrosia villosa*.

Insect species	Sample size (N)	*T. purpurea* No. of pollen grains			*T. villosa* No. of pollen grains		
		Range	Mean	S.D.	Range	Mean	S.D.
Bees							
Apis cerana	10	91–274	184.5	54.3	73–255	178.5	55.9
Apis florea	10	78–231	174.4	47.3	51–239	150.2	52.5
Ceratina smaragdula	10	30–112	71.5	25.2	21–63	47.4	12.5
Xylocopa latipes	10	–	–	–	66–173	110.3	29.7
Xylocopa pubescens	10	–	–	–	43–148	102.2	30.4
Nomia sp.	10	49–136	91.8	26.9	34–131	94.6	26.3
Megachile sp.	10	–	–	–	17–46	32.4	7.3
Wasps							
Eumenes petiolata	10	–	–	–	24–52	40.5	8.2
Butterflies							
Azanus jesous	10	6–24	23.8	7.2	–	–	–
Chilades laius	10	9–38	28.5	7.9	–	–	–
Chilades pandava	10	15–47	35.7	9.0	13–40	29.6	7.0
Euchrysops cnejus	10	10–40	27.6	8.2	–	–	–
Freyeria trochylus	10	–	–	–	7–29	21.3	5.8
Lampides boeticus	10	–	–	–	15–34	26.3	5.2
Spindasis vulcanus	10	7–31	23.7	6.7	–	–	–
Tarucus nara	10	–	–	–	8–35	24.9	7.2
Zizeeria karsandra	10	–	–	–	11–43	25.7	8.3
Zizina otis	10	11–42	30.2	8.7	–	–	–
Borbo cinnara	10	–	–	–	10–31	22.2	6.4

Fruiting and seed ecology. In both species, fruits are characteristically pods producing seeds in a linear manner. After the full-length formation of pods, seeds gradually develop and reach maturity. The total duration of pod formation, seed development and maturation amounted to 3–5 weeks. The pods are initially green and light brown when mature and dry in *T. purpurea* (Fig. 4a-d), and initially green and silvery brown when mature and dry in *T. villosa* (Fig. 6a-e). In both species, the pod is linear, slightly turgid and slightly convex around the seeds but glabrous, 33 ± 4.7 mm long and 3.5 ± 0.5 mm wide in *T. purpurea* (Fig. 4f) and retrofalcate, densely velutinous, 28.4 ± 0.2 mm long, and 5.1 ± 0.2 mm wide in *T. villosa* (Fig. 6i). In both species, the seeds are small and reniform; they are green initially, brown-mottled in mature stage, 4.4 ± 0.5 mm long and 2.8 ± 0.3 mm wide, and 9.8 mg in weight in *T. purpurea;* green to light ash-coloued initially, light- brown-mottled in mature stage, 3.1 ± 0.3 mm long, 2.3 ± 0.4 mm wide and 8.5 mg in weight in *T. villosa*. Seed number per fruit (pod) produced from open pollinations varied greatly in both species. In *T. purpurea*, seedless pods accounted for 25% of all pods sampled. Among seeded pods, one-seeded pods accounted for 9%, two-seeded for 10%, three-seeded for 21%, four-seeded for 19%, five-seeded for 12%, and six-seeded for 4% (Fig. 13). In *T. villosa*, seedless pods accounted for 7% of the all pods sampled. Among seeded pods, one-seeded pods amounted to 2.7%, two-seeded to 5.8%, three-seeded to 9%, four-seeded to 11.3%, five-seeded to 18%, six-seeded to 31%, seven-seeded to 15%, and eight-seeded to 0.2% (Fig. 13).

In both species, mature pods are two-valved and dehisce by spiral twisting to disperse seeds (Fig. 4e); the spiral twisting is very distinct in *T. villosa* (Fig. 6f-h). The twisted remains of pods stay attached to the parent plant. Most dispersed seeds fall in the vicinity of parental plants. Most pods dehisce in March-May, although pod dehiscence occurs throughout the year, due to year-long flowering and fruiting. Dry ambient conditions were found to be ideal for pod dehiscence by spiral twisting. Seeds fallen to the ground are non-dormant and germinate within 8–10 days, if the soil is sufficiently moist, and within 10–14 days, if the soil is moderately moist for both species. Field studies indicated that seed germination and production of seedlings is highest during the rainy season and lowest at other times of the year for both species. Out of all seeds germinated in the natural area, the production rate of seedlings is 56% during rainy season and 13% during winter season in *T. purpurea,* and 87% during rainy season and 29% during winter season in *T. villosa*. Seed germination is completely absent during summer season. Production of new plants from seedlings in both species was found to be related to the continued availability of moisture and resource environment in which they grow. In both species, the plants disappear during summer season in areas with dry soil conditions but their perennial underground root stock remains dormant and sprouts back to produce new foliage and proceed with flowering and fruiting during the rainy season.

