6. Bohs, I., Cyphomandra (Solanaceae). *Flora Neotropica Monograph*, 1999, **63**, New York Botanical Garden.
7. Furini, A. and Wunder, J., Analysis of eggplant (*Solanum melongena*) related germplasm: morphological and AFLP data contribute to phylogenetic interpretations and germplasm utilization. *Theor. Appl. Genet*., 2004, **108**, 197–208.
8. Daunay, M. C. and Lester, R. N., The usefulness of taxonomy for Solanaceae breeders, with special reference to the genus *Solanum* and to *Solanum melongena* L. (eggplant). *Capsicum Newslett*., 1988, **7**, 70–79.
9. Gepts, P., The use of molecular and biochemical markers in crop evolution studies. *Evol. Biol*., 1993, **27**, 51–94.
10. Williams, J. G. K A. R., Kubelik, K. J., Livak, J. A., Rafalski and Tingey, S. V., DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res*., 1990, **18**, 6531–6535.
11. Welsh, J. and McClelland, M., Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res*., 1990, **18**, 7213–7218.
12. Deb, D. B., *Solanum melongena* versus *S. incanum* (Solanaceae). *Taxon*, 1989, **38**, 138–139.
13. Karihaloo, J. L. and Gottlieb, L. D., Random amplified polymorphic variation in the eggplant, *Solanum melongena* L. (Solanaceae). *Theor. Appl. Genet*., 1995, **90**, 767–770.
14. Hasan, S. M. Z. and Lester, R. N., Crosability relationship and *in vitro* germination of F1 hybrids between *S. melongena* L. × *S. panduriforma* E. Meyer (*S. incanum* L. Sensu ampl.). *SABRAO J*., 1990, **22**, 65–72.
15. Hasan, S. M. Z. and Lester, R. N., Comparative micromorphology of the seed surface of *Solanum melongena* L. (eggplant) and allied species. *Pertanika*, 1990, **13**, 1–8.
16. Lester, R. N. and Hasan, S. M. Z., Origin and domestication of the brinjal eggplant *Solanum melongena* from *S. incanum* in Africa and Asia. In *Solanaceae III. Taxonomy, Chemistry, Evolution* (eds Hawakes, J. G., Lester, R. N., Nee, M. and Estrada, N.), The Royal Botanic Gardens, Kew, London, 1991, pp. 369–388.
17. Isshiki, S., Okubo, H. and Fuziada, K., Phylogeny of eggplant and related *Solanum* species constructed by allozyme variation. *Sci. Hort*., 1994, **59**, 171–176.
18. Sakata, Y., Nishio, T. and Mathews, P. J., Chloroplast DNA analysis of eggplant (*Solanum melongena*) and related species for their taxonomic affinity. *Euphytica*, 1991, **55**, 21–26.
19. Sakata, Y. and Lester, R. N., Chloroplast DNA diversity in eggplant (*Solanum melongena*) and its related species *S. incanum* and *S. marginatum*. *Euphytica*, 1994, **80**, 1–4.
20. Sakata, Y. and Lester, R. N., Chloroplast DNA diversity in eggplant (*Solanum melongena*) and related species. *Euphytica*, 1997, **97**, 295–301.
21. Mace, E. S., Lester, R. N. and Gebhardt, C. G., AFLP analysis of genetic relationship among the cultivated eggplant. *Solanum melongena*; and its wild relatives (Solanaceae). *Theor. Appl. Genet*., 1999, **99**, 626–633.
22. Vavilov, N. I., The origin, variation, immunity and breeding of cultivated plants. *Chron. Bot*., 1951, **13**, 1–364.
23. Baksh, S., Cytogenetic studies of the F1 hybrid of *Solanum incanum* L. × *S. melongena* L. variety Giant of Banaras. *Euphytica*, 1979, **28**, 793–800.
24. Daunay, M. C., Lester, R. N. and Laterrot, H., The use of wild species for the genetic improvement of brinjal (*Solanum melongeana*) and tomato (*Lycopersicon esculentum*). In *Solanaceae III: Taxonomy, Chemistry, Evolution* (eds Hawkes, J. G., Lester, R. N., Nee, M. and Estrader, N.), Royal Botanic Garden, Kew, Richmond, UK, 1991, pp. 380–412.
25. Sambrook, J. E., Fritch, F. and Maniatis, T., *Molecular Cloning – A Laboratory Manual*, Cold Spring Harbor Laboratory, New York, USA, 2nd edn, 1989.
26. Jaccard, P., Nouvelles recherches sur la distribution florale. *Bull. Soc.Vaud. Sci. Nat*., 1908, **44**, 23–270.
27. Whalen, M. D., Speciation in Solanum, section Androceras. In *The Biology and Taxonomy of the Solanaceae* (eds Hawkes, J. G., Lester, R. N. and Skelding, A. D.), Linnean Society Symposium Series, London Academic Press, 1979, vol. 7, pp. 581–596.
28. Rodriguez, J. M., Berke, T., Engle, L. and Nienhuis, J., Variation among and within *Capsicum* species revealed by RAPD markers. *Theor. Appl. Genet*., 1999, **99**, 147–156.
29. El, Rabey, H. A., Badr, A., Schafer-Pregl, R. and Martin Salamini, W., Speciation and species separation in *Hordeum* L. (Poaceae) resolved by discontinuous markers. *Plant Biol*., 2002, **4**, 1–9.
30. Karihaloo, J. L., Brauner, S. and Gottlieb, L. D., Allozyme variation in the eggplant. *Solanum melongena* L. (Solanaceae). *Theor. Appl. Genet*., 1994, **90**, 578–883.
31. Decondolle, A., *Origin of cultivated plants*, London, 1904.

