

Research Paper

Pollination Strategies of Some Perennial Weed Species

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Weeds have evolved a number of adaptations to thrive even under adverse conditions. One of the adaptations is their ability to set seeds even under pollination uncertain environments through autogamous self-pollination. Autogamous pollination which provides reproductive assurance (RA) is critical in annual weeds as they get only one chance to set seeds in their life; if they miss this chance their survival is threatened. In perennial weeds, however, RA through autogamous self-pollination is not so critical as they get repeated chances to set seeds; lack of seed set in some years does not affect their survival. There is very little information on pollination strategies of perennial weeds particularly of Indian species. To test this prediction, studies were carried out on five Indian perennial weeds — *Cassia auriculata*, *Ipomoea obscura*, *Oxalis corniculata*, *Plumbago zeylanica* and *Dodonaea viscosa*. Seed set in the populations of *Ipomoea*, *Oxalis* and *Plumbago* was exclusively dependent on autogamous pollination as they did not attract any pollinators and there was no difference in the extent of seed set in bagged and open-pollinated flowers. Autogamy was absent in *Cassia*; it depended exclusively on pollination by *Xylocopa* sp. *Dodonaea* depended on wind for pollen transfer. Thus, in agreement with the expectation, autogamy is not critical in perennial weed species and their pollination strategies vary from complete autogamy to obligate outbreeding, similar to non-weedy species.

Key Words: Autogamous Self-Pollination; Perennial Weeds; Pollination Strategy; Reproductive Assurance

Introduction

Pollination is a prerequisite for seed set and thus plays a critical role in reproductive success of seed plants. A majority of flowering plants make use of animals to achieve pollination while a small proportion of them use wind or water for pollination. Plants have evolved diverse pollination strategies ranging from complete selfing to obligate outcrossing (Richards, 1986). Except fully self-incompatible and dioecious species, most of the others show a mixed mating system permitting both self- and cross-pollinations; the proportion of each is highly variable between populations and species depending on the structure of the flower, breeding system and pollination

environment. Many of the species that depend on animals for pollination, often experience pollination limitation as a result of low density of conspecific plants or scarcity of pollinators in the habitat. Frequent pollination limitation/failure threatens the survival and spread of the populations/species. Under such conditions, many plant species have evolved autogamous self-pollination or apomixis as a means of reproductive assurance (RA). Autogamy happens to be the most frequently evolved strategy of RA in different groups of plants (Kalisz and Vogler, 2003; Goodwillie *et al.*, 2005; Eckert *et al.*, 2006; Levin, 2012; Wright *et al.*, 2013).

Biology of weeds has been the focus of

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extensive studies since long because of the damage they cause to the crop species as well as their impact on the environment and biodiversity (Anonymous, 2000; Simberloff and Rejmanek, 2011; Lockwood *et al.*, 2013). RA is considered to be an important reproductive strategy in weed species as it enables them to set seeds even in pollinator-uncertain environments and thus facilitates their invasion to and colonization of new locations (Baker, 1955; Baker, 1974; Barrett, 2011; Busch and Delph, 2012; see also Cheptou, 2012). However, studies on pollination biology of weed species are very limited when compared to those on their seed biology, although pollination is the basis of seed set. RA through autogamy is expected to be critical in annual weed species as they lack vegetative propagation and get only one opportunity to set seeds in their life; if they miss this opportunity, their survival and spread is threatened. My recent studies on pollination ecology of 15 annual weed species of India showed a high level of autogamy in all the species studied (Shivanna, 2014a). Many of the annual weed species show flexibility in their pollination strategy and show a mixed mating system; apart from autogamy as a means of RA they permit cross-pollination when pollinators are available (Goodwillie *et al.*, 2005). This is the ideal bet-hedging pollination strategy that exploits the advantages of both RA as well as out-crossing.

Perennial weeds, on the other hand, generally have dual reproductive strategy, through seeds as well as vegetative propagules. Unlike annuals they get repeated opportunities for seed production. Their survival and fitness, therefore, are not seriously compromised even if they do not produce seeds in some years. Thus, RA through autogamy is not expected to be a critical adaptation in perennial weeds. Surprisingly, most of the investigations on pollination strategies of weed species have not taken their life span (annual/perennial) into consideration. In many of the publications, the life span of the weed species is not even mentioned (Raimundez-Urrutia *et al.*, 2008; Tripp and Manos, 2008; Hardin *et al.*, 1972). An understanding of weed biology including pollination ecology is important for developing their management strategies (Campbell and Grice, 2000). There are very few studies on pollination strategies of perennial

weeds, particularly of Indian species. With the main objective of testing the prediction that autogamy is not critical for the success of perennial weeds, and gaining a better understanding on their pollination strategies, I investigated pollination ecology and seed set in five common perennial weed species. I essentially asked two questions: 1. Do the species show autogamous pollination and if so, what is the extent of autogamy? 2. What is the mode of pollination if the species is non-autogamous?