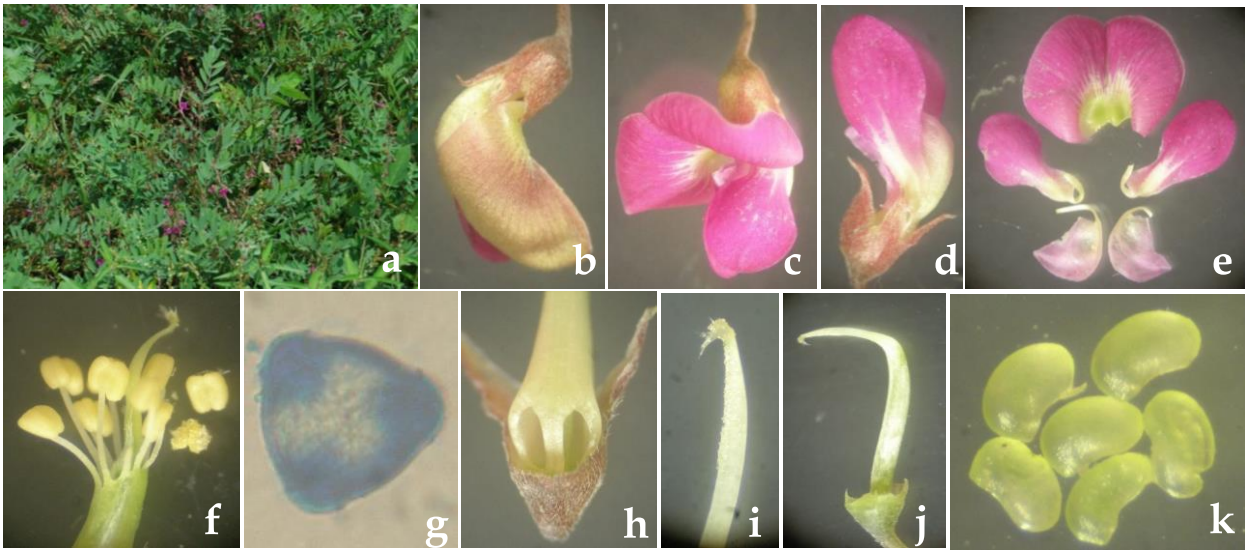

Fig. 1. *Tephrosia purpurea:* **a**. Habit – in flowering phase, **b**. Anthesing bud, **c**. Flower, **d**. Wing and keel petals (stamens and stigma within the keel), **e**. Papilionaceous corolla, **f**. Monoadelphous stamens, **g**. Pollen grains, **h**. Nectar windows, **i**. & **j**. Pistil, k. Ovules.

Fig. 2. *Tephrosia purpurea:* a. Non-resupinate flower (left) and resupinate flower, (right) on the same plant.

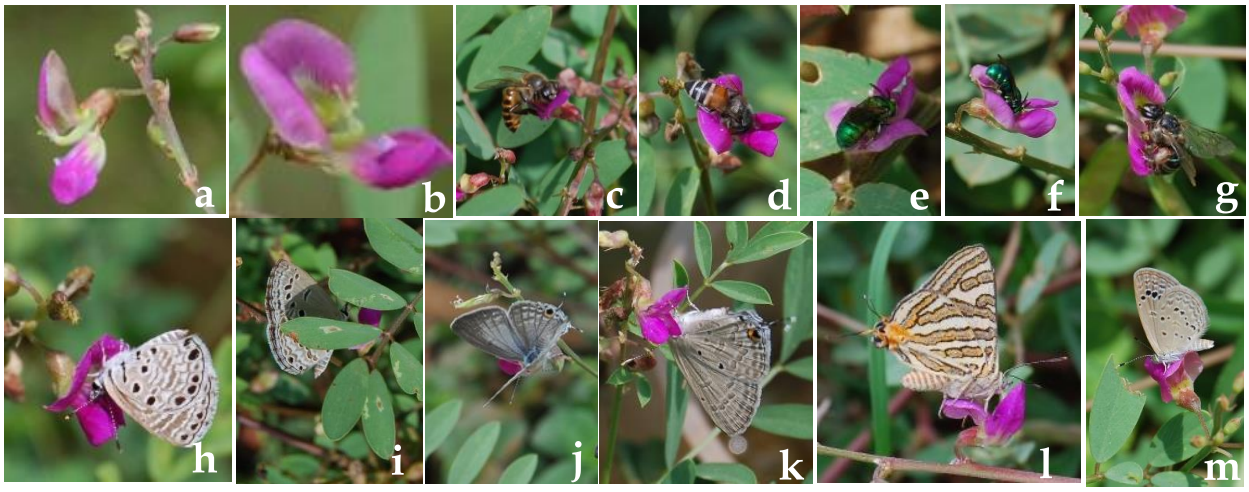

Fig. 3. *Tephrosia purpurea:* **a**. & **b**. Sexual apparatus position after tripping of keel-wing petal complex, **c**-**g**. Bees – **c**. *Apis cerana* collecting nectar from non-resupinate flower, **d**. *Apis florea* approaching keel petals for pollen collection from resupinate flower, **e**. *Ceratina smaragdula* collecting nectar from resupinate flower, **f**. *Ceratina smaragdula* approaching keel petals for pollen collection from non-resupinate flower, **g**. *Nomia* sp. collecting nectar from non-resupinate flower, **h**-**m**. Lycaenid butterflies foraging for nectar from non-resupinate flowers – **h**. *Azanus jesous*, **i**. *Chilades laius*, **j**. *Chilades pandava*, **k**. *Euchrysops cnejus*, **l**. *Spindasis vulcanus*, **m**. *Zizina otis*.

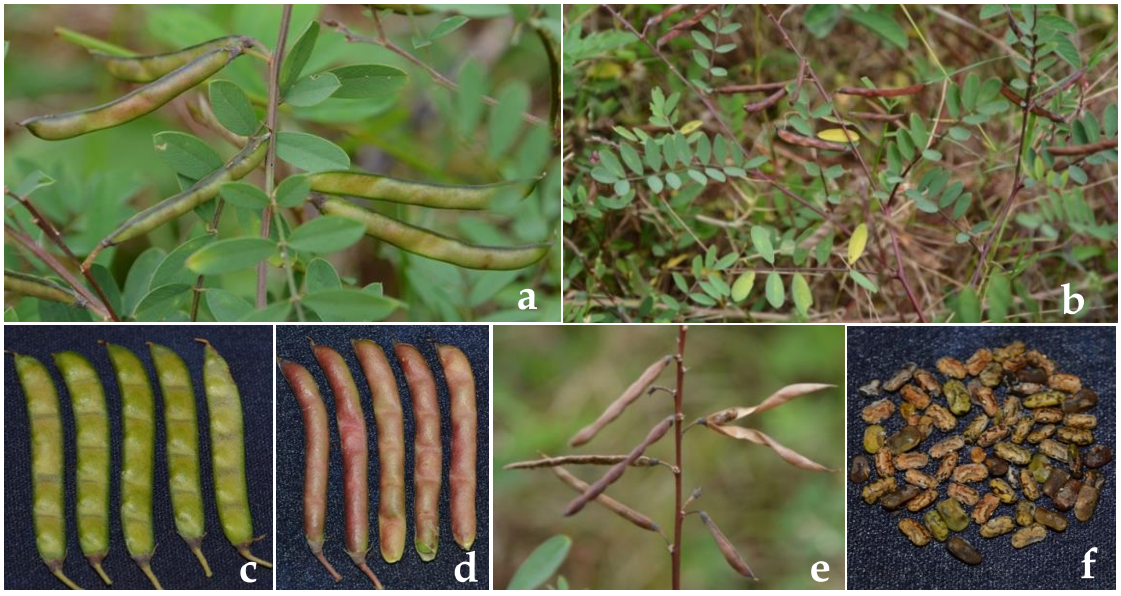

Fig. 4. *Tephrosia purpurea:* **a**. Developing fruits (in green phase), **b**. Mature fruits (in brown phase), **c** & **d**. Stages in fruit development. **e**. Seed dispersal by explosive dehiscence of fruits, **f**. Growing and developed seeds.

