



POLLINATION ECOLOGY OF *RHYNCHOSIA RUFESCENS* (WILLD.) DC. (FABACEAE), A PERENNIAL TRAILING SUB-SHRUB IN THE SOUTHERN EASTERN GHATS, ANDHRA PRADESH, INDIA

SOLOMON RAJU A. J.^{1,*}, VENKATA RAMANA K.²

¹Department of Environmental Sciences, Andhra University, Visakhapatnam 530 003, India

²Department of Botany, Andhra University, Visakhapatnam 530 003, India

*Corresponding author: Telephone: +919866256682; e-mail: solomonraju@gmail.com

(RECEIVED 28 NOVEMBER 2017; RECEIVED IN REVISED FORM 11 JANUARY 2018; ACCEPTED 15 JANUARY 2018)

ABSTRACT – *Rhynchosia rufescens* is a perennial trailing sub-shrub. It flowers during September-February. The flowers are hermaphroditic, self-compatible and have explosive pollination mechanism principally adapted for melittophily. The plant is facultative xenogamous but autogamy essentially requires vectors. Pods dehisce explosively to disperse seeds. Re-growth from root stock and propagation by seed together contribute to build up its populations in soils with sufficient moisture and nutrients.

KEYWORDS – *RHYNCHOSIA RUFESCENS*, HERMAPHRODITISM, EXPLOSIVE POLLINATION MECHANISM, MELITTOPHILY, EXPLOSIVE POD DEHISCENCE.

INTRODUCTION

Franco (1995) noted that *Rhynchosia* species in Brazil are autogamous but self-pollination is limited by spatial segregation between stigma and anthers. Levels of out-crossing are maintained by retention of a pollination mechanism. *Hypanthidium* sp. and *Centris* sp. (Apoidea) are the primary pollinators. Craufurd & Prins (1979) reported that *Rhynchosia sublobata* is self-compatible and pollinated by *Xylocopa* bees. Etcheverry et al., (2011) reported that *R. edulis* and *R. senna* var. *texana* display valvular pollination mechanism; the former is facultatively xenogamous while the latter is obligately xenogamous. There is no other information on the reproductive ecology of any species of *Rhynchosia*. In this framework, the present study was intended to provide the details of the pollination ecology of *Rhynchosia rufescens*.

MATERIALS AND METHODS

The study region occurs in the deciduous forest ecosystem and is an integral part of the Southern Eastern Ghats of Andhra Pradesh (13°40'N latitude and 79°19'E longitude) in Peninsular India. *Rhynchosia rufescens* plants growing in this area were used for this study. The plant grows as small or lush populations in areas of scanty herbaceous vegetation and as scattered individuals in areas where several other plant species show luxurious growth. It grows in flat areas, hill-slopes and hill tops including dry, rocky habitats.

Twenty inflorescences were tagged and followed to record the length of flowering and the number of flowers produced. Anthesis schedule and anther dehiscence were recorded by observing twenty-five marked mature buds in the field. The floral aspects were recorded in detail. Thirty mature but un-

dehiscent anthers from five different plants were used to record the pollen output per anther and flower, and pollen-ovule ratio as in the protocol given in Cruden (1977). The nectar volume, sugar concentration, sugar types and sugar content at flower level, and stigma receptivity were examined as in the protocols described by Dafni et al., (2005). Fruit set rates in different modes of pollination were recorded by following the method described in Dileepu Kumar et al., (2017). The hourly foraging visits of insect species were recorded on 3 or 4 occasions depending on the possibility and data were tabulated for use in further analysis. Insects' foraging behaviour was observed with reference to their role in pollination. Pollen carry over efficiency of each foraging insect species was examined by following the procedure in Dileepu Kumar et al., (2017). Fruit maturation period, fruit and seed dispersal modes, seed germination and seedling establishment were recorded.

RESULTS

Phenology

R. rufescens is a perennial sub-shrub with trailing glandular-hairy branches and branchlets (Fig. 1a). The plant re-grows from perennial root below ground soon after the first rains in June (Fig. 1b), or gradually emerge from the seed during June-September. In dry, rocky localities, seeds germinate late and produce new plants depending on the soil nutrient and moisture environment. Plants growing in moist soils are robust when compared to those growing in dry soils or rocky areas. Flowering occurs during September-January in plants that emerge from perennial root stock, or during late October-early February in plants that emerge from seeds (Fig. 1c). The plants disappear in March-April. The inflorescence is a pedunculate and hairy raceme of 2-6 flowers borne in leaf axils (Fig. 1e).

