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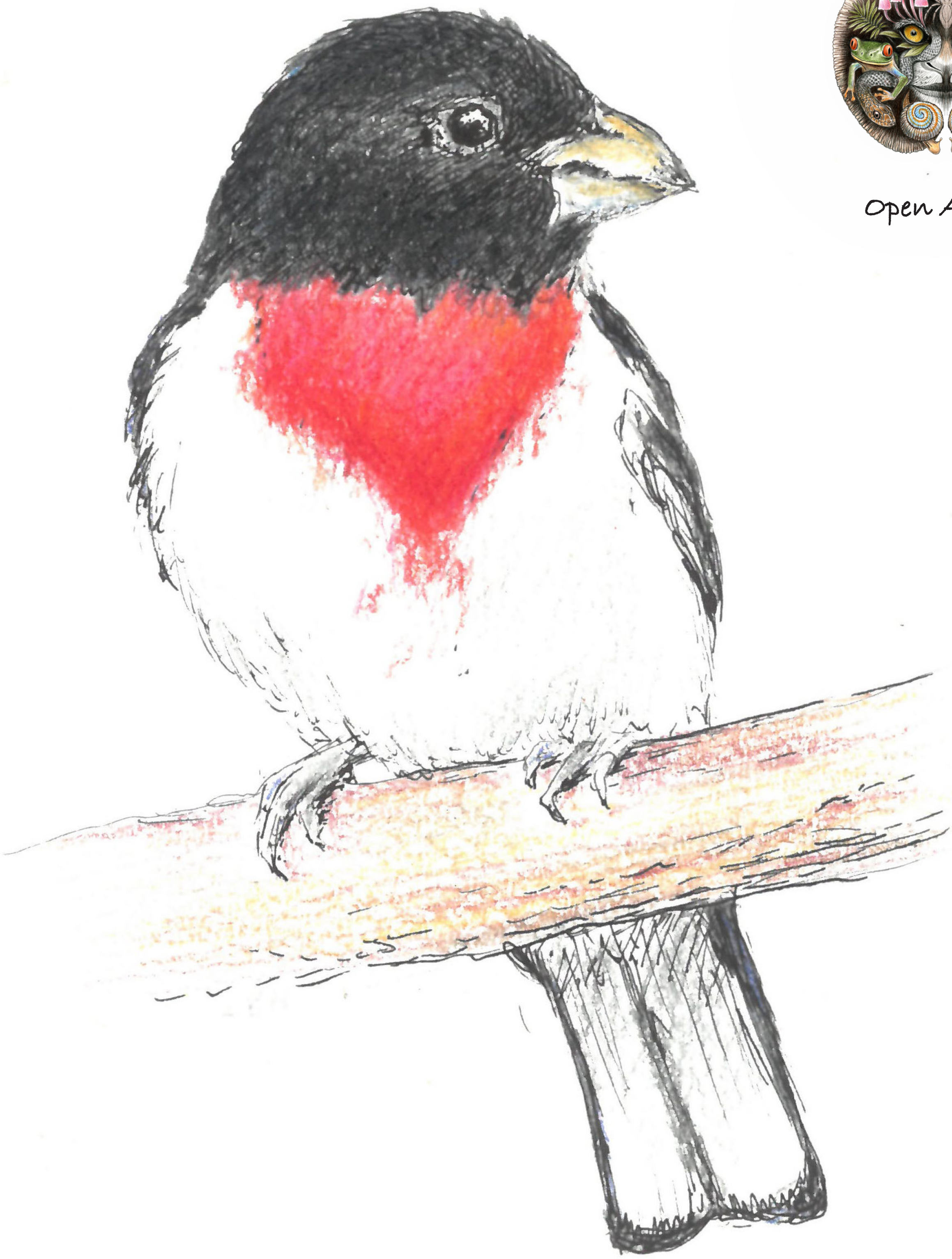
Journal of Threatened Taxa

10.11609/jott.2024.16.5.25119-25282
www.threatenedtaxa.org

26 May 2024 (Online & Print)
16(5): 25119–25282
ISSN 0974-79t07 (Online)
ISSN 0974-7893 (Print)

Open Access



ISSN 0974-7907 (Online); ISSN 0974-7893 (Print)

Publisher
Wildlife Information Liaison Development Society
www.wild.zooreach.org

Host
Zoo Outreach Organization
www.zooreach.org

43/2 Varadarajulu Nagar, 5th Street West, Ganapathy, Coimbatore, Tamil Nadu 641006, India
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Cover: Rose-breasted Grosbeak *Pheucticus ludovicianus*, pen & ink with colour pencil. © Lucille Betti-Nash.

Journal of Threatened Taxa | www.threatenedtaxa.org | 26 May 2024 | 16(5): 25198–25208

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.8534.16.5.25198-25208

#8534 | Received 18 May 2023 | Final received 05 March 2024 | Finally accepted 02 April 2024

ARTICLE

Reproductive biology of *Senna spectabilis* (DC.) H.S.Irwin & Barneby (Fabaceae) - an invasive tree species in the tropical forests of the Western Ghats, India

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Abstract: *Senna spectabilis* (DC.) H.S.Irwin & Barneby is an invasive tree species native to tropical America and is commonly found in the forest areas of Wayanad Wildlife Sanctuary. Its aggressive growth rate and ability to quickly cover up open and degraded lands in forest ecosystems make it challenging to control its spread. Reproductive studies of *S. spectabilis* and its pollen-ovule ratio indicate this species is cross-pollinating. The species is self-compatible, owing to the simultaneous occurrence of xenogamy, geitonogamy and autogamy. This reproductive strategy helps the taxon to colonise degraded areas and invade the forest ecosystem. The anthesis is diurnal and sometimes asynchronous. Peak insect visitors were observed from 0900 h to 1230 h, with the major visitor being *Tetragonula iridipennis*. *Xylocopa violaceae* was also a regular visitor along with resident Formicidae members, such as *Oecophylla smaragdina* and *Myrmicaria brunnae*. They feed on the floral parts, like tender petals and sepals. The reproductive syndrome of this plant favours maximum fertilization.