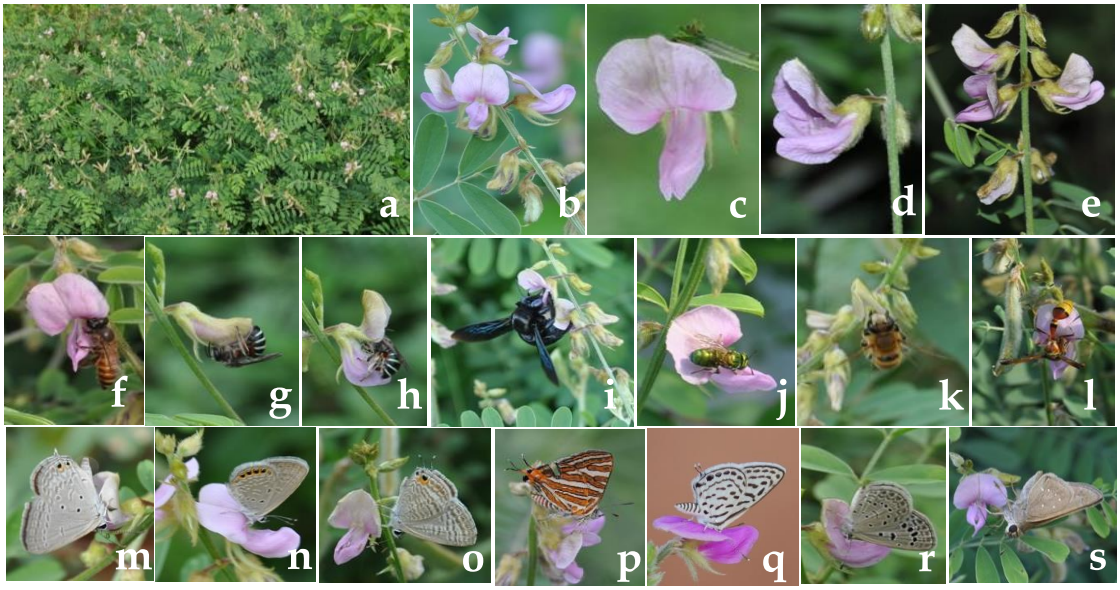

Fig. 5. *Tephrosia villosa:* **a**. Habit – plant in flowering phase, **b**. Flowering raceme, **c**. Flower, **d**. & **e**. Flower closure by evening, **f**-**k**. Bees – **f**. *Apis cerana* probing the flower from the side to access nectar without causing tripping, **g**. *Nomia* sp. probing the mature bud for pollen collection in upside down position, **h**. *Nomia* sp. probing the flower for nectar collection by tripping the keel petals, **i**. *Xylocopa latipes* collecting nectar, **j**. *Ceratina smaragdula* probing the wing-keel petal complex for pollen collection, **k**. *Megachile* sp. probing for nectar collection **l**. *Eumenes petiolata* (wasp) landed on the standard petal to collect pollen, **m**-**r**. Lycaenid butterflies collecting nectar– **m**. *Chilades pandava,* **n**. *Freyeria trochylus*, **o**. *Lampides boeticus*, **p**. *Spindasis vulcanus*, **q**. *Tarucus nara*, **r**. *Zizeeria karsandra*, **s**. Hesperiid butterfly, *Borbo cinnara*.

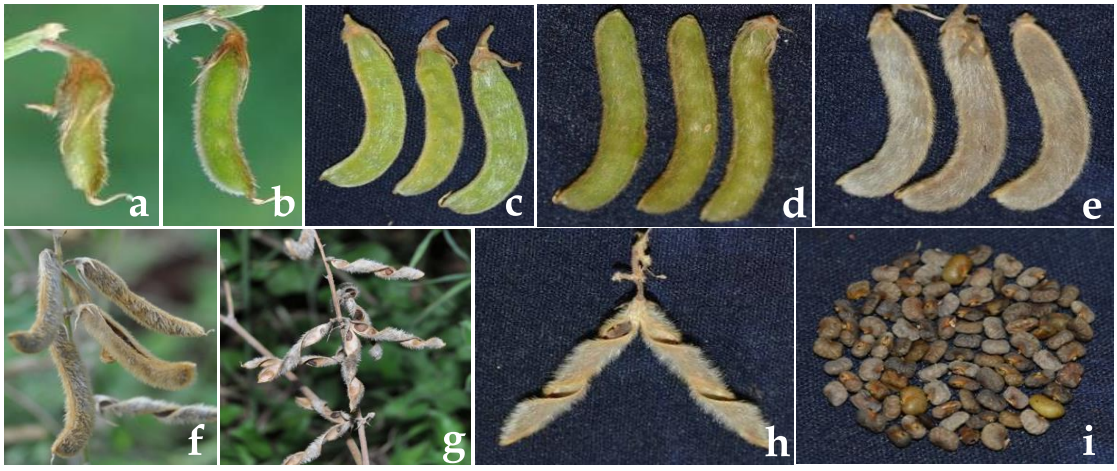

Fig. 6. *Tephrosia villosa:* **a**-**e**. Stages of fruit development and maturation, f. Initiation of fruit dehiscence, **g**. & **h**. Explosive seed dispersal by twisting of fruits in spiral manner, **i**. Growing and developed seeds.

