Monoecy, ambophily and polychory in the endemic deciduous tree species *Croton scabiosus* (*Euphorbiaceae*) of the Southeastern Ghats of Andhra Pradesh, India

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Abstract. A study was undertaken to find out the sexual system, pollination syndrome and seed dispersal modes in *Croton scabiosus*, an endemic deciduous tree from the Southeastern Ghats of Andhra Pradesh, India. The standard field methods/observations were used to examine different aspects of the pollination ecology. The study revealed that *C. scabiosus* blooms and fruits throughout winter, summer and the early phase of the wet season. Sporadic flowering occurs during the late wet season, if soil environment is favorable. The species is monoecious, ambophilous, involving myrmecophily and thripsophily, and polychorous, involving diplochory and hydrochory. *In vitro* pollen germination studies with different nutrient media indicated that sucrose and boric acid had each good pollen germination and tube development, while sucrose together with boric acid showed stimulatory effect in maximizing pollen germination rate and pollen tube length at the optimal concentration. Calcium nitrate, potassium nitrate and magnesium sulphate had dual roles of regulation and enhancement of pollen germination, as well as pollen tube growth, depending on their concentrations.

Key words: anemophily, *Croton scabiosus*, myrmecophily, polychory, thripsophily

Introduction

Reproductive ecology covers all aspects of the reproductive events and their interactions with biotic and abiotic components of the environment (Shivanna & Tandon 2014). Reproduction in flowering plants includes three sequential phases: flowering and pollination, fruiting and seed dispersal, and germination and seedling establishment. Variation in reproductive success results from variation in many ecological factors, including growing conditions, pollinator service, dispersing agents, and pest and disease pressures (Barrett & Eckert 1990). Pollination phase is the most critical event in sexual reproduction and the basis of gene flow and genetic recombination. This process can be effected by abiotic vectors such as wind and water and by various animals, including insects, birds and mammals (Veerabhadraiah 1990; Shivanna & Tandon 2014). Comprehensive understanding of pollination ecology of individual species is needed to become thoroughly familiar with the phenology, floral morphology and sexuality of the species (Shivanna & Tandon 2014), which is essential for the conservation and management of both wild and domesticated species.

Effective pollination is a prerequisite for fruit and seed set in most plants, and information on pollen biology, including pollen viability, pollen germination and pollen tube growth, is required for any rational approach to increase productivity (Bolat & Pirlak 1999; Shivanna 2003). Pollen viability and the efficiency of pollen transfer partially determine the reproductive success of a species (Abdelgadir & al. 2012). It is evaluated by staining techniques, *in vitro* and *in vivo* germination tests and analysis of the final seed set, but choice of the method depends on the plant species being studied (Dafni & al. 2005). In many species, *in vitro* pollen germination depends on addition of key substrates to the germination media (Steer & Steer 1989). Pollen tube growth can affect the outcome, or self- versus cross-pollination, because pollen tubes from self-pollen may grow slower or have higher rates of attrition than those from cross pollen (Aizen & al. 1990). Pollen fertility and viability have a paramount importance in breeding programs. *In vitro* pollen germination studies are important to understand the physiology and biochemistry of pollen germination and tube growth (Moutinho & al. 2001). Pollen germination and pollen tube development are used in determining the importance of the cytoskeleton in cell growth and differentiation (Ma & al. 2000). *In vitro* germination of pollen grains is necessary for various biotechnological manipulations (Abdelgadir & al. 2012). Therefore, studies of pollen viability and pollen germination are very crucial for biotechnological applications and for conservation of plant species, especially the ones which are endemic or threatened.

Plants being stationery require a mobile mode for fruit or seed dispersal. Some fruits have built-in mechanisms to disperse by themselves, whereas others require the help of such agents like wind, water and animals. Wind-dispersed fruits/seeds are light-weight and may also have wing-like appendages that facilitate them to be carried away by the wind. Water-dispersed fruits/seeds are light-weight and buoyant giving them the ability to float. Dispersal by animals involves plant-animal interactions in which reciprocal adaptations between them take place and lead either a change in both or in one of the organisms as a result of interaction with the other (Stiles 1989; Fleming & Estrada 2012).

With this backdrop, the pollination ecology of the deciduous tree species *Croton scabiosus* Bedd. (*Euphorbiaceae*) was contemplated keeping in mind its endemic status, medicinal and commercial value in the Southeastern Ghats of Andhra Pradesh, India (Ahmedullah & Nayar 1986; Devi & al. 2011). Further, IUCN placed this species under vulnerable (Salamma & Rao 2014), considering its threatened status. The present study includes flowering phenology, floral biology, sexual system, pollination syndrome, pollen viability, *in vitro* pollen germination, and fruit and seed dispersal. The information generated on these aspects of *C. scabiosus* is useful to understand its sexual reproduction and seed dispersal modes which contribute to its present population status in natural habitats.

Material and methods

Study area. Two populations of *Croton scabiosus* Bedd. – one with 240 trees in Palakondalu Hill Range (14°28.39 N Lat., 78°49.71 E Long. and 610 m a.s.l.) and another with 360 in the Idupulapaya Reserve Forest (14°19.418 N Lat., 78°31.468 E Long. and 275 m a.s.l.) in Kadapa District, Andhra Pradesh, India – were selected for study in 2014–2018. The two populations are distanced at 50 km. The westward extension of the hill range in Anantapur forms the Seshachalam Hills, which have been recently declared as Seshachalam Biosphere Reserve by the Ministry of Environment, Forests and Climate Change of India. The name *Palakondalu* in local dialect stands for *milk hills,* because of their past use as pastureland, now degraded by pilgrimage and local tourism activities to the Palakonda Rayaswamy Temple and Waterfalls. Idupulapaya Reserve Forest is a rocky terrain with a deciduous forest ecosystem.

Phenology. Field visits were made regularly at monthly intervals to record such phenological events of *C. scabiosus* as leaf fall, leaf flushing, flowering, and seed dispersal. In the flowering season, field observations included flowering patterns at plant and inflorescence level. As the plant was found to be monoecious, the inflorescences were examined for the sexual status of flowers, in order to record the occurrence rate of male and female flowers. At each study site, one hundred inflorescences from ten trees were collected to record the ratio of male and female flowers at inflorescence level, and also to record whether all inflorescences produce both male and female flowers, or not. The ratio of male and female flowers produced at plant level was recorded separately for each flowering season at the two study sites.