Keywords: Breeding, Fabaceae, forest, invasive, reproduction, Wayanad,

Malayalam: ഉഷ്ണമേഖല അമേരിക്കൻ പ്രദേശങ്ങളിലെ സ്വദേശി ആയ സെന്ന സ്പെക്ടാബിലിസ്, ഒരു അധിനിവേശ മരം എന്ന നിലയിൽ വയനാട് വന്യജീവി സങ്കേതത്തിൽ സർവ്വ സാധാരണമായി ഇപ്പോൾ കണ്ടുവരുന്നു. അവയുടെ അതിതീവ്രമായ വളർച്ചാനിരക്കിനുള്ള ശേഷി മൂലം വനത്തിലുള്ളിലെ തുറസ്സായ ഇടങ്ങളിലും, ദുർബ്ബലമായ താഴ്ന്ന പ്രദേശങ്ങൾ ഉൾപ്പെട്ട ആവാസവ്യവസ്ഥകളിലും വളരെ പെട്ടെന്ന് വളർന്ന് ഇത്തരം പ്രദേശങ്ങൾ മൂടിവയ്ക്കാൻ ഉതകുന്ന തരത്തിലുള്ള ശേഷിയും, ഇവയുടെ വളർച്ച നിയന്ത്രണങ്ങൾക്ക് വലിയ വെല്ലുവിളി ഉയർത്തി നിൽക്കുന്നു. പ്രത്യുത്പാദന പഠനങ്ങളും, അവയുടെ പുഷ്പങ്ങളിലെ പരാഗരേണുക്കളും അണ്ഡവും തമ്മിലുള്ള അനുപാതവും പരിഗണിക്കുമ്പോൾ, സെന്നാ സ്പെക്ടബിലിസ് ഒരു പരപരാഗണം നടത്തുന്ന സസ്യമാണ് എന്ന് വ്യക്ത മാകുന്നു. ഒരേ സമയം സ്വപരാഗണവും, ഗൈറ്റനോഗാമിയും സിനോഗമിയും നടക്കുന്ന കാരണത്താൽ തന്നെ സെന്നാ സ്പെക്ടബിലിസ് ഒരു സ്വയം പൊരുത്തപ്പെടുന്ന (സെൽഫ് കോമ്പാറ്റബിൾ) സസ്യജാതി ആയി കരുതാം. ഇത്തരം പ്രത്യുത്പാദന തന്ത്രങ്ങൾ ഈ സസ്യ ജാതിയെ കാട്ടിലെ ദുർബ്ബലമായ ആവാസവ്യവസ്ഥകളിലേക്ക് പെട്ടെന്ന് അധിനിവേശം നടത്തുന്നതിന് സഹായിക്കുന്നു. പുഷ്പങ്ങളിലെ കേസരങ്ങൾ പൂർണ വളർച്ച പ്രാപിച്ച്, ആന്ദർപാളികൾ പൊട്ടി പരാഗരേണുക്കൾ വിതരണത്തിന് തയ്യാറാവുന്ന ആന്തസിസ് എന്ന പ്രക്രിയ സെന്നയിൽ നടക്കുന്നതു പകൽ സമയങ്ങളിൽ ആണ്. പലപ്പോഴും അത് ഒരുപോലെ അല്ല. പ്രാണികൾ സെന്നയുടെ പുഷ്പത്തി ലേക്ക് വരുന്നത് കൂടുതലായി നിരീക്ഷിയ്ക്കപ്പെട്ടതു് രാവിലെ 9 മണി മുതൽ 12 മണി വരെയാണ്. പ്രധാനമായും ടെട്രഗോണലൂ ഇരിഡിപെന്നിസ്, സൈലോകോപ്പ വയോലെസിയേ തുടങ്ങിയവ ആണ് തുടർച്ചയായി പുഷ്പത്തിൽ വന്നിരുന്ന പരാഗികൾ. ഇവ കൂടാതെ സ്ഥിരമായി പുഷ്പത്തിൽ ഇരിക്കുന്ന ഫോർമിസിഡെ വർഗ്ഗത്തിൽ പെട്ട ഈസോഫില്ല സ്മരാഗ്ദിന, മീർമികേറിയ ബ്രൂണെ തുടങ്ങിയ പ്രാണികളെയും പഠനത്തിൽ കണ്ടെത്തി. ഇവ പുഷ്പത്തിൻ്റെ ഇളം ഭാഗങ്ങൾ ആയ ദളങ്ങൾ വിദളങ്ങൾ എന്നിവ ആഹാരമാക്കുന്നു. ഈ സസ്യജാതിയുടെ വ്യത്യസ്തങ്ങളായ പ്രത്യുത്പാദന പ്രവൃത്തികൾ ഇവയ്ക്ക് പരമാവധി ബീജസങ്കലനത്തിന് ഉള്ള സാഹചര്യങ്ങൾ ഒരുക്കുന്നു.

Editor: Vijayasankar Raman, USDA-APHIS-PPQ-NIS, Smithsonian Institution, Washington, DC, USA.

Date of publication: 26 May 2024 (online & print)

Citation: Muraleekrishnan, K., S.C. Viswanath & T.K. Hrideek (2024). Reproductive biology of *Senna spectabilis* (DC.) H.S.Irwin & Barneby (Fabaceae) - an invasive tree species in the tropical forests of the Western Ghats, India. *Journal of Threatened Taxa* 16(5): 25198–25208. https://doi.org/10.11609/jott.8534.16.5.25198-25208

Funding: KERALA FOREST DEPARTMENT through KSCSTE-KFRI.