Flower morphology

The flowers are pedicellate, small, yellow, odorless, papilionaceous, zygomorphic and bisexual. The calyx consists of 5 free, ovate with obtuse apex, densely pubescent sepals. The corolla is bright yellow, pubescent, specialized and consists of the upper standard petal with a nectar guide, two wing petals and two keel petals. Stamens are ten, diadelphous with dithecos anthers (Fig. 2f). The ovary is sub-sessile, green, villous, and lies in the sheath of filaments along the cylindrical part of the keel (Fig. 2h, j). It is monocarpellary and monolocular with one and rarely two ovules arranged on marginal placentation (Fig. 2k). It has a long glabrous style with a capitate wet shiny stigma situated at the same height of the anthers (Fig. 2i).

Floral biology

Mature buds open during 1000-1400 h with peak anthesis during 1100-1200 h (Table 1; Fig. 1d, 2a-d). The keel petals do not unfold and remain in their original position as in mature bud stage (Fig. 2e). All the ten anthers in a flower dehisce at the same time by longitudinal slits in mature bud stage. The number of pollen grains per anther is 642 ± 47.79 and per flower is 6,420. The pollen-ovule ratio is 6,420:1. The pollen grains are monads, spheroidal, $32.64 \pm 5.64 \mu\text{m}$ in size, powdery and tricolporate, angulaperturate with reticulate exine (Fig. 2g). A nectariferous disc is present at the base of the ovary. The initiation of nectar secretion occurs in mature bud stage and its cessation occurs an hour after anthesis. Individual flowers produce $0.8 \pm 0.21 \mu\text{l}$ of nectar with 0.26 mg of sugar. The nectar sugar concentration is 29% (Range 27-30%) consisting of sucrose, glucose and fructose with the first as dominant. Nectar is deeply concealed and it is accessed through two windows between the joined and the free filaments at the flower base. The stigma attains receptivity during anthesis and remains receptive for about three hours when the standard, wing and keel petals gradually move close to each other enclosing the reproductive organs (Fig. 2l-o). The closed flowers remain so even during most part of fruit development. The calyx initially encloses the ovary and subsequently turns pale to dark brown and exposes the ovary since the latter gradually bulges and develops into a seeded pod.



Figure 1. *Rhynchosia rufescens*: a. Habitat, b. Vegetative phase, c. Flowering phase, d. Floral buds in different stages, e. Axillary, racemose inflorescence with 2-6 flowers.



Figure 2. *Rhynchosia rufescens*: a-d. Different stages of anthesis from bud to flower, e. Stamens and stigma enclosed by keel and wing petals, f. Diadelphous condition of stamens together with stigma, g. Pollen grain, h. Gynoeccium, i. Capitate stigma, j. Ovary covered with hair, k. Ovule, l-o. Different stages of flower closure, p. *Apis dorsata* probing the flower for nectar, q. *Ceratina* sp. collecting pollen, r. *Ceratina* sp. collecting nectar.

Table 1. Anthesis as a function of time in *Rhynchosia rufescens*.

Pollination mode	No. of flowers pollinated	No. of fruits formed	Fruit set (%)
Autogamy (un-manipulated and bagged)	25	0	0
Autogamy (hand-pollinated and bagged)	60	12	20
Geitonogamy	60	31	52
Xenogamy	60	48	80
Open-pollination	756	652	86

Pollination mechanism

The reproductive column is held under pressure within the keel petals and is exposed when the pollinator presses against the wing and the keel petals. When bees land on the wing petals, the latter cause the keel petals to release the reproductive column explosively. Consequently, the reproductive column snaps forward against the standard petal, causing most of the pollen to be instantly released and to come into contact with the ventral side of the bee body. Since the incurved stigma is situated above the height of the anthers, it strikes the bee body first, due to which cross-pollination occurs if the bee previously visited other flowers and carried pollen on its ventral side. If it is the first visit for the bee to a flower, then it effects self-pollination upon explosive release of reproductive column from the keel. With the departure of the bee from the flower, the reproductive column does not return back to its former position but the keel moves forward partly covering the stamens and stigma. The downward movement of keel petals occurs in each subsequent foraging visit. If the flower is untouched or tripping to keel did not occur, the reproductive column is never exposed and remains enclosed in the keel boat. Such flowers fall off subsequently upon withering without fruit set.