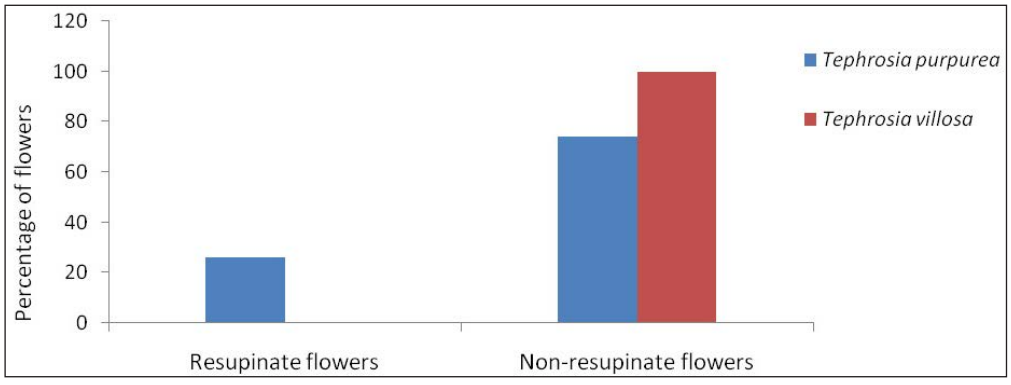

Fig. 7. Percentage of resupinate and non-resupinate flowers in *Tephrosia purpurea* and *T. villosa.*