Competing interests: The authors declare no competing interests.

Author details: K. Muraleekrishnan is a Ph.D. scholar from KSCSTE-KFRI attached to the University of Calicut. Dr. Sanal C Viswanath was the co-researcher and expert in tree breeding and genetics. Dr. TK Hrideek is the chief executive officer of the State Medicinal Plants Board Kerala on deputation from KSCSTE KFRI where he held the office of the head of the Forest Genetics and Tree Breeding Department. Currently, he also holds full additional charge of Oushadhi, the Pharmaceutical Corporation Kerala Limited of the Government of Kerala. He is a plant geneticist and tree breeder with extensive field and laboratory experience in both cultivars and wild plants in the humid tropics of Kerala.

Author contributions: All authors equally contributed in fieldwork, travel and data collection. MK and SCV did data compilation and analysis under the supervision of HTK. MK led the writing with inputs from SCV and HTK. All authors equally contributed to proofreading; and have read and approved the final file.

Acknowledgements: We would like to express our sincere gratitude to director of KSCSTE-Kerala Forest Research Institute for their generous provision of research facilities. Furthermore, I would like to extend my heartfelt appreciation to the Kerala Forest Department, Government of Kerala, for their assistance in conducting this research. I express my heartfelt appreciation to PhD scholars, Suby, B. Preetha and Sinny Francis, from the Department of Forest Genetics and Tree Breeding of KSCSTE Kerala Forest Research Institute.

INTRODUCTION

Exotic species must reproduce successfully in new areas to establish self-replacing populations. Therefore, reproductive characteristics and reproductive success are crucial factors in the invasion of plants. Biological invasions are considered the second largest threat to the environment, next to habitat destruction. According to Inter-governmental Science-Policy Platform on Biodiversity and Ecosystem Services IPBES (2019), one-fifth of Earth's surface, including global biodiversity hotspots, is under biological invasion risk. Richardson et al. (2014) studied tree invasions, their patterns and processes and discussed the challenges facing researchers and managers. Tree invasions are being studied from different perspectives due to their increased importance in recent decades as more species are becoming invasive and larger areas of land are being invaded, resulting in larger impacts and increasing complexity of management challenges (Richardson & Rejmánek 2011; Rejmánek & Richardson 2013).

Senna spectabilis (DC.) H.S.Irwin & Barneby is an invasive tree in the forest areas of Wayanad Wildlife Sanctuary, part of the Western Ghats, India. It has an aggressive growth rate and the ability to quickly occupy open and degraded forest areas. Furthermore, *S. spectabilis* has a trait of suppressing the regeneration of native species due to allelopathic effect, which can increase their extinction risks. *S. spectabilis* spreads aggressively in disturbed and open forests, vacant spaces, parks, riverbanks, and plantations but not in closed canopies (Irwin & Barneby 1982), which is typical of most invasive plant species. Invasive plants are exotic species introduced in new areas that reproduce and disperse efficiently to the extent that they spread rapidly. Some of the plant traits related to seedling emergence, growth form, growth rate, breeding system, dispersal, and environmental tolerance are important in predicting whether a species will become invasive (Thuiller et al. 2006; Kleunen & Johnson 2007; Pysek & Richardson 2007). Seed production is essential for the establishment of self-sustaining populations and the subsequent naturalization of introduced species. However, seed production relies on the pollination ecology and breeding system of the plants introduced, and the environmental conditions of the recipient area (Richardson et al. 2000). Thus, floral traits linked to the functioning of the flower and dependence on pollinators, as well as pollinator attraction, will determine the final reproductive success of the plant. Field surveys on the occurrence of *S. spectabilis* showed that in areas it has invaded, particularly forest areas, this species is markedly abundant and out-competes other plants. It has significantly reduced overall species abundance and diversity and has impacted forest ecosystems and the natural reversion of vegetation in degraded lands.

This study aimed to find out the reproductive characteristics, including pollination mechanisms and breeding systems, of *S. spectabilis*. Identifying the reproductive traits alone cannot control the invasion but understanding the ecology of *S. spectabilis* in introduced areas is important in controlling the spread. We, therefore, examined the reproductive biology of *S. spectabilis,* by studying its: (i) floral biology through the description of floral morphology, the pattern of production and concentration of nectar, and stigmatic receptivity periods, (ii) pollination system and foraging behaviour of visitors, (iii) breeding system through hand pollination experiments, and (iv) reproductive success estimated as the proportion of the total number of fruits over the total number of flowers. These observations analyse the factors that aid the rapid spreading of *S. spectabilis* and may help develop eradication strategies for this species in forest ecosystems.

MATERIALS AND METHODS

Study Species

Senna spectabilis (Fabaceae: Caesalpinioideae), according to Irwin & Barneby (1982) it is commonly seen in the region of northeastern Brazil, where it is known as *Cassia*. It occurs naturally from southwestern Mexico to southern tropical America. It has been widely introduced and naturalized in many tropical countries (https://powo.science.kew.org/). Wayanad Wildlife Sanctuary is one of the aggressive growth habitats of *S. spectabilis,* which is considered invasive. It is a medium to large-sized tree growing up to 60 feet high, but is often much smaller. This species is extremely fast-growing, flowers, and sets seeds profusely. In India, it was introduced as an ornamental plant in the botanical gardens and is distributed in Mysuru in Karnataka, Wayanad in Kerala, Rishikha in Sikkim, Coimbatore and Sathyamangalam in Tamil Nadu, and Howrah in West Bengal. This species is reported in the forest areas of Sathyamangalam, suburban areas of Coimbatore and Wayanad Wildlife Sanctuary (Satyanarayana & Gnanasekaran 2013) and has been confirmed to have a high potential to flourish rapidly and produce numerous viable seeds. The plant, which was first introduced to Wayanad Wildlife Sanctuary in the early 1980s, has invaded approximately

23% of the sanctuary's total area in 40 years (Anoop et al. 2021).