Breeding systems

In mature buds, anthers dehisce but autonomous autogamy does not occur. Fruit set is absent in un-manipulated autogamy, 20% in hand-pollinated autogamy, 52% in geitonogamy, 80% in xenogamy and 86% in open-pollination (Table 2).

Bee pollinators and pollination

The flowers were foraged by bees for pollen and nectar, and by butterflies for nectar only. The bees included *Apis dorsata*

Table 2. Results of breeding systems in *Rhynchosia rufescens*

Time (h)	No. of flowers anthesed	Percentage of anthesis
08:00	0	0
09:00	0	0
10:00	10	16
11:00	17	27
12:00	26	42
13:00	6	10
14:00	3	5
15:00	0	0

No. of mature buds tagged: 62

(Fig. 2p), *Ceratina perforatrix* (Fig. 3b,c), *Ceratina* sp. (Fig. 2q,r), *Nomia* sp. (Fig. 3a) (Apidae) and *Megachile* sp. (Fig. 3d) (Megachilidae) while the butterflies were *Catopsilia pomona* (Fig. 3e), *Eurema hecabe* (Fig. 3f) (Pieridae), *Leptotes plinius* (Fig. 3g), *Freyeria trochylus* (Fig. 3h), *Lampides boeticus* (Fig. 3i), *Chilades pandava* (Fig. 3j) (Lycaenidae) and *Borbo cinnara* (Fig. 3k) (Hesperiidae) (Table 3). The bees foraged during 1030-1730 h while butterflies foraged during 1130-1630 h; both categories of insects showed more foraging activity during 1230-1330 h. Bee foraging visits constituted 70% and butterflies 30% of total visits made in a given day during peak flowering phase (Fig. 4). All bee species were regular and consistent foragers throughout the flowering season. In case of butterflies, they were occasional in their visits during the initial and final phase of flowering, while they were regular and consistent during the peak phase. The bees were quite effective in flower-tripping while foraging for nectar and effect pollination. The butterflies were ineffective in tripping but foraged for nectar from both un-tripped as well as tripped flowers; they effected pollination only when foraging on tripped flowers from the front side. Bees and butterflies visited the flowers of the same and different conspecific plants promoting cross-pollination rate. The body washings of bees and butterflies showed that they carried pollen to different extents; bees showed a pollen carrying capacity ranging from 341 to 1230 grains while butterflies, carried from 23 to 83 pollen grains especially on their proboscis (Table 4). These data indicated that both bees and butterflies are pollen carriers and effect pollination to different extents.

Fruiting behavior

The fruits mature within three weeks. They are green initially and brown to dark brown when ripe and dry (Fig. 5 a, b). It is non-fleshy, hairy, oblong, rounded with apiculate apex. Pods commonly produced one seed each, rarely two (Fig. 5c).

Seed ecology

Dry pods with bi-valvate configuration dehisce elastically ejecting the seeds (Fig. 5e). Seeds are greyish to brown or black, sub-globose, transversely elliptic, finely pubescent and strophiolate (Fig. 5d). The growth of seedlings is subject to the availability of moisture and nutrients in the soil; they produce mature plants within two months and commence flowering and fruiting if the soil is litter-rich and sufficiently wet. The perennial underground root sprouts (Fig. 5f) and produces new foliage and subsequently display flowering and fruiting. Seeds germinate during the rainy season, which starts in June (Fig. 5g, h).

DISCUSSION

Rhynchosia rufescens grows in deciduous forest with both wet or dry and rocky sites. Leaf flushing is followed by flowering and fruiting. Individual plants produce a few inflorescences, each with only two to six flowers which are prominent by their yellow colour but not by their position since they occur within the foliage. The flowers with anther dehiscence in mature bud stage and stigma receptivity during anthesis display homogamy. Hand-pollination tests indicated that autogamy occurs only when pollination is manipulated indicating the occurrence of vector-mediated autogamy. It appears that the stigma although receptive blocks the germination of the self-pollen while it is in keel petals and essentially requires the rupture of its surface by a pollinator while tripping the keel to allow the self- or cross-pollen to germinate. This stigmatic regulatory function appears to have evolved to discourage selfing and promote out-crossing. Such a situation is reported in certain Phaseoleae (Shivanna & Owens, 1989). Apart from autogamy, *R. rufescens* also sets fruit through geitonogamy and xenogamy and is facultatively xenogamous.