Materials and Methods

Study Site and Species

Studies were carried out on weed populations growing on fallow lands and waste lands in and around Odekar farm (13° 32' 35" N, 77° 2' 53" E, elevation 825 m msl) located about 20 km from Tumkur, Karnataka State, in south India. It is a semi-arid region made up of largely cultivated and fallow fields. Cultivation is mostly confined to rain-fed coarse cereals. The annual rainfall ranges from 500-700 mm.

Studies were carried out on five perennial weed species: *Cassia auriculata*, *Dodonaea viscosa*, *Ipomoea obscura*, *Oxalis corniculata* and *Plumbago zeylanica*. All the species commonly grow in fallow and waste lands particularly in south India. Some of them have been reported to be economically useful (Rani *et al.*, 2009; Singh *et al.*, 2010; Sekar *et al.*, 2012). Their phenology and floral structure relevant to pollination are given below:

Cassia auriculata L. (Fabaceae, Caesalpinioideae), is a hardy perennial shrub reaching up to a height of 2 m. It is common on waste lands and road sides. It flowers sporadically throughout the year but profuse flowering is seen during February-April. Flowers are borne on racemose inflorescences at the tips of branches. The flowers are zygomorphic with typical features of Caesalpinioideae (Fig. 1A). The stamens are 10 of which three are staminodes. Of the remaining seven stamens, the anthers of four are smaller and produce sterile pollen whereas the anthers of three stamens are larger and produce functional pollen. The anthers exhibit poral dehiscence. The pistil shows typical enantiostyly; the

style is deflected either to the left or the right of the median plane. The flowers of the same plant show both types of style curvature.

Ipomoea obscura (L.) Ker.-Gawal (Convolvulaceae) is a straggling creeper native to tropical Africa but naturalized in most parts of India. Flowers are white and arise as single cymes on 3-4 cm long pedicel in the axils of each leaf; however, only two flowers develop in each cyme. The corolla is differentiated into a lower, narrow tube of about 1.2 cm and terminal expanded part (Fig. 1 B). The stamens are four of which two are longer (10-11 mm) and the other two are slightly shorter (8-9 mm). The pistil is 9-10 mm long with a terminal white stigma positioned at the level of longer anthers.

Oxalis corniculata L. (Oxalidaceae) is a creeping herb with ascending shoots. Flowers are yellow and arise in terminal clusters of 2-5; they are about 7 mm long and 7 mm wide (Fig. 1C). Stamens are in two whorls of 5 each. Carpels are 5, syncarpous but the style of each carpel is distinct and terminates in a capitate stigma located at the level of longer anthers (Fig. 1D). A few small droplets of nectar could be seen at the base of the ovary.

Plumbago zeylanica L. (Plumbaginaceae) is a straggling shrub that grows in fallow fields and wastelands. Flowers are borne on racemose inflorescences; 2-4 flowers open on each day in each inflorescence. The flowers are white, about 2.5 cm long and 1.5 cm wide. Sepals are covered with stalked sticky glands. Petals are 5, fused at the lower part to form a narrow corolla tube and the terminal parts are free and expanded. Anthers are 5, pinkish and are located at the tip of the corolla tube. All the anthers are arranged close to each other enclosing five erect stigmatic lobes located at the level of the anthers. Small amount of nectar (<1µl) is present at the base of the corolla tube only in about 50% of the flowers. Anthers dehisce 1-2 h after anthesis and spread out slightly exposing white pollen grains. The stigmatic lobes also spread out and come in contact with dehisced anthers.

Dodonaea viscosa (L.) Jacq. (Sapindaceae) is an evergreen, hardy perennial shrub widespread in

countries of warm tropical regions of Southern hemisphere. In India, it is commonly found in drier open fields and fallow lands. The sexuality of the species seems to vary; it has been described as dioecious, monoecious and polygamous in populations growing in different countries (see Rivers, 1971). Flowering occurs sporadically in a few plants from January but peaks during June-July. Both male and female plants produce flowers in loose clusters at the tips of fresh branches. Each panicle bears about 15 flowers in male plants and about 10 flowers in female plants. Flowers are greenish, without any petals and are not showy. Each male flower produces 4/5 free, greenish perianth lobes and 8-10 anthers arranged close to each other in the form of a cup. There is a small pistillode at the centre of male flowers but female flowers do not bear any staminodes. The pistil is 3-carpelled with two ovules in each carpel. The ovary is 3-4 mm long, with a robust style terminating in a trifold stigma. The style emerges from the bud even before the stigmatic lobes become visible. Fruits are 3-winged papery capsules; unlike flowers, fruits are conspicuous and remain on the plant until the next flowering season (Fig. 1E). Only one or two seeds develop from each fruit.