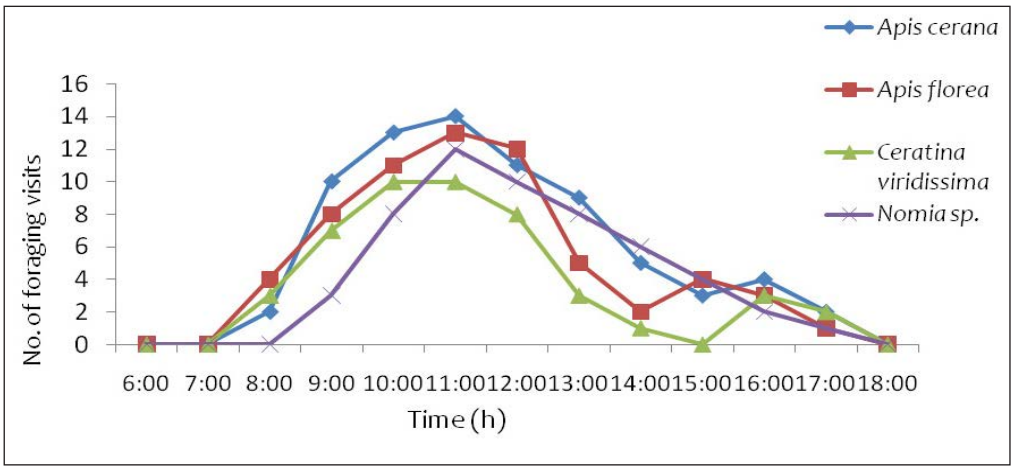

Fig. 8. Hourly foraging activity of bees on *Tephrosia purpurea.*

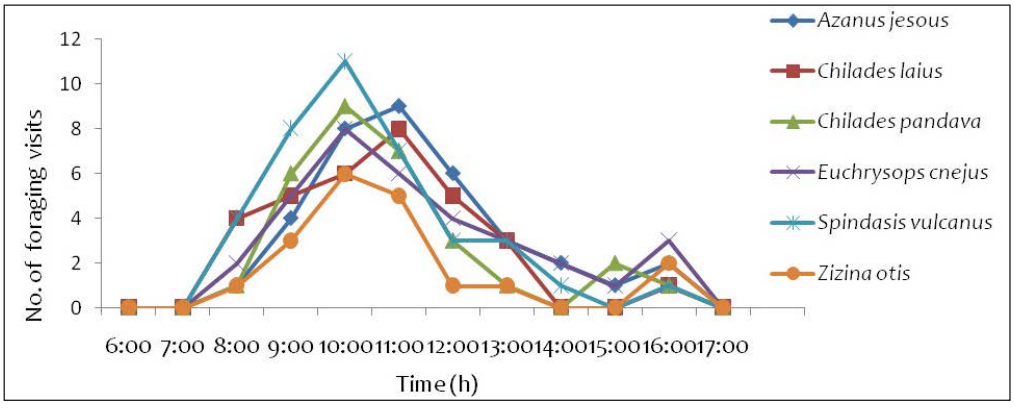

Fig. 9. Hourly foraging activity of butterflies on *Tephrosia purpurea.*

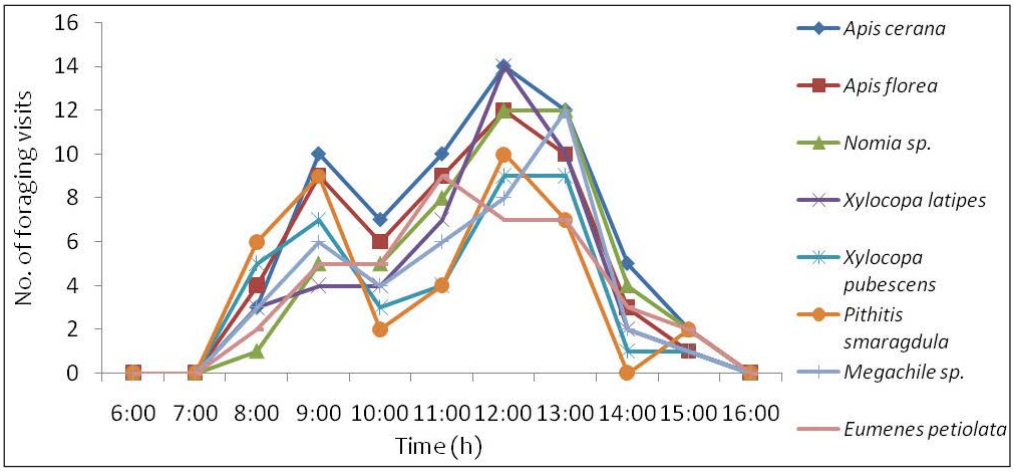

Fig. 10. Hourly foraging activity of bees and wasps on *Tephrosia villosa.*

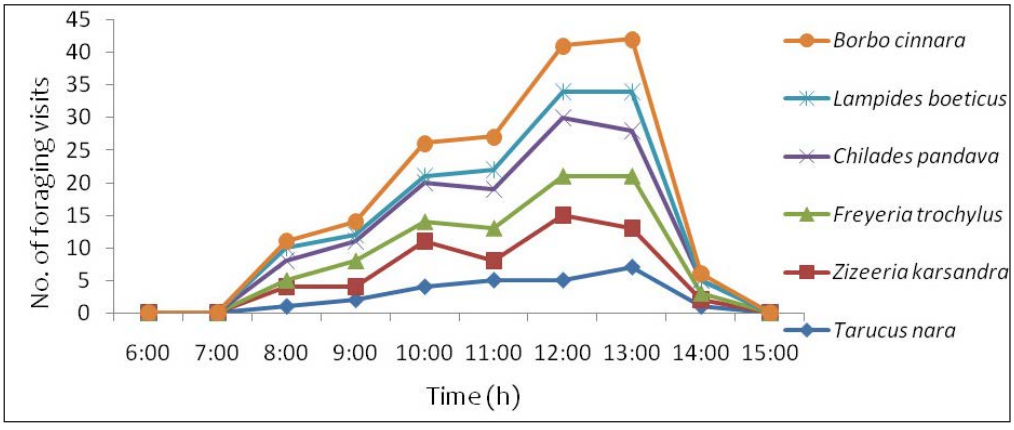

Fig. 11. Hourly foraging activity of butterflies on *Tephrosia villosa.*

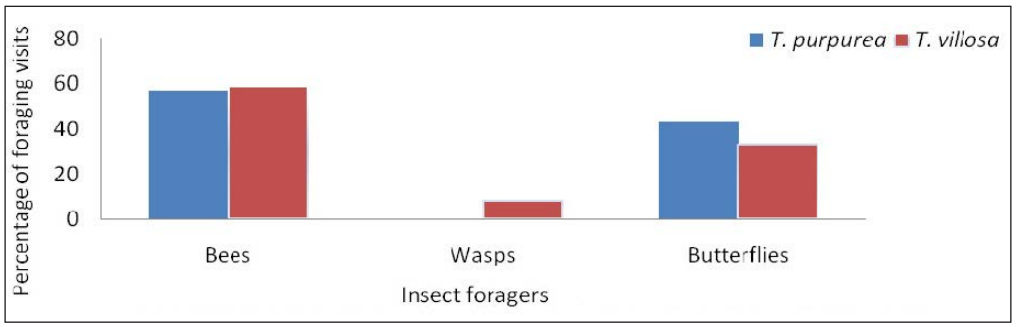

Fig. 12. Percentage of foraging visits of insects on *Tephrosia purpurea* and *T. villosa.*

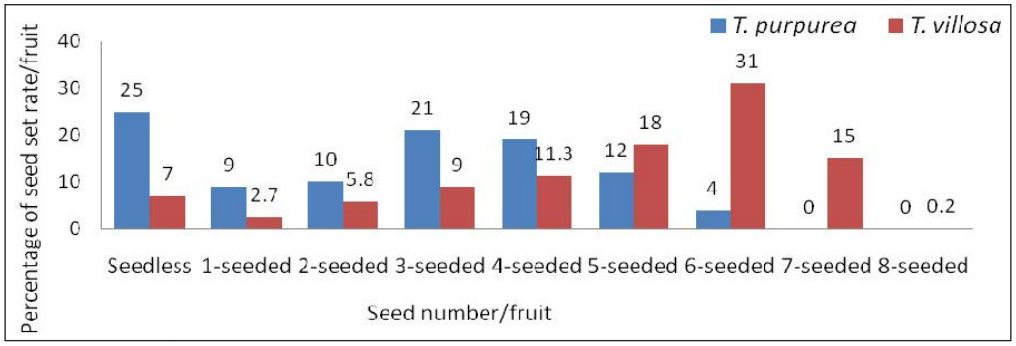

Fig. 13. Percentage of seed set rate/fruit in *Tephrosia purpurea* and *T. villosa.*

Discussion

Venkateswarlu & Rao (1963) reported that *T. purpurea* grows in varied habitats and forms. Mishra & Sen (1986) reported the occurrence of two distinct populations in *T. purpurea* growing in Jodhpur, which is an arid zone in India. These authors used the nomenclature of L-population with long leaves and large seeds and S-population with small leaves and small seeds. Furthermore, they also stated that L-population blooms and fruits during the rainy season (August-September) while S-population blooms during September-October and fruits during November-January. Annadurai & Velayudhan (1986) noted that *T. purpurea* flowers in September-March in South India. In the present study, *T. purpurea* and *T. villosa* have grown in varied habitats, mostly under semidry soil conditions. In these habitats, all observed populations are similar in leaf and seed characteristics, with only one population type, despite their occurrence in varied habitats ranging from wet to dry soil environments. These species display vegetative growth, flowering and fruiting throughout the year, if the soil is moderately or sufficiently moist. However, the plants maximize their vegetative growth and sexual reproduction during the rainy season, due to availability of sufficient moisture and nutrient resources. Seed production is continuous in plants growing in areas with moist soils; seeds germinate throughout the year, except for in summer season. These species grow side by side in the same habitat and also form pure populations closely covering the soil in the same or different habitats. However, when these species grow in the same habitat, *T. villosa* is highly prolific, while *T. purpurea* is either sporadic or form small populations. This indicates that the former species has the ability to outcompete the latter by quickly utilizing the available nutrient resources in the soil. These findings substantiate the generalized statements by Weigel & Nilsson (1995) supported by Amasino (2004) that vegetative growth and sexual reproduction are controlled by several intrinsic as well as extrinsic factors.

Sen (1977) wrote that *T. purpurea* produces flowers with different colours ranging from red to white. Mishra & Sen (1986) reported that *T. purpurea* with L-populations and S-populations growing in the Jodhpur region of India exhibit different anthesis schedules, the former in daytime, in full sunlight, and the latter in the evening hours. In the present study, *T. purpurea* flowers are characteristically dark pink, while those of *T. villosa* are light pink; this indicates that the flower colour is a stable character and uninfluenced by the habitat of their occurrence. In case of *T. purpurea*, a few individuals in one location have been found to produce only white flowers, indicating that this floral variety could be a mutant phenotype and, hence, can be named an *albino* variety; however, further study is suggested for its confirmation. In these species, anthe-

sis schedules vary, it is in the early morning in *T. purpurea* and in the late morning to noon in *T. villosa*. In both species, the flowers are borne in pseudo-racemes produced terminally and in leaf axils. Earlier studies into *T. purpurea* by different authors have not reported any occurrence of resupinate flowers in this species (Sen 1977; Mishra & Sen 1986; Kumari & Sharma 2017). In the present study, *T. purpurea* has been found to produce resupinate and non-resupinate flowers, with the latter as the most common type. *T. villosa* produces exclusively non-resupinate flowers.