Study Sites

Reproductive studies were conducted at the model site established at Muthanga Forests, Wayanad Wildlife Sanctuary. It is contiguous to the protected areas of Nagarhole National Park and Bandipur Tiger Reserve of Karnataka on the north-east and Mudumalai Wildlife Sanctuary of Tamil Nadu on the south-east and is located at 11.5777–11.9701 ^{0}N and 75.9896–76.4364 ^{0}E. Wayanad Wildlife Sanctuary has an area of 344.44 km^2. The biodiversity-rich sanctuary is an integral part of the Nilgiri Biosphere Reserve. Other study sites are Meppadi and Kalpetta forest ranges of South Wayanad Territorial Forest Division of Kerala, India.

Data Collection

The plant species for the study was selected after carrying out a field study in Wayanad. Field investigations and experiments were conducted from September 2019 to January 2020 and from October 2020 to January 2022. Following a preliminary field study of the flowering seasons of the selected species, regular field studies were carried out to collect information and data on the reproductive aspects. The functional events of individual flowers, sexual status, floral rewards and their details, breeding system, flower visitors and their behaviour and pollination role, natural fruit and seed output rates, and duration of fruit maturation were carefully observed, and seed dispersal aspects were examined. Floral structural and functional aspects were studied, as per the methods of Raju & Reddi (1994), Raju & Rao (2004), and Dafni et al. (2005).

Flower Morphology

The details of flower morphology, such as flower sex, shape, size, colour, odour, sepals, petals, stamens and ovary, as well as the position of stamens were described. The morphology and dimensions of the inflorescence were studied from the fresh inflorescence as well as those fixed in formalin-aceto-alcohol under a microscope. The order of wilting or dropping off of floral parts was recorded. These details of the selected plant species were provided due to inadequate and confusing taxonomic descriptions.

Pollen-Ovule Ratio

The pollen-ovule ratio was determined by dividing the average number of pollen grains per flower by the number of ovules per flower. The value thus obtained was taken as the pollen-ovule ratio (Cruden 1977).

Nectar Characters

The presence of nectar was determined by observing the mature buds and open flowers. When the nectar secreted was found to be in a measurable quantity, the volume of nectar from 10 flowers of 10 trees were determined. Then the average volume of nectar per flower was determined and expressed in µl, following Dafni et al. (2005). The flowers used for this purpose were bagged at the mature bud stage, opened after anthesis, and the nectar was squeezed into micropipettes for measuring the volume of nectar. Nectar sugar concentration was determined using a handheld sugar refractometer.

Stigma Receptivity

The stigma receptivity was observed visually and by the H_2O_2 (Hydrogen peroxide) test. In the visual method, the stigma's physical state (wet/dry) and the unfolding of its lobes were considered to record the commencement of receptivity, withering of the lobes was taken as loss of receptivity. The stigma receptivity period was recorded using the H_2O_2 test (Dafni et al. 2005). This test is widely followed, although it does not indicate the exact location of the receptive area. In this study, the period of slow release of bubbles from the surface of the stigma following the application of H_2O_2 was taken as stigma receptivity.

Anther Dehiscence

Anthesis was initially recorded by observing markedly mature buds in the field. Later, the observations were repeated three to four times on different days to provide an accurate anthesis schedule for this species. Similarly, the mature buds were followed to record the time of anther dehiscence. It is confirmed by observing the anthers using a 10x hand lens.

Breeding Systems

In *S. spectabilis*, mature flower buds of some inflorescences on different individuals were tagged and enclosed in paper bags. A fixed number of flowers from different inflorescences were bagged or tagged and followed further to study whether the pollination is vector-dependent and to understand the flower abortion rate. Another set of flowers was used for experiments on apomixis, self-pollination, and cross-pollination, such as geitonogamy and xenogamy, to collect data for understanding the breeding behaviour. All these categories of flower pollination were followed for the fruit set. If the fruit set was present, the percentage of the fruit set was calculated for each mode.

Plant-Pollinator Interaction

Flower visitors were also observed concerning their mode of approach, landing, probing behaviour, forage collected, and contact with sex organs of flowers to effect pollination, and inter-tree foraging activity. Foraging visits made by major pollinators were recorded on selected inflorescences.

Pollen Viability

The viability of pollen at the time of dehiscence was tested using 1% acetocarmine, considering stained grains as viable and shrivelled grains as non-viable (Radford et al. 1974; Koshy & Jee 2001). The viable pollen in the 40x microscopic field was counted and expressed as a percentage of the total. In vitro germination of pollen was tested in five different germination media. Fresh mature anthers were collected from the field at anthesis, and pollen grains were carefully dusted on cavity slides containing germination media. One hour after inoculation, the number of pollen grains germinated, and the number of grains per field of view, were recorded. Pollen grains were considered to have germinated when the pollen tube length was greater than the diameter of the pollen grain (Tuinstra & Wedel 2000). Pollen diameter and tube length were observed under an image analyzer (Leica Q 500 MC) at 40 x magnifications.

RESULTS

Floral Biology

The phenological observations have indicated that the peak flowering of *S. spectabilis* typically commences in September and extends until December. The inflorescence takes the form of a raceme, either terminal or axillary, featuring corymbose panicles that are approximately 10–15 cm in length. Each panicle contains 120–140 flowers, with peduncles measuring 2–3 cm in length and pedicels also measuring 2–3 cm in length. The bracts are narrowly ovate or lanceolate with an acute or sub-acuminate apex, and are caducous. The plant possesses five sepals, which are unequal in size and reflexed. The outer two sepals are green and ovate, measuring about 5.5 x 3 mm, with a concave shape and pubescent surface. The inner three sepals are petaloid, rotund or ovoid in shape, measuring 9–10 x 10–13 mm, with inconspicuous veins and a pubescent surface. The plant also has five unequal petals, which are ovoid in shape and measure 2–2.5 cm in length. The petals have a short claw at the base and a smooth margin. There are two types of stamens present: seven fertile stamens and three sterile stamens or staminodes. The fertile stamens are equal in size and have a glabrous surface, with filaments measuring approximately 3 mm in length and anthers measuring approximately 5 mm in length. The anthers are biporose at the apex and reflexed.