Flowers pollinated by the bee family Apidae are typically zygomorphic, bright yellow or blue, with nectar guides, and frequently carry hidden rewards such as those of the Lamiaceae, Scrophulariaceae, Fabaceae and Orchidaceae (Faegri & van der Pijl, 1979). *R. rufescens* has papilionaceous corolla; the flag petal serves as a visual attractant, wing petals provide landing platform and keel petals protect the entire reproductive column and are typical of pollination by bees. Within the sub-family Papilionoideae, primary and secondary pollen presentations have been reported. In primary pollen presentation, pollen is delivered directly from the anthers to the vector's body while in secondary pollen presentation, pollen grains are delivered first on a floral part such as the keel petals and then on the body of the vector, implying a more accurate delivery of pollen



Figure 3. *Rhynchosia rufescens*: a. *Nomia* sp. collecting nectar, b. *Ceratina perforatrix* collecting nectar, c. *Ceratina perforatrix* collecting pollen, d. *Megachile* sp. collecting nectar, e. *Catopsilia pomona*, f. *Eurema hecabe* (Pierid), g. *Leptotes plinius*, h. *Freyeria trochylus*, i. *Lampides boeticus*, j. *Chilades pandava* (Lycaenids), k. *Borbo cinnara* (Hesperiid).

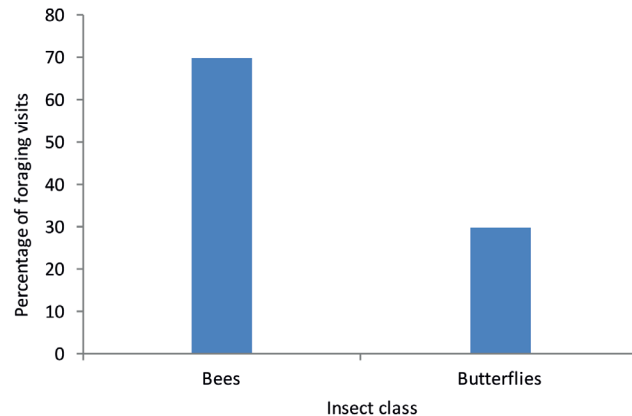


Figure 4. Percentage of foraging visits of bees and butterflies on *Rhynchosia rufescens*.

on the vector's body (Howell et al., 1993). These pollen presentation patterns are associated with valvular, pump, explosive and brush pollination mechanisms, all of them associated with a particular floral architecture and kinetics. In the valvular type, pollen presentation is primary, whereas in the other three mechanisms, it is secondary (Yeo, 1993). In the explosive mechanism, commonly only one pollination event occurs while in the other three mechanisms, repeated visitation is possible (Westerkamp, 1997). *R. rufescens* flowers have primary pollen presentation associated with an explosive pollination mechanism whose efficiency depends

Table 3. List of insect foragers on *Rhynchosia rufescens*.

Order	Family	Genus	Species	Foraging schedule (h)	Forage collected
Hymenoptera	Apidae	<i>Apis</i>	<i>dorsata</i> F.	1030-1730	Pollen, Nectar
		<i>Ceratina</i>	<i>perforatrix</i> Smith	1030-1730	Pollen, Nectar
		<i>Ceratina</i> sp.	-	1030-1730	Pollen, Nectar
		<i>Nomia</i> sp.	-	1030-1730	Pollen, Nectar
	Megachilidae	<i>Megachile</i> sp.	-	1030-1730	Pollen, Nectar
Lepidoptera	Pieridae	<i>Catopsilia</i>	<i>pomona</i> F.	1130-1630	Nectar
		<i>Eurema</i>	<i>hecabe</i> L.	1130-1630	Nectar
	Lycaenidae	<i>Leptotes</i>	<i>plinius</i> F.	1130-1630	Nectar
		<i>Freyeria</i>	<i>trochylus</i> Freyer	1130-1630	Nectar
		<i>Lampides</i>	<i>boeticus</i> L.	1130-1630	Nectar
	Hesperiidae	<i>Chilades</i>	<i>pandava</i> Horsfield	1130-1630	Nectar
		<i>Borbo</i>	<i>cinnara</i> Wallace	1130-1630	Nectar

Table 4. Pollen recorded in the body washings of bee foragers on *Rhynchosia rufescens*.