Flower morphology and floral biology. Fifty male and fifty female flowers were collected from ten trees and used for describing such floral morphological details as size, colour and shape, and to measure the essential and non-essential floral parts. Floral morphology and functional details of each flower sex were described separately. Close observations of male and female flowers indicated presence of nectar only in the male flowers. The nectar secreted by those flowers could not be measured due to its secretion in trace amounts. Ten inflorescences were tagged prior to initiation of flowering and followed to record the daily anthesis rate and duration of anthesis. As the inflorescences produced flowers both in the forenoon and afternoon hours, the percentage of anthesis at each hour and in the forenoon and afternoon period was calculated.

Determination of pollen output. Pollen output was determined by taking 20 mature buds from male flowers of ten plants. The anthers collected from the sample flowers were placed in a Petri dish. Then, one by one the anthers were taken out and placed on clean microscope slides (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× eyepiece). 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 output per anther was multiplied by the number of anthers in the flower for obtaining the mean number of pollen grains per male flower. Another set of ten dehisced anthers was placed into a Petri dish and the removed pollen was examined under microscope for recording the pollen grain features.

Determination of stigma receptivity. Ten female flowers from each of five trees were used to test stigma receptivity. It was tested with hydrogen peroxide from the mature bud stage to flower wilting as per Dafni & al. (2005) to record the duration of stigma receptivity period during flower life.

***In vitro* pollen viability**. Fresh dehisced anthers were put in a Petri dish and stored in the laboratory. The pollen grains were placed on a glass slide and 2,3,5-triphenyl tetrazolium chloride was applied to observe whether they got stained or not. If stained, it was assumed that the pollen grains were viable. Using the collected pollen, this procedure was followed and pollen viability was tested at hourly intervals for fifteen hours. As there was no pollen viability after 14 hours, the test was terminated.

***In vitro* pollen germination.** Fresh flowers were collected and transferred to polythene bags. Different concentrations of sucrose, boric acid, potassium nitrate, calcium nitrate and magnesium sulphate, and also sucrose in combination with boric acid were used to test the *in vitro* pollen germination. For each solution and each concentration, ten grooved slides were half-filled with the chosen solution and pollen was placed there. Then, the slides were kept in Petri dishes lined with moist filter paper and examined under Olympus microscope to record the percentage of germination of pollen grains and also the pollen tube length, following the method of Shivanna & Rangaswamy (1993). A pollen grain was considered germinated, if the pollen tube length became at least twice the diameter of the pollen grains (Gupta & al. 1989).

Assessment of the breeding system. The plants were tested only for geitonogamy and xenogamy, as there was no possibility for autogamy due to production of unisexual flowers. One hundred mature female flower buds from twenty trees were tagged and enclosed in paper bags for each mode. The female flowers were hand-pollinated with the pollen of male flowers on the same plant and bagged to test geitonogamy. The female flowers were hand-pollinated with the pollen of male flowers from different conspecific plants and bagged to test xenogamy. Thirty inflorescences with 150 developing/maturing female buds were tagged separately at Palakondalu and Idupulapaya sites in the January-June flowering season. Furthermore, 75 developing/maturing female buds were tagged at Idupulapaya site in the September-October flowering season. All these bagged and tagged buds were followed for calculation of fruit and seed set percentage in all three modes of pollinations.

Pollination syndrome and pollinators. The floral features of male and female flowers, both essential and non-essential, were carefully observed to define the pollination syndrome. Field observations during the entire study period at the two study sites indicated that the plant was not visited by any insect species during its flowering season, despite the presence of insects in the habitat at that time. However, there were ants crawling on the plants during the flowering period. Their activity on the plants was carefully observed to record whether they have any role in pollination, at least in geitonogamy. Furthermore, the male flower buds were found to be infested with thrips, which used them for breeding. They emerged during the anthesis of male flowers and concentrated on pollen collection. Then, they were watched to see if they also make visits to the female flowers of the same plant in search of forage and thus effect pollination.

Observations on the activity of sunbirds. Field observations indicated that sunbirds visited the plants during the flowering season. They were observed closely to record whether they visit the flowers or not. However, the observations indicated that they were visiting the plants to collect instars of larvae of an unknown butterfly species from the new foliage and that was recorded.

Plant species in flowering in association with *Croton scabiosus*. The two study sites were carefully surveyed to record the plant species in flowering phase along with *C. scabiosus*. Furthermore, all these plant species were observed in the field to record whether foragers were visiting the flowers or not, in order to confirm the availability of insect fauna in the habitat of *C. scabiosus*. The flowers of these plant species were watched to record their sexual system and whether they were nectariferous or not.

Fruiting ecology and seed dispersal. Field observations were made regularly to record the fruit maturation period of fertilized female flowers. Fruit and seed characteristics were described in detail. Fruit dehiscence mode and seed dispersal modes were described based on field observations. Ants were found to be involved in seed dispersal on the ground; they were followed to record their role in seed dispersal.