In the subfamily Faboideae, ciliate style, ciliate and penicillate stigma, and pollen-brush types have been described (Lavin & Delgado 1990). In the present study, *T. purpurea* and *T. villosa* are homogamous, the flowers in the former species possess a simple stigma with a few cilitate hairs, while those in the latter possess a capitate stigma with penicillate hairs extending beyond its surface. This study substantiates the report by Brummitt (1980) that *T. villosa* flowers possess penicillate stigmas. Furthermore, the simple stigmas with ciliate hairs and capitate stigmas with penicillate hairs and their placement above the anthers appear to be adaptations to prevent the rupture of stigmatic surface for achieving cross-pollination in preference to self-pollination. In *Canavalia gladiata*, the protandrous flowers with simple stigma situated at the height between the long and short stamens essentially require stigmatic rupture to achieve cross-pollination in preference to self-pollination, which occurs after flower tripping by subsequent visits made by bees. Kumari & Sharma (2017) reported that *T. purpurea* is protogynous and the stigma with long papillae secreting copious exudates extends beyond the anthers and subsequently bends downwards to capture pollen from the dehisced anthers, which results in autogamy in case of non-availability of pollinators. Moreover, these authors also mentioned that stylar and stigmatic curvatures represent a form of flexistyly, which is quite different from the mechanisms of flexistyly functional in certain genera in *Lamiaceae, Malvaceae, Valeriaceae,* and *Zingiberaceae* (Zhang & al. 2003; Khajuria & al. 2011; Wani & al. 2011). In the present study, the flowers of *T. purpurea* have not been found to show any stylar movements and changes in stigmatic curvature, and also the stigmatic papillae do not secrete any exudate. Still, the flowers display simultaneous maturation of anthers and stigma but the pollen remains with the anthers, despite their dehiscence, without any contact with the ciliate stigma. Since the style is above the anthers and the curved stigma is away from the anthers, there is no possibility for their contact with each other in flowers that were not tripped by biotic or abiotic agents. Flower-tripping is essential for the occurrence of self- or cross-pollination; tripping effected by wind, rain and temperature results in autogamy, by thrips in autogamy and geitonogamy, and by insects in self- and cross-pollination. *T. villosa* flowers with slight bending of style and stigma curvature extending beyond the height of stamens present the same situation and require flower-tripping by biotic or abiotic agents for occurrence of pollination.

Aronne & al. (2012) classified the pollination mechanisms in the *Fabaceae* family into explosive, valvular, piston, and brush type. The presently studied *Tephrosia* species manifested the explosive and valvular mechanisms: the former in *T. purpurea* and the latter in *T. villosa*. In *T. purpurea*, the keel petals with the sex organ complex are held under tension, and following tension release by vector-caused tripping, the sex organs snap forward against the standard petal, causing all pollen to eject instantly and remain exposed permanently during the flower life. In this process, the curved ciliate stigma positioned away from the dehisced anthers strikes the ventral surface of the insect first and then the anthers; this could result in cross-pollination, if the insect has pollen from earlier visited flowers or, else, self-pollination may occur depending on the placement of pollen on the insect body during the tripping process. Staminal complex remaining outside the keel petals and the style and stigma staying close to the standard petal do not facilitate the insects in probing for nectar; this observation is supported by the rare visits of nectar-seeking insects to the tripped flowers. Such probing behavior is consistently exhibited by insects in the non-resupinate flowers. Insects use resupinate flowers for pollen only, because they lack the power of distinguishing between non-resupinate and resupinate flowers and approach both types of flowers by landing on the standard petal and moving towards the keel petals for pollen collection in both tripped and untripped flowers. Therefore, the nectar and pollen foraging behaviours in both types of flowers of *T. purpurea* results in sternotribic pollination only, which involves pollen wastage during flight and most of it remains available for grooming by the insects with their legs. Functionality of the explosive pollination mechanism in *T. purpurea* is exclusive-

ly dependent on external agents. Still, concealment of stamens within the keel until it is tripped might be an adaptation to protect pollen from rainfall, and make it available only when it is tripped by pollinating agents. Concealment of stamens and also of the stigma within the keel after anthesis appears to be a mechanism evolved to protect the pollen from moisture during low ambient temperatures and dew at night and also to maintain pollen fertility because it will be affected after contact with water on rainy days and cool humid days; this protection is very much required for the flowers which were not tripped on the day of anthesis (Peter & al. 2004). In *T. villosa*, the flowers with an unsealed upper rim of the keel petals open along its total length, when the keel petals are pushed downwards by the visiting insect, close back, when the insect departs from the flower, and simultaneously the staminal complex with pistil returns back to the keel petals. Insects visiting the flowers of this species do not distinguish between tripped and untripped flowers and, hence, visit the tripped flowers repeatedly for forage, owing to which the pollination rate is increased. This species produces only non-resupinate flowers and insect visits to tripped and untripped flowers for nectar and/or pollen always end up in sternotribic pollination. Moreover, the valvular mechanism characterized by concealment of the sex organ complex before and after flower-tripping inside the keel petals appears to be an advanced mechanism evolved to protect the pollen from moisture during low ambient temperatures and dew at night and also to maintain pollen fertility until the flowers close back, which occurs at the end of the day (Peter & al. 2004). Therefore, valvular pollination in *T. villosa* appears to be a more advanced mechanism than explosive pollination operational in *T. purpurea*. Etcheverry & al. (2012) reported that flowers offering nectar and pollen, or only pollen as a reward, tend to have higher P/O ratios than those offering only nectar as a reward for pollination services. Species offering only pollen produce it in greater quantities because it is necessary for them to compensate for the amount of pollen consumed by pollinators. Small (1988) stated that the *Medicago* species of the tribe Trifolieae, with an explosive pollination mechanism display the lowest pollen-ovule ratios. Lopez & al. (1999) recorded an explosive pollination mechanism with the highest pollen-ovule ratios in certain genera of the *Fabaceae* such as *Cytisus, Pterospartum, Teline, Ulex, Stauracanthus,* and *Cytisophyllum*. Etcheverry & al. (2012) noted that the *Fabaceae* plants with their explosive pollination mechanism had intermediate pollen-ovule ratios. In the present study, *T. purpurea* with an explosive pollination mechanism and *T. villosa* with a valvular pollination mechanism have moderate pollen-ovule ratios, comparable to those of the species with a facultative xenogamous breeding system, as prescribed by Cruden (1977); the production of slightly high pollen-ovule ratio in these species is perhaps a response to high pollen collection activity by foraging insects, which effects sternotribic pollination involving wastage and loss of pollen. The fruit set and seed set rates in all tested modes of pollination indicate that both *Tephrosia* species are self-compatible and self-pollinating, though all pollination modes are vector-dependent, whether biotic or abiotic, and predominantly outcrossing, which is characteristic of the functionality of facultative xenogamy.