Insect species	Sample size (N)	Number of pollen grains		
		Range	Mean	S.D
<i>Apis dorsata</i>	10	560-1230	805.1	277.82
<i>Ceratina perforatrix</i>	10	435-892	676.9	149.51
<i>Ceratina</i> sp.	10	467-782	626.7	110.26
<i>Nomia</i> sp.	10	341-568	476.5	84.81
<i>Megachile</i> sp.	10	420-589	510.3	49.55
<i>Catopsilia pomona</i>	10	58-79	67.3	6.84
<i>Eurema hecabe</i>	10	62-83	74.5	6.58
<i>Leptotes plinius</i>	10	45-81	65.6	14.22
<i>Freyeria trochylus</i>	10	41-78	62.8	12.39
<i>Lampides boeticus</i>	10	32-67	54.1	10.87
<i>Chilades pandava</i>	10	31-69	51.5	12.15
<i>Borbo cinnara</i>	10	23-56	43.7	11.52

on the ambient temperature and relative humidity. Since *R. rufescens* flowers during winter and commences anthesis from late morning onwards to maximize the efficient functioning of the explosive pollination mechanism. Further, the bees also begin their foraging activity immediately after anthesis and continue forage collection until the flowers close back. The concealment of the stamens within the keel petals until it is tripped will secure pollen from unusual rains and ambient moisture conditions during the flowering season.

Baker & Baker (1983) stated that flowers with long corolla tube possess more sucrose in their nectar while those with short tubes possess more hexoses. In *R. rufescens*, the corolla tube is short and produces sucrose-rich nectar. Baker & Baker (1990) reported that flowers pollinated by long-tongued bees produce sucrose-rich nectar. *R. rufescens* flowers with sucrose-rich nectar is utilized exclusively by long-tongued bees, *Apis*, *Ceratina*, *Nomia* and *Megachile*. Bee-flowers tend to produce a small volume of nectar with high sugar

concentration (Cruden et al., 1983). Honey bees prefer nectar sugar concentrations of 30-50% (Baker & Baker, 1983). *R. rufescens* produces a small volume of nectar with 29% sugar concentration and its energy yield is in accordance with the requirement of energy by bees. Apart from bees, butterflies also visit the flowers for nectar especially during the peak flowering season but they do not cause tripping of keel boat to result in pollination. Therefore, butterflies are principally nectar robbers but nectar depletion by them promotes bee foraging activity which in turn enhances pollination rate.

Arroyo (1981) stated that in the Papilionoideae the P/O varies according to the pollination mechanism. The plants with explosive mechanism have a low P/O because a single pollinator visit is needed for efficient pollen transfer; this low P/O is a consequence of the highly specialized, irreversible pollination mechanism, which allows only one effective exchange of pollen with pollinators. Etcheverry et al., (2011)



Figure 5. *Rhynchosia rufescens*: a. Single-seeded pod, b. 2-seeded pod, c. Seeds, d. Mature, dry fruits ready for shedding, e. Production of new shoots from the perennial root stock during rainy season, f. & g. New plants from seeds.

stated that the *Rhynchosia* species which they studied had explosive pollination mechanism associated with intermediate pollen-ovule ratios. *R. rufescens* shows high P/O ratio which could be a consequence of pollen collection activity by bees. Tran & Cavanagh (1984) reported that in Leguminosae, seeds of many taxa exhibit physical dormancy due to the presence of a water-impermeable seed coat. With this dormancy, they remain viable for a long period of time. In *R. rufescens*, seeds exhibit physical dormancy during the dry season and respond to rainfall during the wet season. Many seeds germinate during the rainy season but their continued growth is related to soil moisture and nutrient environment. Since the plant is a perennial, its underground root stock also produces new growth during rainy season. The explosive pod dehiscence in this species does not spread the seeds far away from parental sites but rain water disperses them to new places. Therefore, *R. rufescens* is not a very widespread species and primarily inhabits dry and rocky areas with scanty herbaceous vegetation. Remanandan (1981) suggested that *Rhynchosia*, being closely related to the genus *Cajanus*, can be used to provide substantial contributions to crop improvement in pigeon pea. Furthermore, some species of *Rhynchosia* have been experimented in India to provide physiological resistance against insect pests such as pod-borer and pod-fly in pigeon pea. In *R. rufescens*, the seeds are not infested by any insect and further research on this may be useful to identify and select desirable genotypes in this plant that give physiological resistance against pod or seed pests in order to use them for crop improvement in pigeon pea or other legumes.