Results

Plant and flowering phenology. *Croton scabiosus* is a small deciduous tree which grows up to 3–5 m high. The stem bark is rough, deeply fissured, blackish brown. Leaves are alternate, lamina ovate-rhomboid, with silvery scales on both surfaces, and coriaceous. Field observations on leaf fall, leaf flushing and flowering period at Idupulapaya (Fig.1a) and Palakondalu (Figure 1b) sites indicated that these three phenological events occurred simultaneously at the two sites. Leaf fall and flushing events occured in succession, without any time gap, while flowering began in synchrony with leaf flushing in January (Fig. 1c-e). Leaf fall occurred in November-December, while leaf flushing occurred in January-March. Flowering occurred in January-June, with peak flowering in March. However, the population at Idupulapaya site also flowered in September-October, with profuse flowering on some branches and sporadic flowering on others. The inflorescence was a terminal simple raceme consisting of female flowers at the base and male flowers above them; the length of the raceme varied from 5.5 cm to 13.2 cm, depending on the number of produced flowers (Fig. 1f). However, some inflorescences consisted either of male or of female flowers but not of both. The percentage of these three types of inflorescences was 60 % for those with male and female flowers, 35 % for those with only male flowers and 5 % for those with only female flowers at plant level. All three types of inflorescences initiated anthesis acropetally. In inflorescences with female and male flowers, anthesis began in the female flowers and was followed by anthesis in the male flowers after completion of the anthesis of all female flowers. All three types of inflorescences continued anthesis without any break and the total duration of flowering in them varied from 8 to 10 days. In inflorescences with female and male flowers, the number of female flowers varied from 5–10, while that of male flowers varied from 15 to 20. In inflorescences with male flowers only, their number varied from 20 to 35, while in inflorescences with only female flowers, their number varied from 11 to 22. At plant level, the ratio of male-to-female flowers was 9:1 in the January-June flowering season at Palakondalu. The corresponding ratio was 6:1 in the January-June flowering season and 2:1 in the September-October flowering season at Idupulapaya. The three types of inflorescences flowered synchronously and displayed availability of the self-pollen for stigmas of female flowers on the same plant; this facilitated geitonogamous pollinations.

Flower morphology. The plant is monoecious, with both male and female flowers on the same/different inflorescences of the same plant. The male and female flowers are sepalous but small in the former, and larger in the latter. The male flowers are petaliferous, while the female flowers are apetaliferous. The flowers of both sexes produced in January-March are slightly odoriferous, those produced in April-June are odour-

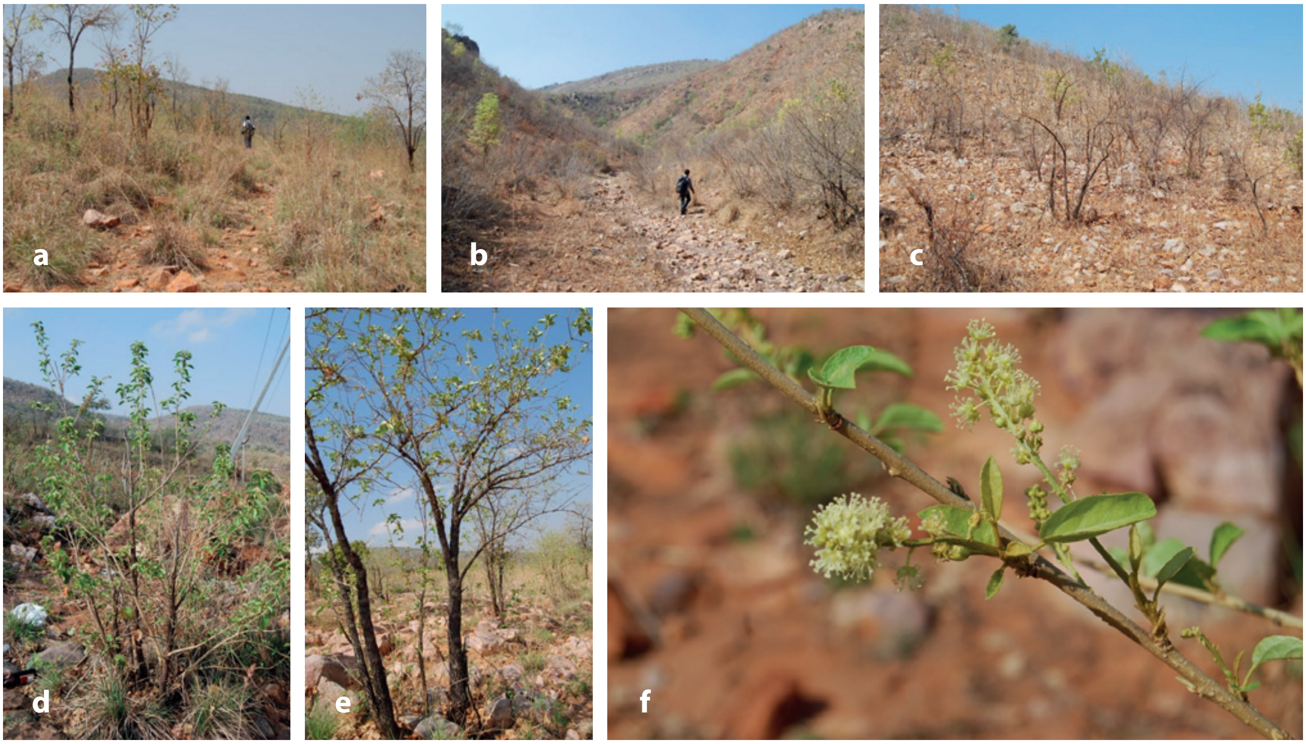

Fig. 1. *Croton scabiosus*: a. Idupulapaya study area; b. Palakondalu study area; c. Leaf fall stage; d. Leaf flushing stage; e. Flowering initiation; f. Twig with flowering inflorescences.

less and those produced in September-October are slightly odoriferous too.

Anthesis. Mature buds opened throughout the day, from 06:00 to 18:00 h, with a break for three hours from 11:00 h to 13:00 h. Of all mature buds opened in a day, 77 % open in the forenoon, and 33 % open in the afternoon. At forenoon, 28 % of the buds open at 07:00 h, 22 % at 08:00 h, 18 % at 06:00 h, 8 % at 09:00 h, and 2 % at 10:00 h. In the afternoon, 8 % of the buds open at 17:00 h, 5 % each at 16:00 h and 18:00 h and 2 % each at 14:00 h and 15:00 h (Table 1). Individual male and female mature buds take approximately one hour to complete unfolding of sepals and/or petals to expose the stamens or styles and stigmata (Fig. 2a-c).

Male flowers. The flowers are pedicellate, small (7 mm long and 6 mm wide), greenish yellow, dish-shaped and actinomorphic. They are produced in 3–4 flowered clusters in each node at the upper part of the raceme. The calyx consists of 5 sepals (2 mm long) which are free, hairy and green. The corolla consists of 5 petals (6 mm long) which are delicate, free, slightly concave and greenish yellow, with two deep green longitudinal lines. The androecium is represented by 15 to 20 free yellowish green 4–5 mm long exserted

Table1. Percentage of anthesis as a function of time in *Croton scabiosus*.