The anther is dehisced by apical slits, which open or close according to ambient humidity. The sterile stamens, or staminodes, are each 4 mm long, glabrous, and deeply cordate at both ends. The ovary is curved, 2 mm long, style up to 2.3 cm long, glabrous, stigma fringed with cilia. Style is bent downwards. The sickle-shaped pistil projects into the fertile stamens. The average number of pollen grains per anther is 6580 ± 5.20, which has moderate viability. The pods are pendulous, 17–25 x 1–1.50 cm long, shortly stipitate, linear-cylindric, 100–108 seeded, nearly terete, turgid, septate, and dehiscing along one margin. Seeds are orbicular, 4–6 x 3–5 mm,

Table 1. Observations on floral characters of *Senna spectabilis*.

Floral Characters	Observations
Flowering period	September to December
Flower colour	Rich yellow to Dark-veined
Odour	Present
Nectar	Present
No. of primary branch	16 ± 1.73
No. of inflorescence/branch	2262.75 ± 527.74
No. of flowers/inflorescence	120–140
Sepals/ flower	5
Petals/ flower	5
No. of anthers/ flower	7 fertile stamens, 3 sterile staminodes
No. of pollen grains /anther	6580 ± 5.20
No. of ovules/ flower	80–120
Pollen/ ovule ratio	59.81
Length of stigma ± style (in cm)	2.35 ± 0.19
Length of ovary (in cm)	0.2
Anthesis time	0600–0900 h
Anther dehiscence time	0800–1200 h
Nectar sugar concentration (%)	4.11 ± 0.79
Pollen type	Tri-colporate
Pollen size	35.05 ± 2.19 µm
Stigma type	Above anther level
Fruit setting / inflorescence	10.55 ± 0.95
No. of seeds / pod	108.91 ± 9.69

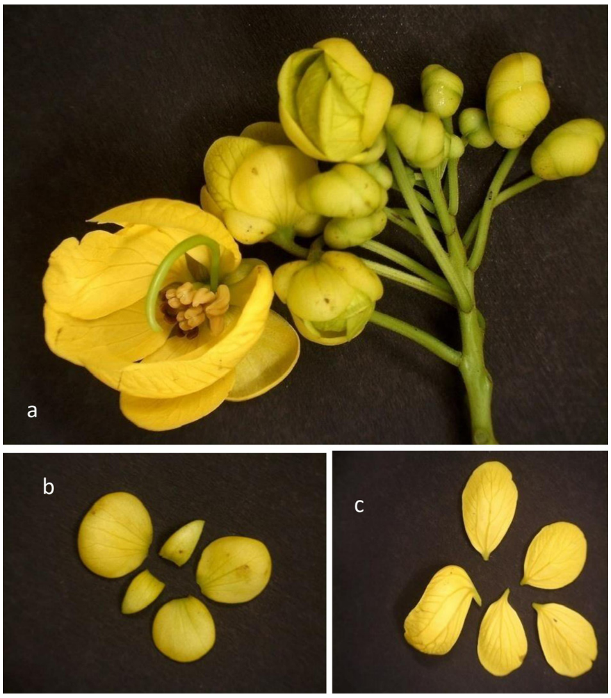

Image 1. Floral morphology of *Senna spectabilis*: a—inflorescence | b—sepals | c—petals. © K. Muraleekrishnan.

brown, and rugulose. Floral morphology observations are detailed in Table 1. The dimensions of the floral parts of *S. spectabilis* are given in Image 1.

Anthesis and Pollination

The duration of anthesis was from 0600 h to 0900 h, and anther dehiscence started at 0800 h and continued up to 1200 h. The stigma became receptive at 0800 h. The anthesis process is diurnal and sometimes asynchronous, which means some flowers are completely open by 1000 h, while some flowers start opening early. The flowers remain open until the next day, probably due to increasing temperature favoring the anthesis. The anthesis exhibited two days of positive stigmatic receptivity under this condition. The flowers open partially on the first day. Then they gradually open fully and expose the sexual whorls for visitors. A fluid-like substance in the basal portion of the flower and tender floral parts of newly opened flowers were used for sugar concentration, and the mean nectar sugar concentration is 4.11 ± 0.79 brix. No distinct nectaries or extra floral nectaries were found. According to Marazzi (2013) extra floral nectar was absent in the case of *S. spectabilis* var. *excelsa*. The peak arrival time of insect visitors was observed from 0900 h to 1230 h.

Dammar Bee is a major visitor to *S. spectabilis* while Violet Carpenter Bee is a regular visitor. Some Formicidae

Table 2. List of Flower foragers on *Senna spectabilis*.