Annadurai & Velayudhan (1986) reported that *Tephrosia purpurea,* with a special type of flower structure, forms a suitable micro-environment for foraging, breeding and oviposition sites for the thrips species *Megalurothrips distalis* and *Frankliniella schultzei* in South India. These thrips oviposit around the keel petals and, after an incubation period of 4–5 days, the larvae emerge in good coincidence with the production of maximum amount of floral nectar. They feed on both nectar and pollen, effecting pollination. Their movements inside the keel petals enable pollen transfer to the receptive surface of the stigma. In the present study, the same species of thrips have been also found to use *T. purpurea* and *T. villosa* as breeding and foraging sites and entail self-pollination through their movements inside the flowers and between flowers of the same plant by transferring pollen to the receptive stigma. The fruit and seed set rates evidenced in the bagged flowers are indicators of their role in pollination. Similarly, Annadurai & Velayudhan (1986) reported that thrips contribute up to 80 % of the fruit set in *T. purpurea*. In the present study, the percentage of thrips-infested buds in both *Tephrosia* species is lower (25–28 %) and, hence, thrips appear to have only a minor role in effecting pollination in these species.

Aronne & al. (2012) noted that the specialized pollination mechanisms reported in the *Fabaceae* family depend on pollen vectors, because they cannot self-activate the tripping process to effect even autogamy. The papilionaceous corolla is considered to be a general adaptation to pollination by Hymenoptera. In the

present study, *T. purpurea* with its explosive pollination mechanism attracts only honey bees, small carpenter bees, sweat bees and lycaenid butterflies, while *T. villosa* with its valvular pollination mechanism also attracts large carpenter bees, leaf-cutter bees, potter wasps, and hesperiid butterflies in the habitats of these species. The principal reason could be the small, odourless and pink flowers. However, in *T. villosa*, the flowers are relatively large and more attractive due to their light-pink colour and, hence, these flowers attract more species of insects than those of *T. purpurea*. However, the habitats of these plant species have several other species of flower-visiting insects which, though appropriate for the flowers of *Tephrosia* species, never visited them. In this context, it seems appropriate to mention the observations of Hingston & McQuillan (2000) that the *Indigofera* species with their purple zygomorphic flowers are not attractive to bees, as compared to the yellow flowers of other *Fabaceae* members. The present study indicated that the daily schedules of insect foraging activities coincide well with the anthesis schedule and the availability of standing crop of forage in both *Tephrosia* species; the peak foraging activity also coincides well with the availability of maximum number of flowers with full content of nectar and pollen. Bees are appropriate foragers in tripping the floral mechanisms ensuring pollination in these species. However, honey bees and sweat bees occasionally employed illegitimate foraging behavior to access the floral rewards, which indicates that they are not very skillful in handling the flowers of these species. Adult carpenter bees are too large for the flowers to hold them and, in effect, the flowers swing back and forth after their landing and departure; however, juvenile carpenter bees are quite appropriate foragers for these flowers, though they never visited them during the observation period. All bees recorded on the flowers of *Tephrosia* species tend to visit as many plants as possible in a single bout, because of the production of a few flowers daily by individual plants and non-availability of the earlier day flowers for forage because of their closure at the end of the day of anthesis. Such a foraging behavior of bees and also of wasps appears to have an important role in enhancing cross-pollination. The bees carry pollen ventrally and individual bee species vary in their pollen carrying capacity, which could be related to pollen loss during flight and pollen grooming by them for use in brood provision. In both *Tephrosia* species, being light in weight, butterflies are inappropriate to trip the flowers but they access nectar and, hence, act as nectar robbers. The butterflies effect pollination depending on their posture to access nectar in tripped flowers of *T. purpurea,* because the staminal complex and pistil stay exposed until flower closure. Their role as pollinators in *T. villosa* is totally ruled out as the staminal complex and pistil are closed by the keel petals in tripped and untripped flowers until flower closure.

Kumari & Sharma (2018) reported that in *T. purpurea* the pod set and seed set rates in bagged and open-pollinated flowers are nearly the same, suggesting facultative autogamy. In the present study, in *T. purpurea*, the pod and seed set rates in both bagged and open-pollinated flowers were at moderate levels, the high reproductive output in the latter suggesting the function of facultative xenogamy. The pod and seed set rates in *T. villosa* in these modes of pollination are slightly higher as compared to those in *T. purpurea,* but the reproductive output indicates that it is also facultatively xenogamous. The fruit and seed set rates in these species indicate that autonomous autogamy is not operational and the evidenced fruit and seed set is a function of the thrips breeding and feeding activity on floral rewards. Moreover, the fruit and seed set evidenced in open-pollinated flowers is a function of vector-mediated pollination. In both modes of pollination, the seed set rates are not commensurate with the number of ovules produced by the individual flowers. Seedless pods in both species indicate that the fertilized ovules speed up the seed production only after complete development of the pod. Empty pods, ill-formed seeds and variation in the number of seeds produced in individual pods suggest that there is an internal regulation by plants to allow cross-pollinated flowers to produce more seeds and abort selectively the genetically inferior pollen resulting from self-pollination, probably mostly from autonomous autogamy. This finding is in agreement with Stephenson & Bertin (1983), who reported that self-pollinated flowers produce always fewer seeds that are more likely to be aborted than the fruits from cross-pollinated flowers. Still, the observed low fruit and seed set rates in open-pollinated flowers of both *T. purpurea* and *T. villosa,* despite the functionality of facultative xenogamous mating system, may be attributable to the presence of untripped flowers, extent of cross-pollination rate, short tenure of pollen viability, number of forag-