Time (h)	Number of flowers anthesed	Percentage of anthesis
0600	7	18
0700	11	28
0800	8	22
0900	3	8
1000	1	2
1100	0	0
1200	0	0
1300	0	0
1400	1	2
1500	1	2
1600	2	5
1700	3	8
1800	2	5

Sample size: 10 inflorescences – 6 inflorescences with female and male flowers, 2 only with female and 2 only with male flowers.

stamens. Anthers are light yellow, bilocular, basi-versatile, extrorse and dehisce through longitudinal slits after anthesis. The anthers of all stamens in a flower dehisce over a period of about 2 hours. The pollen

Fig. 2. *Croton scabiosus*: a. & b. Anthesis of female flowers; c. Acropetal anthesis of male flowers; d. & e. *Camponotus* sp. collecting nectar from female flowers; f. & g. Pollen; h. & i. *Nectarinia asiatica* (male) searching for butterfly larva from the leaves; j. Fully developed fruits from female flowers.

output is 219.4 ± 24.7 per anther and 3219 to 4388 per flower, due to variation in the number of produced stamens. The pollen-ovule ratio also varies accordingly, and the pollen is copiously produced at inflorescence and plant level. The pollen grains are monads with ornamented exine, light yellow, spheroidal, inaperturate, 44.8 ± 4.06 µm in diameter, dry and powdery. The five nectaries, situated opposite to the sepals at the base, secrete nectar in traces. The flowers that open in the forenoon fall off by the end of the day, while those that open in the afternoon fall off before noon on the following day.

***In vitro* pollen viability and *in vitro* germination**. The pollen grains are viable for 14 hours from the time of anther dehiscence (Fig. 2 f,g). The percentage of viable pollen grains is 93 % after one hour, 64 % after four hours, 42 % after eight hours, and 13 % after 12 hours of anthesis. The pollen grains are not viable after 14 hours of anthesis. The viable pollen grains from male flowers of the previous day are available for pollination of female flowers until the morning of the following day, because of the continuous anthesis throughout the day.

The effect of pollen germination percentage and tube length varied with each nutrient medium used for *in vitro* study. In a 5 % sucrose solution, 39 % of pollen germinated with a maximum tube length of 373 µm. In a 10 % sucrose solution, 45 % of pollen germinated with a tube length of 429 µm. In a 20 % sucrose solution, 33 % of pollen germinated with a tube length of 283 µm. The percentage of pollen germination gradually increased from 37 to 51 % and the pollen tube length increased from 171 to 323 µm in 15 to 100 ppm boric acid concentrations, while pollen germination and tube length decreased in 200 and 300 ppm boric acid concentration. The percentage of pollen germination is 61 %, with a tube length of 315 µm in 10 % sucrose + 20 ppm boric acid solution and 41 % pollen germination with a tube length of 306 µm in 10 % sucrose + 25 ppm boric acid solution. In a 10 % sucrose and 50 ppm boric acid solution, 10 % sucrose and 100 ppm boric acid solution, 10 % sucrose and 200 % ppm boric acid solution, the percentage of pollen germination gradually decreased from 29 % to 19 % and pollen tube length from 157 to 104 µm respectively. In KNO_3 concentrations of 50, 100, 200, and 300 ppm, the percentage of pollen germination gradually decreased from 29 % to 17 % and the pollen tube length from 168 to 111 µm, respectively. In 50 ppm Ca $(No3)_2$ concentration, the percentage of pollen germination is 24 %, with a tube length of 134 µm, while in 100, 200, 300 ppm Ca $(No3)_2$ concentrations, the percentage of pollen germination gradually decreased from 33 % to 18 % and the pollen tube length from 218 to 147 µm, respectively. In 50 ppm

$MgSo_4$ concentration, the percentage of pollen germination is 34 %, with a tube length of 160 µm, while in 100, 200, 300 ppm $MgSo_4$ concentrations, the percentage of pollen germination gradually decreased from 40 % to 11 % and the pollen tube length from 213 to 101 µm, respectively (Table 2).

Female flowers. The flowers are short-stalked, small (6.8 ± 0.6 mm long and 5.4 ± 0.4 mm wide), greenish yellow, oval shaped and actinomorphic. The calyx consists of 5 sepals (3.4 ± 0.7 mm long and 1–2 mm wide) which are free, hairy and green. The ovary is sessile, globose, warty, tri-carpellary, densely hairy and light green to brownish green. Each carpel is unilocular and has one epitropous ovule with axile placentation. The styles are three, 4.8 ± 0.5 mm long, light yellow and each style is bifurcated into stigmata. Seldom, the ovary is tetra-carpellary, with four styles and eight stigmata due to bifurcation of the styles. Stigmas attain receptivity with anthesis and stay receptive to pollen for 6–8 hours. Nectar is not secreted, due to absence of nectaries at the base of the ovary. The flowers that open in the forenoon fall off by the end of the second day, while those that open in the afternoon fall off before noon of the third day.

Breeding system. The plant does not set fruit through autogamy because the flowers are unisexual. Hand-pollination tests indicated that geitonogamy and xenogamy are functional. Fruit set is 23 % in geitonogamy and 35 % in xenogamy. Seed set is 52 % in geitonogamy and 87 % in xenogamy. In open pollinations, the fruit set is 46 %, while seed set is 45 % in the January-June flowering season at Palakondalu study site; the corresponding values for the same flowering season are 53 % and 51 % at Idupulapaya study site. The fruit and seed set recorded in the September-October flowering season are 39 % and 40 %, respectively, at Idupulapaya study site (Table 3).