	Scientific name	Common name	Visiting status
1.	*Tetragonula iridipennis* Smith	Dammar Bee	Regular
2.	*Xylocopa violaceae*.	Violet carpenter bee	Regular
3.	*Amata huebneri* Boisdual	Wasp Moth	Occasional
4.	*Bocana manifestalis* Walker	Moth	Occasional
5.	*Camponotus mitis* Smith	Carpenter Ant	Regular
6.	*Myrmicaria brunnea* Saunders	Hunchback Ant	Resident
7.	*Oecophylla smaragdina* Fabricius	Weaver Ant	Resident
8.	*Tapinoma melanocephalum* Fabricius	Ghost Ant	Occasional
9.	*Borbo cinnara* Wallace	Rice Swift	Occasional
10.	*Musca domestica* L.	Housefly	Occasional
11.	*Halyomorpha halys* Stal	Stink Bug	Occasional
12.	*Coptosoma* Laporte	-	Occasional

members, like Weaver Ant and Large Myrmicine ant, are residents of the flowers of this species. They feed on the floral parts, like the tender petals and sepals, even during night hours. Rice Swift is an occasional visitor. Other visitors, such as Stink Bugs and Wasp Moths, came to consume the sap from tender pedicels and branches. The list of flower visitors is recorded (Table 2, Image 2). The Indian Stingless Bee, a major visitor, starts its nectar-foraging activity, from 0800 h to 1230 h, and resumes forging from 1600 h to 1730 h. The Violet Carpenter Bee species foraged during 1000 h to 1130 h. Dammar Bee, a very frequent visitor, only visited open flowers. This foraging behaviour is thought to be boosting the chances of cross-pollination.

Breeding Systems

Studies carried out on artificial breeding experiments and observations of natural and open pollination showed that 20% of fruits were set in crossing experiments such as hand-geitonogamy, while 25% were set in hand-xenogamy and 20% of fruits in autogamy. The natural and open pollination from our tagged flowers set 30% of fruits (Table 3). The fruit set per inflorescence in open pollination is 10.55 ± 0.96. The number of flowers per inflorescence is 114 ± 4.27. After observing 20 trees and their tagged uniform inflorescence, 10% of fruits were found to be finally maturing following the abortion of immature flowers, immature fruits and unripe fruits. The examination of futile percentage also demonstrates that 13.58% of opened flowers were lost, while 90.84% represents the final ripened pod futile percentage (Table 4). Despite these findings, the remaining 10% of

Image 2. Some of the floral visitors of *Senna spectabilis*: a—Formicidae | b—Dammar Bee | c—Housefly | d—Wasp Moth | e—Stink bug | f—Coptosoma. © K. Muraleekrishnan.

Table 3. Modes of breeding pattern in *Senna spectabilis*.

	Treatments	n	No. of flowers		Fruit set (%)
			Pollinated	Set fruit	
1.	Autogamy	20	8	4	20
2.	Geitonogamy	20	11	4	20
3.	Xenogamy	20	9	5	25
4.	Apomixis	20	-	-	0
5.	Open	20	16	6	30

Table 4. Flower and fruit set per inflorescence.

Tree no.	Flower			Fruit-pod		
	Bud	Young	Opened	Bud	Young	Opened
1	140	124	120	76	24	12
2	138	137	121	68	16	15
3	139	128	114	59	17	10
4	132	130	116	72	20	13
5	128	119	114	60	28	12
Mean	135.40	127.60	117	57.40	21	12.40
Futile (%)		5.70	13.58	57.60	84.49	90.84