Methods

Floral phenology was studied by tagging flower buds (N=30) and monitoring them from anthesis until their senescence. Autogamous self-pollination was studied through bagging of flowers. In *I. obscura*, the flowers were bagged individually one day before anthesis. In the remaining species the inflorescences/terminal branches bearing clusters of flower buds were bagged. All opened flowers and very young fruits, if any, were removed before bagging and the number of flower buds in each inflorescence/branch was recorded. For open pollination, individual flowers/inflorescences/flowering branches were tagged and monitored.

One set of bagged and open flowers were excised after the flowers started senescing and their stigmas observed under a stereomicroscope for the presence of pollen grains. Pollinated pistils were preserved in 70% ethanol and used to study pollen germination and pollen tube growth using aniline blue



Fig. 1. (A) A flowering twig of *C. auriculiformis*. (B) A patch of *I. obscura* in flowering; inset is a flower from which a part of the corolla tube is removed to show the position of the anthers and the stigma. (C) and (D). *O. corniculatus* A cluster of plants with ascending branches (C) and a flower magnified (D). (E). *D. viscosa*, a fruiting branch

fluorescence method (Shivanna and Rangaswamy, 1992). The pistils were cleared with 6N NaOH for 8-12 h at room temperature (about 25°C), mounted in a drop of water soluble aniline blue; applied gentle pressure on the cover slip to spread the tissue and observed under the fluorescence microscope using UV filter combination (excitation filter 330-400 nm, barrier filter 420 nm). The remaining flowers (both bagged and tagged) were left on the plants to record fruit set.

Floral visitors were monitored at 30 min time slots each hour during the 8-10 h period for which the flowers remained open, at least for 2 consecutive days for each species. In *C. auriculata*, the flowers remain open for 2-3 days and the floral visitors were monitored at 30 min time slots each hour in the mornings (06.00-10.00 h) and evenings (14.00-18.00 h). *D. viscosa* is believed to be wind pollinated, although bees have been reported to visit the flowers

to collect pollen (Rani *et al.*, 2009). However, no rigorous studies have been carried out on pollination biology of the species. To confirm wind pollination, two types of bags were used to enclose female flower clusters. One set of flowers was covered with pollen proof bags made of glossy paper and the other set was covered with bags made up of net cloth (aperture size about 2 mm²) that permits the passage of air borne pollen but prevents the entry of insects. The flowers were then monitored for fruit and seed set. In another experiment, microscope slides coated with white petroleum jelly were hung near the female flowers to trap air borne pollen grains. The slides were collected 24 h after exposure and a drop of 10 % glycerin was placed on the petroleum jelly-coated surface of the slide and a cover glass was lowered to observe the presence of pollen under the microscope. The pollen grains trapped on the slides were identified with the help of reference slides of *D. viscosa* pollen.

The differences in pollination efficacy and fruit set (%) between bagged and open-pollinated flowers were analyzed by pair wise t-test. The percentage values for pollination and fruit set were ascertained for their normal distribution and root square arsine transformed before the analysis. All data were analyzed using SPSS 16 statistical package (SPSS Inc., Chicago, IL, USA).

Results

Except *O. corniculatus*, none of the other species studied showed vegetative propagation. Three of the species *I. obscura*, *O. corniculatus* and *P. zeylanica* showed autogamous self-pollination (Table 1) as a result of close proximity of the anthers and the stigmas. Pollination efficiency in bagged flowers was as good as in the open flowers and the differences were not statistically significant. All the three species showed good pollen germination on the stigma and pollen tube growth in the style in both autogamous and open-pollinated flowers. During the eight hours of observation of each species, I did not observe any regular floral visitors to the flowers of these species

indicating that autogamy is the only mode of pollination in the populations studied. All the autogamous species produced good seed set and to the similar extent in both bagged and open flowers (Table 2).

There was no autogamy in *C. auriculata* (Table 1). None of the bagged flowers were observed to be pollinated and no fruits were realized (Table 2). Even in open flowers, only one third of the flowers got pollinated indicating inefficient pollination. Carpenter bee (*Xylocopa* sp.) was the only floral visitor recorded and its frequency was very low. Although the flowers remained open for two days, nearly half of the flowers did not receive any visitor. Even those that received the visitor, not more than one visit to each flower was recorded during the observation period. Even the fruit set in open-pollinated flowers was less than one third (Table 2), in agreement with the low extent of pollination.