Pollination syndrome and pollinators. Such characters as flowers packed in terminal racemose inflorescences, small male and female flowers, well exposed stamens with basi-versatile anthers, reduced sepals and petals, and light, non-sticky, spheroidal pollen grains with clavate ornamentation in male flowers, well exposed styles and stigmas, reduced sepals, and absence of petals in female flowers have been considered as adaptations for anemophily. Production of nectar in male flowers is an entomophilous character, while absence of nectar production in female flowers is an anemophilous character. These characters collectively indicate that the plant is exclusively anemophilous. The habitat of the plant at the two study sites is

Table 2. ***In vitro*** **pollen germination of** ***Croton scabiosus*** **in different media.**

Medium	No. of pollen grains observed	No. of pollen grains germinated	*Pollen germination (%)	**Pollen tube length (µm)
Sucrose				
5 %	165	39	39	373
10 %	229	45	45	429
20 %	115	33	33	283
Boric Acid (PPM)				
15	175	65	37	171
20	203	87	43	228
25	230	102	44	267
100	241	123	51	323
200	276	111	40	303
300	268	99	37	260
Sucrose (%) + Boric acid (PPM)				
10 + 20	258	158	61	315
10 + 25	272	113	41	306
10 + 50	235	73	31	229
10 + 100	204	60	29	157
10 + 200	210	46	22	145
10 + 300	236	44	19	104
KNo3 (PPM)				
50	201	58	29	168
100	215	55	25	157
200	196	46	21	121
300	208	35	17	111
Ca (No3)2 (PPM)				
50	216	51	24	134
100	224	74	33	218
200	185	43	23	184
300	197	35	18	147
MgSo4 (PPM)				
50	178	61	34	160
100	200	81	40	213
200	172	40	23	113
300	220	25	11	101

*, ** – values rounded off to the nearest digit.

Table 3. Results of breeding systems in *Croton scabiosus*.

Treatment	No. of pollinated flowers	No. of flowers set fruit	Fruit set (%)	Seed set (%)
Geitonogamy	100	23	23	52
Xenogamy	100	35	35	87
Open-pollination				
Palakondalu (January-June flowering season)	150	69	46	45
Idupulapaya (January-June flowering season)	150	80	53	51
Idupulapaya (September-October flowering season)	75	29	39	40

a deciduous ecosystem and most of the plant species are leafless and non-flowering, especially in March-May. This physical state of the habitat in the flowering season of the plant is found to be conducive to anemophily. Furthermore, the nectariferous male flowers and soft and succulent sepals and tissues around ovary base in female flowers attract *Camponotus* ants at both study sites (Fig. 2 d,e). These ants are resident foragers and crawl all over the plant. They crawl on the inflorescences and collect sap from succulent parts of the female and nectar from the male flowers. In this foraging activity, they have found to be transferring pollen from male flowers to simultaneously available female flowers on other inflorescences of the same plant; their foraging activity results in geitonogamy. The thrips species, *Haplothrips* sp. (Thysanoptera: Thripidae) use the male buds for breeding at both study sites. The larvae emerge from the eggs in synchrony with anthesis and nectar production in male flowers. The larvae and adults forage for pollen and nectar produced by the male flowers, but they never go towards the female flowers on the same plant as they do not produce nectar and pollen. Therefore, thrips have been found to be mere foragers on the male flowers but collection of nectar by them could enhance the foraging activity of ants to promote geitonogamy.

Plant species in the flowering phase in association with *Croton scabiosus*. At both study sites, certain plant species flowered simultaneously and attracted mostly bees to their flowers. At Palakondalu site, *Boswellia serrata* (Burseraceae), *Cochlospermum religiosum* (Cochlospermaceae), *Cleistanthus collinus* (Euphorbiaceae), *Pterocarpus santalinus* (Fabaceae), *Ceiba pentandra* (Bombacaceae), *Mimosa intsia* (Mimosaceae), *Ochna obtusata* (Ochnaceae), and *Gardenia gummifera* (Rubiaceae) flowered simultaneously with *Croton scabiosus*. At Idupulapaya, *Maerua apetala* (Capparaceae), *Cochlospermum religiosum* (Cochlospermaceae), *Gardenia gummifera,* and *G. latifolia* (Rubiaceae) flowered simultaneously with *Croton scabiosus* (Table 4). Of these, *C. collinus*, *P. santalinus* and *Gardenia* species occur in ample populations, while all other plant species consisting of a few individuals have scattered occurrence. *B. serrata, C. religiosum* and *C. collinus* are leafless in their flowering phase, while all others have foliage in that phase. At Palakondalu, all plant species, except *M. intsia,* are deciduous trees. *M. intsia* is a deciduous straggler. At Idupulapaya site, all four tree species flowering along with *C. scabiosus* are deciduous trees. All plant species, except *C. collinus,* are hermaphroditic, while *C. collinus* is monoecious; all are nectariferous and nectar is measurable in all of them, except in *M. intsia,* which produces it in traces. Bat foraging activity has been observed at night time on *C. pentandra* and *Gardenia* species. At day time, all plant species have been visited consistently by bees, *B. serrata* also by butterflies and *M. apetala* and *G. latifolia* also by birds. In the habitats at the two study sites, the plant species flowering simultaneously with hectic foraging activity of animals, especially bees, on them are considered an indication of availability of pollinator animals. However, none of these animals have attempted to visit the flowers of *C. scabiosus,* despite the displayed flowers on terminal racemose inflorescences against the newly emerging and emerged foliage and, hence, this plant species is adapted for anemophily.

Activity of sunbirds. The sunbirds, *Nectarinia asiatica* and *N. zeylonica,* were found collecting larval instars of a butterfly species as a source of food at the two study sites (Fig. 2h,i). The birds were regular and busily engaged in the collection of butterfly larvae in the flowering season. As this plant was available with considerable populations at the two study sites, the

Table 4. List of associated plant species flowering simultaneously along with *Croton scabiosus*.

Family	Plant species	Flowering Season	Study sites Palakon-dalu	Study sites Idupula-paya	Habit	Foragers
Burseraceae	*Boswellia serrata**	March-June	+	-	Deciduous tree	Bees, butterflies
Capparaceae	*Maerua apetala*	April -July	-	+	Deciduous tree	Bees, birds
Cochlospermaceae	*Cochlospermum religiosum**	February-August	+	+	Deciduous tree	Bees
Euphorbiaceae	*Cleistanthus collinus**	February-October	+	-	Deciduous tree	Bees
Fabaceae	*Pterocarpus santalinus*	March-May	+	-	Deciduous tree	Bees
Bombacaceae	*Ceiba pentandra*	December-April	+	-	Deciduous tree	Bats, bees
Mimosaceae	*Mimosa intsia*	Year-long	+	-	Deciduous straggler	Bees
Ochnaceae	*Ochna obtusata*	March-July	+	-	Deciduous tree	Bees
Rubiaceae	*Gardenia gummifera*	December-May	+	+	Deciduous tree	Bats, bees
	Gardenia latifolia	December-May	-	+	Deciduous tree	Bats, birds, bees

* Leafless during flowering season; *Cleistanthus collinus, Pterocarpus santalinus* and *Gardenia* species had considerable population while all other species manifsted a few individuals with scattered distribution.

sunbirds used it as an important source of solid food, especially in the dry season, because it was the only plant species which displayed simultaneously both new foliage and flowering at the two study sites. The sunbirds never visited the flowering inflorescences of the plant, although male flowers provided nectar in traces.