ing visits made by the pollinating insects, flower closure by the end of the day of anthesis, and energy resources available to the plant during flowering and fruiting phase. However, the low natural fruit/seed to flower ratios do not affect the colonizing success rate of the *Tephrosia* species, because individual plants have inherent ability to flower and fruit throughout the year, ensuring constant production of seeds in order to expand their distribution range in varied natural, human and agricultural habitats. The pod and seed features are almost similar in both *Tephrosia* species. The difference between these species is that the pods are glabrous in *T. purpurea* and densely velutinous and retrofalcate in *T. villosa*. Kumari & Sharma (2018) reported that in *T. purpurea*, individual flowers produce on the average 4.6 ovules and individual pods produce on the average 13.37 seeds. This finding is totally incorrect because seed number per pod depends on the number of ovules produced per flower. In the present study, it was found that *T. purpurea* produces 1–6 seeds per pod against 5–7 ovules produced per flower, while *T. villosa* produces 1–8 seeds per pod against 5–8 ovules per flower. Kumari & Sharma (2018) stated the fruit and seed size and number per pod in *T. purpurea*. The pods are on the average 3.41 mm long, each measuring a mean size of 3.79 mm, with individual seeds weighing 12.18 mg on the average. On the contrary, in the present study it was found that *T. purpurea* pods have comparably a slightly shorter mean length, while the seeds had a lower weight. *T. villosa* pods are small in length, with individual seeds of lower weight, as compared to those in *T. purpurea*. Mishra & Sen (1986) reported two population types in *T. purpurea*, L-population with large cylindrical seeds showing black, black-mottled, brown, brown-mottled and yellow-less- mottled seed coat and S-population with small round seeds displaying yellow-mottled, brown-mottled and purple-mottled seed coat. In the present study, it was found that *T. purpurea* with a single population type produces slightly smaller seeds, with uniform colour pattern typified by green in the growing stage and brown-mottled in the mature stage. Similarly, the *T. villosa* seeds are smaller than those of *T. purpurea* and the seed coat colour is green to light-ash in the growing stage and light-brown-mottled in the mature stage.

In both *Tephrosia* species, the mature pod is dry and remains attached to the parent plant by a pedicel. The pod is autochorous and breaks open into two halves by spiral twisting; this spiral twisting is very prominent in *T. villosa*. The summer season with its high ambient temperatures and low relative humidity is the ideal season for effective explosive seed dispersal. In both species, the seeds at the upper end of the pod are dispersed before those at the base and, accordingly, the time gradually increases for dispersal of seeds from apex to base. However, seed dispersal through autochory is limited to a few meters away from the parental plants, although wind currents depending on the ambient temperatures and humidity levels may also aid a long-range dispersal (Bansal & Sen 1981). Furthermore, long-range dispersal is effected by rain water and this mode might be the main reason for the colonization of different habitats by the *Tephrosia* species. Dispersal far away from the parental sites reduces competition between parents and offspring on germination, thus improving reproductive capacity. The most vigorously growing seedlings establish and enjoy advantages in nutrients or light over their siblings (Willson & al. 1990). Shra & Sen (1984) reported that *T. purpurea* exhibits vivipary or early germination in their pods when seeds dry slowly due to rains in the ripening period. These authors also noted that the amount of water lost from seeds depends on the ambient relative humidity during the time of seed drying. The present study has not observed any vivipary in *T. purpurea* and *T. villosa,* even during the heavy rain period for more than twenty continuous days and despite the fact that the study zone was a non-arid zone.

In both *Tephrosia* species, despite the ability of seeds to germinate as soon as they fall on the ground, their germination and subsequent production of new plants is greatly influenced by soil moisture and nutrient levels. Ideal soil conditions occur during the rainy season and it is this season that enables most of the germinated seeds to produce new plants. Seeds dispersed at the end of the summer season begin to germinate as soon as the rainy season sets in. Seeds germinate within eight to 14 days, provided the soil is sufficiently or moderately moist and charged with nutrients, but their continued growth and development is largely related to their efficiency in utilizing the available nutrients over other co-occurring seedlings or plants growing simultaneously with them, and further availability of soil moisture and nutrients. In addition to propagation by seeds, both species resprout from the dormant underground perennial root stock in a favorable moisture and nutrient environment in

the soil, in order to repeat their life cycle. Therefore, the dual modes of propagation by these species ensure their recolonizing of the same habitat and invasion of new habitats.

Soni & al. (2006) and Pavana & al. (2007) pointed out that *Tephrosia purpurea* is used as an important constituent in several herbal medicines for treatment of various human diseases. In the study area, local people had no knowledge of the uses of *T. purpurea* and *T. villosa* in traditional medicine. Still, these species can be considered multi-purpose legume species, because they are drought-tolerant and fix atmospheric nitrogen by use of rhizobial bacteria in their roots, and are useful in soil improvement and erosion control (Bosman & De Haas 1983; Zhi & Pedley 2010). Furthermore, these species support bees, wasps and butterflies by providing pollen and/or nectar as long as they are in flowering. Apart from these uses, *T. purpurea* acts as a larval host plant for the lycaenid butterfly, *Catochrysops crabo* (Jothimani & al. 2014). Therefore, *T. purpurea* and *T. villosa* can be considered for inclusion in the eco-restoration programs intended for semidry and dry regions, due to their drought resistance and ability to improve soil fertility and erosion control.

Conclusions

Tephrosia purpurea and *T. villosa* are year-long bloomers. *T. purpurea* produces resupinate and non-resupinate flowers, presenting an explosive pollination mechanism, while *T. villosa* produces exclusively non-resupinate flowers, presenting a valvular pollination mechanism. Pollination mechanisms and occurrence of pollination in these species are functional through flower-tripping effected by wind, rain and temperature and resulting in autogamy, by thrips resulting in autogamy and geitonogamy, and by bees and wasps in self- and cross-pollination. These species are self-compatible, self-pollinating and facultatively xenogamous. Fertilized ovules speed up seed production only after complete development of pods. Empty pods, ill-formed seeds and variation in the number of seeds produced in individual pods evidenced in these species appear to be a consequence of internal regulation by them to allow cross-pollinated flowers to produce more seeds and abort selectively the genetically inferior pollen resulting from self-pollination. The low fruit and seed set rates recorded in open-pollinated flowers of both species appear to be related to the presence of untripped flowers, extent of cross-pollination rate, short tenure of pollen viability, number of foraging visits made by pollinating insects, flower closure by the end of the day of anthesis, and availability of energy resources. Both species are autochorous and seeds germinate, if the soil has moisture and nutrients. The plants also sprout from the perennial root stock, usually during the rainy season. Therefore, the dual modes of propagation ensure that these species re-colonize the same habitat and invade new habitats. These species are drought-tolerant and fix atmospheric nitrogen by using rhizobial bacteria in their roots, thus they are useful in soil improvement and erosion control. Hence, they can be included in the eco-restoration programs intended for semidry and dry regions.

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