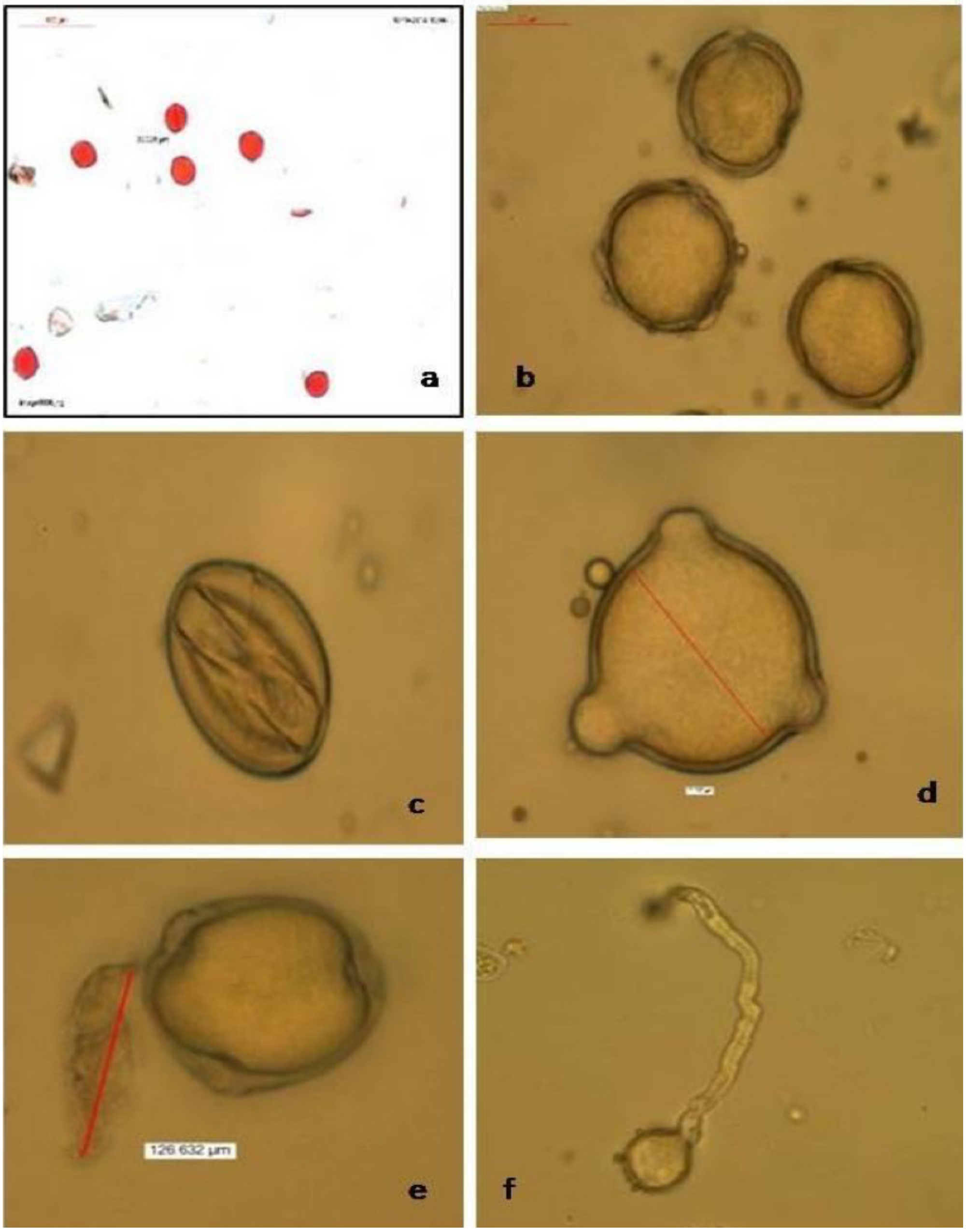

Image 3. Stages of pollen germination: a—Viable pollen (stained red in acetocarmine) | b–d—Pollen germination | e,f—Pollen tube development. © K. Muraleekrishnan.

Table 5. Composition of the pollen germination media.

Composition	1	2	3	4	5	6	7	8	9
Sucrose (g)	10	10	10	0	10	5	5	5	5
Boric acid (g)	0.01	0.01	0	0.01	0	0.01	0.01	0	0
Calcium nitrate (g)	0.03	0	0.03	0.03	0	0.03	0	0.03	0
Distilled water (ml)	100	100	100	100	100	100	100	100	100
Germination %	100	91	72	32	64	75	71	54	44
Duration (min)	20	20	20	30	20	20	20	20	20

Table 6. Nectar sugar concentration in *Senna spectabilis*.

Time of testing	0530	0600	0700	0800	0900	1000	1100	1200	1400
Brix %	3.02± 0.14	3.30± 0.57	3.58± 0.40	4.32± 0.34	4.90 ± 0.26	5.00 ± 0.12	5.10 ± 0.17	4.38± 0.57	3.42± 0.86

ripened pods proved sufficient for additional dispersal mechanisms and the successful invasion of this particular tree species. The results of the breeding system indicated that the flowers are self-compatible and self-pollinating, and they also facilitate cross-pollination. Being an out-crosser and a self-pollinating species, *S. spectabilis* has different ways to reproduce in this invasion area.

Pollen Viability

Fresh pollen grains of *S. spectabilis* show 30% viability when stained with acetocarmine (1%). In vitro germination was found to be 32–100 % when the pollen grains dusted in different media were observed under the microscope after 20 min. (Table 5; Image 4). The highest germination was obtained in medium 1 (100%). The lowest germination was obtained in medium IV (32%) which does not contain sucrose.

DISCUSSION

Information on floral characters and pollination systems is important in the breeding system, especially in the case of *Senna spectabilis* which poses a major threat and has a negative impact on the structure and diversity of the forest and its ecosystem. In order to manage this species in the invaded forest areas, observation of reproductive biology is very important. The diurnal anthesis period of this species is characterized by the simultaneous presence of flowers and flower buds at various stages of development on the same inflorescence, as observed in *Sesbania virgata* (Cav.) Pers. Additionally, an extended duration of flower opening has been observed to promote pollinator activity throughout the day (Souza et al. 2016). In the case of *S. spectabilis*, the flowers remain open until the following day, which may facilitate cross-pollination by providing a continuous supply of pollen as a resource for flower visitors across different plants and flowers.

The flowers of the Fabaceae family possess specific and highly efficient pollination mechanisms that rely on various biotic vectors, including bees and birds (Rasmussen 2013). The present study has identified the Dammar Bee, a widespread species in India, and the Violet Carpenter Bee, as the primary pollen vectors. These species have been confirmed as pollinators based on their pollen load and their role in seed setting (Rasmussen 2013).

Research findings indicate that *Senna* pollen-collecting bees employ a technique of extracting pollen by vibrating the middle “feeding” stamens, which they firmly grasp with their legs (Marazzi & Endress 2008). In their investigation into the diversity and evolution of a trait associated with ant-plant interactions involving extra floral nectaries in Senna (Leguminosae), Marazzi et al. (2013) deliberately excluded *S. spectabilis* from their study due to the absence of ants in the vicinity of its floral buds or leaves. However, extensive field observations revealed the presence of abundant Formicidae species, which were observed to be permanent residents of these flowers and actively feeding on delicate floral components. These ants displayed both diurnal and nocturnal activities. Additionally, a moth species, *Bocana manifestalis*, was observed on the flowers during the night.

This species has poricidal dehiscence of anthers, minute terminal stigmas and curved styles. Pollens are released when anthers are vibrated by the bees (Buchmann 1974). These floral features showed that this species has buzz pollination syndrome. According to Almeida et al. (2015), *S. spectabilis* is listed as an Enantiostylous type of species. They classified Cassiinae species into seven types based on morph distribution among plants and grouped species with different flower morphologies and diverse reproductive strategies of these types.

Senna spectabilis belongs to Type 5, which is classified as the Amiciella group. The model species for this group is *Chamaecrista amiciella*. The characteristic pattern of these species involves the deposition of pollen grains on the dorsal portion of the pollinator after they have passed through all the extensions of a modified, tube-shaped petal (Almeida et al. 2013). The pollen produced by the pollination anthers is deposited opposite the stigma. The Amiciella type is considered the second most complex, as it exhibits similar mechanisms to the Ramosa type (Type 7), with the exception of the use of a group of petals (only one petal fulfills this role). This type is unique to *Chamaecrista* and *Senna* species. In the case of *S. spectabilis*, the pollen grains are deposited on both the dorsal and ventral sides as a result of the body-washing behavior of a dammer bee. The number of pollen grains is higher on the ventral side. Pollen serves as the most sought-after floral reward, providing vital nutrition for many insects, particularly Apidae, beetles, flies, thrips, springtails, as well as some orthopteroids and butterflies (Anderson 1996). Pollen is highly nutritious and contains essential and quasi-essential amino acids (Haydak 1970). In the case of *S. spectabilis*, pollen is also the primary reward due to the low concentration of nectar sugar and the absence of proper nectar secretion in this flower (Table 6).