Fruiting ecology and seed dispersal. The pollinated and fertilized female flowers develop continuously so as to produce fruits in a time span of 16–20 days (Fig. 2j). The fruits are initially green, then light yellow and finally brown, when mature. The calyx with inconspicuous sepals does not grow further to enclose the fertilized ovary and subsequently becomes a residual part at the base of the fruit. The styles and stigmas do not fall off to the ground and remain in place at the apex of the fruit. The fruits are schizocarpic capsules; globose or sub-globose, 8.1 ± 0.4 mm long, 9.4 ± 0.6 mm wide, with warty outer walls. Some fruits produce 2–3 seeds in the tri-carpellary female flowers and 2–4 seeds in the tetra-carpellary female flowers. Each carpel grows into a thick-walled mericarp, which consists of a pericarp, two septal parts and one dry seed with a caruncle. The fertilized female flowers produce mostly three seeds, irrespective of the number of produced ovules. The seeds are small, grey to black coloured, oblong, 4–6 mm long and have a small caruncle of creamy white fleshy structure, sugary micropylar region and copious endosperm inside. Fruits with 3 or 4 mericarps explode to disperse seeds indicating that the plant is autochorous. However, the seeds fall to the ground away from the parental plants. The fleshy and sugary caruncle of the fallen seeds is used as a food reserve by *Camponotus* ants. The ants have been observed to eat the caruncle at the dispersal site very seldom. However, they move the seeds to their nests, eat the caruncle at the nest entrance and leave the seeds outside entrance, or move them into the nest, eat the caruncle and leave the seeds inside, which subsequently appear to be buried in the shallow soil or left in rock crevices or cracks. Seed movement from parental sites to nest sites of ants has been considered as a function of myrmecochory. Furthermore, the lightweight seeds are dispersed by wind in the dry season and by rain water in the wet season due to their buoyancy. As the study sites are rocky with little soil, wind and rain water are considered effective in dispersing the seeds to various distances. Therefore, the plant is autochorous (self-dispersal), myrmecochorous (dispersal by ants), anemochorous (dispersal by wind) and hydrochorous (dispersal by water).

Discussion

Croton scabiosus is an annual bloomer and displays phenological events from November to July at both study sites. Leaf fall and flushing occur in succession, but flowering also begins along with leaf flushing and extends until June. Fruits grow very quickly and are produced continuously from January to July. Mention deserves the fact that the plant goes into second flowering in September-October at Idupulapaya site; this flowering is not intense and occurs only on some branches. Field studies indicate that at that study site, the rocky terrain has relatively more soil which holds more moisture, as compared to Palakaondalu study site. Such soil conditions appear to trigger out second flowering, which may help compensate for a low fruit or seed crop (if there is such in the first flowering season), or produce a greater fruit or seed crop. Such second flowering has been reported for *Pongamia pinnata* by Solomon Raju & Purnachandra Rao (2006).

Rai & al. (2012) listed the floral characteristics of the *Croton* genus. The basic inflorescence is racemose and varies from spiciform to paniculiform; the staminate flowers are borne in the distal part, while the pistillate flowers are borne in the proximal part. The staminate flowers are usually double-whorled, while the pistillate flowers are usually single-whorled. The styles are three, free and bifid or multifid, with decurrent stigmas. In the present study, it has been found that *C. scabiosus* produces a terminal spiciform racemose inflorescence, with male flowers in the distal part and female flowers in the proximal part. Staminate flowers are double-whorled, with one whorl of sepals and one whorl of petals, while pistillate flowers are single-whorled, with sepals only. The styles are three, free and each style is bifid with decurrent stigmas. The petaliferous male flowers and apetalous female flowers in *C. scabiosus* are in agreement with Webster (1994), who maintained that most species of *Croton* and other genera in the Crotonoideae subfamily produce petaliferous staminate flowers and apetalous pistillate flowers.

In *C. scabiosus*, *in vitro* pollen germination studies with different nutrient media have indicated that sucrose and boric acid individually show good pollen germination and pollen tube development. The effect of a nutrient medium consisting of sucrose and boric acid on pollen germination and pollen tube development is very prominent, because sucrose maintains the osmotic pressure and acts as a substrate for pollen metabolism (Shivanna & Johri 1989), while boron is directly involved in the synthesis of pectin and its location, and also in the localization of callose in the wall of pollen tubes (Stanley & Loewus 1964; Acar & al. 2010). Furthermore, boron could have a protective effect in preventing excessive polymerization of sugars in the sites of sugar metabolism (Scott 1960). Boron, added in the form of boric acid, is essential for *in vitro* culturing of pollen in most species and it has been found that elimination of boric acid from the culture medium often leads to tube bursting (Wang & al. 2003). Boron deficiency results in low pollen viability, poor pollen germination and reduced pollen tube growth (Nyomora & Brown 1997). The study suggests that though the effect of either sucrose or boric acid have individually shown good results, sucrose together with boric acid highly promote pollen germination and pollen tube development, because boron forms a complex with sugar and this sugar-borate complex is known to be capable of better translocation than non-borate, non-ionized sugar molecules (Sidhu & Malik 1986). In nature, in most plant species, the stigmas and styles provide boron to facilitate sugar uptake and pectin synthesis in the growing pollen tubes (Richards 1986). Therefore, this study shows that the sugar-borate complex has a stimulatory effect on the pollen germination rate, as well as on pollen tube length in *C. scabiosus*.