The population of *D. viscosa* used in the present studies was largely dioecious. Of the 77 plants scored, 37 were males, 35 were females and the remaining 5 plants were essentially males but produced a small

Table 1: Pollination efficiency in bagged and open-pollinated flowers

Species	Bagged flowers		Open-pollinated flowers		t value
	N	No. pollinated (%)	N	No. pollinated (%)	
<i>Cassia auriculata</i>	26	0(0)	27	9(33.33)*	
<i>Ipomoea obscura</i>	25	23(92)	24	22(91.66)	0.046 P=0.917
<i>Oxalis corniculatus</i>	26	24(92)	27	26(96.29)	0.84 P=0.43
<i>Plumbago zeylanica</i>	24	21(87.5)	24	22(91.66)	1.06 P=0.35

*Not estimated as the value for bagged flowers was zero

Table 2: Fruit set in bagged and open-pollinated flowers

Species	Bagged flowers		Open-pollinated flowers			t value
	N	No. fruits set (%)	N	No. fruits set (%)	No. pollinated (%)	
<i>Cassia auriculata</i>	117	0(0)	122	38	(31.14)*	
<i>Ipomoea obscura</i>	45	37(80.43)	42	37	(83.33)	1.192 P=0.297
<i>Oxalis corniculatus</i>	96	84(87.50)	87	78	(89.65)	0.32 P=0.75
<i>Plumbago zeylanica</i>	94	67(71.27)	132	103	(78.03)	0.94 P=0.41

*Not estimated as the value for bagged flowers was zero

proportion of bisexual flowers. The flowers of *D. viscosa* do not attract any visitors. Male flowers produce powdery pollen grains and the stigma is well exposed. Pollen grains were trapped on slides coated with petroleum jelly (16.5/cm² N=8). Wind pollination was confirmed by bagging experiments also. Average number of fruits/cluster in flowers allowed to open pollinate was 57.91% whereas it was 38.87% in net-covered bags (pore size 2 mm²). Lower fruit set in net-bagged flowers indicates that the bag prevented to some extent the flow of wind-borne pollen. However, the differences were not statistically significant ($t = -1.609$; $df = 10$; $p = 0.139$). In both the open-pollinated and net-covered flowers 95-97% of the fruits contained seeds. Some of the flowers covered with pollen-proof bags also yielded 4.8 % fruits; however, none of them contained any seeds indicating their parthenocarpic development.

Discussion

The results of the present investigation clearly show that pollination strategies of perennial weed species range from autogamy to absolute outcrossing. Three of the five species studied showed autogamous self-pollination. The flowers of none of them received any visitors indicating that their natural seed set in the populations studied is exclusively through selfing. As the floral structure of these species does not prevent pollinators' visits, it is possible that other populations growing elsewhere may show mixed mating system, if effective pollinators are present in the habitat.

C. auriculata does not show autogamous self-pollination. Even its pollination efficiency under natural conditions is low. The details of floral specialization especially the elaboration of the anthers is well-studied in *Cassia* spp. (Dulberger, 1981; Pardha Saradhi and Mohan Ram, 1981; Chauhan *et al.*, 2003). It shows typical poral dehiscence of anthers and requires buzz pollination. Even when the insect visits the flower, the stigma may not come in contact with the body of the insect all the time because of the orientation of the stigma. Low level of pollination efficiency and fruit set in *C. auriculata* may partly be due to the elaboration of the floral structure and partly due to

low frequency of insect visits. In agreement with the low level of pollination efficiency, the extent of fruit set was also low. However, since each plant flowers for over two months and produces a large number of flowers resulting in over 100 fruits, each with 5-10 seeds, fruit set is not a constraint in this species. *D. viscosa* is largely dioecious and thus there is no scope for autogamy. Pollination is exclusively mediated through wind.

Limited information available on a few other perennial species also supports the present observations; their pollination strategy varies from complete autogamy to complete outbreeding. *Trichodesma zeylanicum* shows obligate autogamy as its anthers and stigma are not exposed for floral visitors (Shivanna, 2014b). Many of the perennial weed species show mixed mating system, permitting both autogamy and cross-pollination when pollinators are available (Aluri *et al.*, 1997). Indian populations of *Lantana camara*, an invasive and widespread perennial weed in the old-world tropics, are pollinated by thrips (Mathur and Mohan Ram, 1978). Australian populations of this species, however, are reported to be self-incompatible and pollinated by a number of insects, particularly bees (Goulson and Derwent, 2014). *Eichhornia crassipes* (water hyacinth), another invasive weed, is a heteromorphic self-incompatible species but most of the populations are made up of only one morph, largely the mid-style morph (Barrett, 1977). The species shows prolific vegetative propagation and effective dispersal of vegetative propagules through water; it hardly reproduces through seeds under field conditions (Mulcahy, 1975; El Seed and Obeid, 1975). Similarly eight rhizomatous perennial weed species of Canada are not autogamous although the species that grow in groups (caespitose) are autogamous (Mulligan and Findlay, 1970). Thus, autogamy is not critical in perennial weed species and their pollination strategies, similar to non weedy species, are highly variable.

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