Tamnet et al. (2011) studied on optimization of the preservation of pollen grain germination of *S. spectabilis*. For the study, they selected this invasive tree species, which is a large species of bee flora facing extinction threat in the Adamawa region of northern Cameroon. They claimed to have conducted the study to help beekeepers. They tested in vitro germination and storage of pollen. The results reveal that its pollen germinates preferentially up to 38.36% in Brewbaker medium enriched with the optimal concentration of 25% sucrose. Pollen was stored at 10°C and 20°C and germinated at length during 22 weeks of storage.

In vitro germination was found to be good in the present study, and 32 to 100% germination was found in different media, which is also proven in the experiments (Image 3). During field observations for pollinator interactions, the Indian Honey Bee *Apis cerana indica* was always found to be hovering around the flowers of *S. spectabilis* and visiting only the associate plants, but it never made a single visit to *S. spectabilis* flowers. Further observations and research experiments are required to find out the reason behind it, as this could be due to a lack of sufficient forage or the presence of any repellent factors. It also possesses a self-pollination mechanism. Autogamy is a reproductive characteristic of invasive and pioneer species that occupy clearings and forest edges (Williamson 1996; Holsinger 2000). Here, the case of *S. spectabilis* occurred in areas similar to clearings, such as massive bamboo flowering in open areas, other open areas of deciduous forest patches and the edges of Vayal ecosystems. In breeding experiments, 25–20% of fruit sets occurred, and autogamy also accounted for 20% of fruit sets. It reveals that *S. spectabilis* possesses a mixed reproductive system composed of cross-pollination and autogamy. This system is probably related to its success as an invasive species, which helps it spread and colonise new habitats.

Baker & Baker (1979) observed that maintaining a particular balance between self-compatibility and cross-pollination is beneficial to weeds. The author states that once a seed is dispersed to a distant place, the formation of a new population will depend on the self-pollination capacity of the species. *S. spectabilis* is autogamous and an out-crosser, which appears to be a good strategy when combined with its ability to invade degraded lands such as open forest areas. Several invasive plants have been described as self-compatible in the introduced ranges (Rambuda & Johnson 2004; Kleunen & Johnson 2007; Stout 2007; Rodger et al. 2010; Hao et al. 2011), and this has been proposed as an advantage for successful invasion (Williamson & Fitter 1996; Pannel & Barret 1998).

Invasive species generally have a high sexual reproductive capacity, the ability to reproduce asexually, the capability to grow rapidly from seed to sexual maturity, great dispersal and colonization efficiency, a high tolerance to environmental heterogeneity and disturbances, a high adaptation to environmental stress, and a greater competitive capacity than native species (Sakai et al. 2001; Vila & Weiner 2004; Werner & Zahner 2009). As an invasive tree species in forest areas of Wayanad Wildlife Sanctuary, forest officials and locals try to eradicate this species by cutting the tree. However, the tree re-sprouts profusely. During a period of five years, this tree was observed to have grown more branches after re-sprouting, while each branch produced flowers vigorously in three years. Re-sprouting ability is a positive reflection of its invasiveness.

Research conducted on invasive Australian Acacias by Milton & Hall (1981) elucidated that this species possesses various reproductive characteristics that potentially contribute to their invasiveness. These traits include extensive and enduring floral displays, pollination syndromes that cater to a wide range of pollinators, early production of a substantial quantity of long-living and highly viable seeds, leading to the formation of extensive seed banks, adaptations for seed dispersal, and mass germination. These findings were also observed in *S. spectabilis*, which displayed comparable behavior and responses. The study revealed that the high rate of seed production in *S. spectabilis* can be attributed to various factors, including the pollen viability and vigour of the pollen tube, the timing of anther dehiscence and stigma receptivity, the presence of multiple pollinators, and adequate pollen rewards. The pods of *S. spectabilis* were observed to contain an average of 108.91 ± 09.69 seeds. Notably, the plant exhibited no sexual incompatibility or pollination difficulties. The reproductive syndrome of *S. spectabilis* is conducive to achieving maximum fertilization.

CONCLUSION

Reproductive studies of *Senna spectabilis* and its pollen-ovule ratio indicate that this species is a cross-pollinating species. This species is self-compatible, as xenogamy, geitonogamy and autogamy are observed in field experiments. This reproductive strategy helps the tree colonise degraded areas and invade the forest ecosystem. Reproductive successes of this species also depend on its production of large amounts of flowers during its peak phenophase. Flowers, pollen grains, fruit set—everything facilitates the invasive nature of this tree.

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NAAS rating (India) 5.64

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ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

May 2024 | Vol. 16 | No. 5 | Pages: 25119–25282
Date of Publication: 26 May 2024 (Online & Print)
DOI: 10.11609/jott.2024.16.5.25119-25282

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New country record of *Trimeresurus uetzi* Vogel, Nguyen & David, 2023 (Reptilia: Squamata: Viperidae) from India
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New record of Giant Redeye *Gangara thyrsis thyrsis* (Fabricius, 1775) (Lepidoptera: Hesperiidae) from Garhwal region of western Himalaya, India
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***Strobilanthes khasyana* (Acanthaceae): an addition to the flora of Nagaland, India**
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***Sonerila konkanensis* Resmi & Nampy (Melastomataceae) – an addition to the flora of Karnataka, India**
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