Jones & Lunt (1967) reported that calcium is one of the most important cations involved in cell metabolism and also in maintaining membrane integrity and permeability. Miller & al. (1992) demonstrated that calcium concentration plays a crucial role in maintaining the pollen tube growth. Taylor & Hepler (1997) maintained that pollen germination and pollen tube growth are significantly regulated by the transport of inorganic ions, such Ca^{++} and K^{+}, across the plasma membrane of pollen and/or pollen tubes. Fan & al. (2001) noted that external supply of K^{+} ions enhance the rate of pollen germination as well as pollen tube growth in *Arabidopsis*. Moore & Jung (1974) stated that NO^{-}_{3} and Mg^{++} enhance the tube growth in *in vitro* pollen germination of sugarcane. Prajapati & Jain (2010) indicated that calcium, magnesium and nitrate play a key role in pollen tube growth of *Luffa aegyptica*. The results of *in vitro* pollen germination studies of *C. scabiosus* verify that calcium nitrate, potassium nitrate and magnesium sulphate play dual roles in regulation and enhancement of pollen germination and pollen tube length, depending on their concentrations. Therefore, the results of *in vitro* pollen germination studies in different nutrient media with different concentrations for *C. scabiosus* agree with the corresponding studies on different plant species by Bhattacharya & Mandal (2004), Biswas & Mandal (2014) and Dutta & Mondal (2014). Moreover, this information is useful to study the chemical aspects of styles and stigmata in reference to their role in pollen germination and tube development for the fertilization of ovules in *C. scabiosus*.

In the *Euphorbiaceae* family, dioecy and monoecy are widespread sexual systems, but monoecy is most common and also more advanced sexual system (Webster 1994). The sexual systems functional in the genus *Croton* are almost unknown. A monoecious sexual system has been reported in *Croton bonplandianum* (Reddi & Subba Reddi 1985; Biswas & al. 2012) and *Croton suberosus* (Narbona & Dirzo 2010). However, Dominguez (1985) and Dominguez *et al.* (1989) reported that *C. suberosus* is monoecious. In the present study, *C. scabiosus* has been found to be monoecious, with inflorescences consisting only of male flowers, only of female flowers and of both male and female flowers, but most inflorescences produce both flower sexes. These three types of inflorescences seem to explain the possibility for reversal of the sexual systems from monoecy to dioecy and vice-versa, according to the prevailing ecological conditions, as noted by Webster (1994) for *Croton, Euphorbia* and *Phyllanthus,* and other genera.

Machado & Lopes (2004) reported that the *Croton* species have a floral morphology characteristic of the melittophilous pollination syndrome; however, these authors also mentioned anemophily as functional in certain species of this genus. Reddi & Subba Reddi (1985) maintained that *Croton bonplandianum* is pollinated by insects, especially honey bees and also by wind, which is not effective, because pollen shedding occurs in the evening hours, when atmospheric conditions are unfavourable for effective dispersal of pollen. Biswas & al. (2012) stated that *C. bonplandianum* is pollinated by ants, beetles and flies. Dominguez (1985) and Dominguez & al. (1989) reported that *C. suberosus* is visited by a wide range of insects, including wasps, bees, ants, beetles, and butterflies, but the authors have not mentioned whether they are pollinators or not. The nectar produced by the flowers rather attracts herbivore predators than stands as reward for pollinators. The predatory wasp, *Polistes instabilis,* defends this plant, while foraging for nectar. Narbona & Dirzo (2010) wrote that this predatory wasp kills herbivorous insects and defends the foliage, while collecting nectar from the flowers of *C. suberosus*. The wasp is the most effective pollinator, while bees are ineffective pollinators. Steiner (1983) maintained that *C. draco* is pollinated by a variety of bees, flies, and butterflies in Panama. Dominguez & Bullock (1989) and Dominguez & al. (1989) reported that *C. pseudoniveus* is anemophilous, despite the presence of nectaries in both staminate and pistillate flowers. These studies indicate that entomophily and anemophily are both functional in the different species of *Croton*.

In the present study, *C. scabiosus* flowers have characters that conform to anemophily (Faegri & van der Pijl 1979). These include a terminal spiciform racemose inflorescence with small male flowers in the distal part facilitating effective pollen dispersal by wind, and female flowers in the proximal part, male flowers consisting of reduced sepals and petals, exserted stamens with basi-versatile anthers and light, non-sticky, spheroidal pollen grains with clavate ornamentation, and apetalous female flowers with reduced sepals and well exposed free styles and stigmas. The day-long pollen viability and short period of stigma receptivity also support the anemophilous pollination syndrome. Furthermore, anthesis taking place almost throughout the day and the habitat of the plant population experiencing high winds due to canopy-free and mostly foliage-free plant species in the flowering season of *C. scabiosus* make anemophily very effective, which is reflected in the high fruit set and seed set rate. Webster (1994) reported that spicate inflorescences facilitate anemophily effectively. Reduction in flower size and in the number of floral parts are adaptations for anemophily. He also pointed out that many entomophilous species have spicate inflorescences, in order to facilitate the occurrence of anemophily in addition to entomophily. In *C. scabiosus*, nectar production in the male flowers and succulent sepals and tissues around ovary in the female flowers appear to be relic characters indicating the evolution of anemophily from entomophily. However, *Camponotus* ants use the nectar in male flowers and the succulent tissue in female flowers as food sources and effect geitonogamy by transferring pollen from male flowers to female flowers on the same plant. Therefore, *C. scabiosus* is ambophilious, because anemophily and myrmecophily occur, the former effects both geitonogamy and xenogamy, while the latter effects only geitonogamy.

In *C. scabiosus*, the male flower buds serve as breeding sites for *Haplothrips* sp. That thrips species moves out when flower buds unfold and expose the sex organs. In the flowering phase, thrips feed on both pollen and nectar. Thrips reduce the standing crop of nectar which is secreted only in trace amount at flower level in the male flowers and these depleted levels of nectar promote ant activity between male and female flowers and in effect pollination rate increases. Mention deserves the fact that thrips do not visit the female flowers and, hence, remain as mere foragers. However, their nectar feeding activity indirectly promotes geitonogamy by ants. Myrmecophily is least costly for the plant as it produces only trace amounts of nectar at flower level and also ants only walk on the flowers and inflorescences for which low energy is required (Schurch & al. 2000).