COCLUSIONS

Rhynchosia rufescens is a hermaphroditic, self-compatible and facultative xenogamous species. The flowers have primary pollen presentation with explosive pollination mechanism adapted primarily for melittophily. Pods dehisce explosively to disperse seeds. Re-growth from root stock and propagation from seeds collectively contribute to its present population size in the study area.

ACKNOWLEDGEMENTS

We thank the Andhra University, Visakhapatnam, India, for providing all facilities to carry out this research work.

REFERENCES

- Arroyo M.T.K., 1981. Breeding systems and pollination biology in Leguminosae. In R.M. Polhill and P.H. Raven (Eds) *Advances in Legume Systematics*, pp. 723-769, Part 2, Royal Botanical Gardens, Kew, London.
- Baker H.G., Baker I., 1983. Floral nectar sugar constituents in relation to pollinator type. In C.E. Jones and R.J. Little (Eds) *Handbook of Experimental Pollination Biology*, pp. 117-140, Scientific and Academic Editions, New York.
- Baker H.G., Baker I., 1990. The predictive value of nectar chemistry to the recognition of pollinator types. *Israel Journal of Botany* 39, 157-166.
- Craufurd R.Q., Prins W.H., 1979. Munkolo (*Rhynchosia sublobata*), a promising pasture legume for Zambia. *Tropical Grasslands* 13, 45-52.
- Cruden R.W., 1977. Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. *Evolution* 31, 32-46.
- Cruden R.W., Hermann H.M., Peterson S., 1983. Patterns of nectar production and plant-pollinator co-evolution. In B. Bentley and T. Elias (Eds) *The Biology of Nectaries*, pp. 80-125, Columbia University Press, New York.
- Dafni A., Kevan P.G., Husband B.C., 2005. *Practical Pollination Biology*. Enviroquest Ltd., Canada.
- Dileepu Kumar B., Sandhya Deepika D., Solomon Raju A.J. 2017. Reproductive biology of the hill glory bower,

Clerodendrum infortunatum L. (Lamiaceae). *Annali Di Botanica* 7, 43-56.

Etcheverry A.V., Aleman M.M., Figueroa-Fleming T., Lopez-Sphar D., Gomez C.A., Yanez C., Figueroa-Castro D.M., Ortega-Baes P. 2011. Pollen: ovule ratio and its relationship with other floral traits in Papilionoideae (Leguminosae): an evaluation with Argentine species. *Plant Biology (Stuttgart)* 14, 171-178.

Faegri K., van der Pijl L., 1979. *The Principles of Pollination Ecology*. Pergamon Press, Oxford.

Franco A.L.M., 1995. *Pollination ecology and reproductive biology of seven species of Phaseoleae (Fabaceae)*, Campinas, SP. Ph.D. Thesis, University of Campinas, pp.123.

Howell G.J., Slater A.T., Knox R.B., 1993. Secondary pollen presentation in angiosperms and its biological significance. *Australian Journal of Botany* 41, 417-438.

Remanandan P., 1981. The wild gene pool of *Cajanus* at ICRISAT, Present and Future. Proc. Intl. Workshop on Pigeon-peas, Vol. 2 15-19, 1980, Patancheru, Andhra Pradesh, India.

Shivanna K.R., Owens S.J., 1989. Pollen-pistil interactions (Papilionoideae). In C.H. Stirton and J.L. Zarucchi (Eds) *Advances in legume biology. Monographs of Systematic Botany*, Missouri Botanical Garden 29, 157-182.

Tran V.N., Cavanagh A.K., 1984. Structural aspects of dormancy. In: D.R. Murray (Ed) *Seed physiology*, Vol. 2, Germination and Reserve Mobilization, pp. 1-44, Academic Press, Sydney.

Westerkamp C., 1997. Keel blossoms: bee flowers with adaptations against bees. *Flora* 192, 125-132.

Yeo P.F., 1993. *Secondary pollen presentation. Form, function and evolution*. Springer, New York, USA.