In the habitats at the two study sites in the flowering season of *C. scabiosus*, certain deciduous tree species and one deciduous straggler also bloom, some with foliage and some without foliage. All these species produce nectar in measurable amounts, but only in trace amount in *Mimosa intsia*; all of them, except *Cleistanthus collinus*, are hermaphroditic. *C. collinus* is monoecious and belongs to the *Euphorbiaceae* family. Nocturnal foragers, bats and day-time foragers such as bees, butterflies and sunbirds, occur in the habitats and visit these tree species according to their preference, but none of them has visited *C. scabiosus* even by mistake, indicating that the latter is a typical anemophile, supplemented with myrmecophily.

Furthermore, *C. scabiosus* is a larval host plant for a butterfly species (unidentified). New foliage available in the flowering season is used by this butterfly for breeding. Different stages of instars feeding on this foliage attract sunbirds, which regularly feed on them and also carry them in their beaks to the nest. Apparently, feeding on the larvae, sunbirds (*Nectarinia asiatica* and *N. zeylonica*) control this butterfly pest, otherwise the plant would be leafless and photosynthesis would become unavailable for the continued growth and development of fertilized ovules to fruits and seeds.

Webster (1979) noted that most *Euphorbiaceae* members produce 3-carpellate schizocarpic fruits, which are a primitive type in this family. These fruits dehisce explosively into three mericarps, each with two seeds. The seeds are carunculate in all subfamilies, except in *Phyllanthoideae*. The presence of caruncle is a characteristic feature of seeds from explosively dehiscent fruits. All these characters are evidenced in *C. scabiosus,* but the flowers are either tri-carpellary with three ovules, or tetra-carpellary with four ovules and, accordingly, the produced seeds are 2–3 in the former type and 2–4 in the latter type. Berg (1975) reported that seed dispersal in *Euphorbiaceae* is usually autochorous, by explosion of the ballistic fruits; however, in certain species it is diplochorous, in combination with myrmecochory. He suggested that the autochorous tricoccous fruits represent a primitive state and myrmecochory evolved subsequently. Garrison & al. (2000) maintained that diplochorous seeds are initially dispersed ballistically, by the explosive dehiscence of the fruit produced by elastic contraction of its tissues. Lisci & al. (1996) wrote that the seeds dispersed ballistically are dispersed further by myrmecochory involving ants as seed dispersers, which are attracted to a seed appendage referred to as *elaiosome*, formed by the lipid-rich caruncle. Wolff and Debussche (1999) noted that elaiosome frequently acts as a nutritional reward for ants. Culver & Beattie (1980) stated that ants collect the elaiosome-bearing seeds and take them to their nests, where elaiosome is consumed by workers and/or larvae. After consuming the elaiosome, the seed is left in a chamber underground. In this study, *C. scabiosus* is diplochorous indicating that it has evolved this mode of dispersal from autochory. After seed dispersal, the seeds reach the ground by gravity at some distance and then ants attracted to the caruncle of the seed consume it on the spot or move the seeds away to their nests, where they consume the caruncle and leave the seeds undamaged. Since the ground is rocky with little soil content, the ant nests occur either in shallow areas or in rocky crevices or cracks. In effect, the seeds brought to the ant nests are not deeply buried and this promotes a seed germination rate in viable seeds under favorable soil conditions. Diplochory in this plant is a two-step sequence, each involving a different dispersal agent. The combination of two dispersal mechanisms provides greater benefits for the seeds than a single mode of dispersal. The first dispersal mechanism moves the seeds away from the influence of parental plants and thus reduces parental and sibling competition, while the second dispersal mechanism moves the seeds to safe sites (Van der Wall & Longland 2004). Furthermore, anemochory is also functional in the dry season, due to the light weight of seeds, and hydrochory operates in the wet season, due to the buoyancy of seeds. Therefore, *C. scabiosus* is polychorous, involving diplochory, anemochory and hydrochory.

Conclusions

Croton scabiosus is a monoecious deciduous tree species that shows phenological events from November to July. The plant characteristically produces a terminal spiciform racemose inflorescence consisting of small male flowers in the distal part facilitating effective pollen dispersal by wind, and female flowers in the proximal part. The male flowers define anemophily by their reduced sepals and petals, exserted stamens with basi-versatile anthers and light, non-sticky, spheroidal pollen grains with clavate ornamentation, and the apetalous female flowers with reduced sepals and well exposed free styles and stigmas, day-long pollen viability, and short period of stigma receptivity. Furthermore, day-long anthesis, canopy-free and windy habitat and some simultaneously flowering tree species lacking foliage in their flowering season enable the functioning of anemophily more effectively, which is reflected in the high fruit and seed set rate. The *in vitro* pollen germination studies have indicated that sucrose and boric acid individually contribute to good pollen germination and tube development, but sucrose together with boric acid have a stimulatory effect in maximizing pollen germination rate and pollen tube length at optimal concentrations. Calcium nitrate, potassium nitrate and magnesium sulphate have a dual role of regulation and enhancement of pollen germination and pollen tube growth, depending on their concentrations. Nectar production in the male flowers and succulent sepals and tissues around ovary in the female flowers apparently indicate the evolution of anemophily from entomophily in this species. However, *Camponotus* ants use the nectar in male flowers and the succulent tissue as food sources and effect geitonogamy. Therefore, *C. scabiosus* is ambophilous, with anemophily as the main pollination syndrome. *Haplothrips* sp. uses the male flowers of *C. scabiosus* for breeding in the flowering phase, they feed on both pollen and nectar; thus, the reduced level of standing crop of nectar enables the nectar-feeding ants to promote geitonogamy. Furthermore, *C. scabiosus* serves as a larval host plant for a butterfly species (not identified). However, sunbirds control this butterfly pest by feeding on the larvae and indirectly enable the plant to produce photosynthate substances, which are essential for the continued growth and development of fertilized ovules to fruits and seeds. *C. scabiosus* produces 3- or 4-carpellate schizocarpic fruits. Seed dispersal involves diplochory and hydrochory.

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