ACKNOWLEDGEMENTS. We thank the Indian Council of Agricultural Research for financial support. We acknowledge IIVR for providing necessary facilities.

Received 16 August 2005; revised accepted 19 December 2005

Reproductive biology of *Crataeva religiosa* Forst.

Shashi Bala Sharma, Anita Rana and S. V. S. Chauhan*

Department of Botany, School of Life Sciences, Dr B. R. Ambedkar University, Agra 282 002, India

***Crataeva religiosa* flowers profusely during March to May, when it sheds all its leaves. The flowers are large, hermaphrodite, actinomorphic and complete. Flowers open in the evening between 1900 and 2030 h followed by anther dehiscence at 1930–2100 h. Flowers offer pollen and nectar to the visitors, which include honey bees, moths, butterflies, bugs and birds. The plant is self-incompatible and obligate out-crosser. Fruit-set is restricted to only 22%. The beauty of the flowers as well as fruit production are adversely affected by the formation of floral galls induced by the insect, *Neolasioptera crataevae* Mani, order Diptera.**

Keywords: *Crataeva religiosa*, floral galls, *Neolasioptera crataevae*, reproductive biology.

CRATAEVA RELIGIOSA Forst. (family Capparidaceae) is a large tree distributed in the tropical zone and is common throughout India, Myanmar and Sri Lanka, either wild or cultivated[1]. It is cultivated in the gardens for its ornamental as well as medicinal value[2]. Despite its usefulness, less

*For correspondence. (e-mail: svs250@rediffmail.com)

attention has been paid to its floral biology, breeding systems and pollination mechanism. These are essential for the conservation, improvement and establishment of cultivation to increase the frequency of occurrence of this species. Keeping these facts in view, a detailed study on the reproductive biology of *C. religiosa* plants growing in different parts of Agra city has been made.

Flowering phenology was observed at plant and inflorescence level with reference to day-to-day flowering pattern in twenty-five marked plants. For the latter, 200 inflorescences, selected at random from different individuals were tagged before the initiation of flowering. These individuals were followed daily and the number of open flowers was recorded. The open inflorescences were then removed to avoid recounting the next day. The tagged inflorescences were followed until they ceased flowering. One hundred flowers were sampled to record the floral morphology and pollen characters. Anthesis, anther dehiscence and stigma receptivity were studied using various methods as described by Shivanna and Rangaswamy[3]. The number of pollen grains/anther/flower was determined from 25 flowers following Cruden[4]. Pollen size was measured with an ocular micrometer under light microscope following the procedure of McKone and Webb[5]. The number of pollen grains divided by the number of ovules per flower will yield the pollen–ovule ratio[4]. Pollen viability was assessed by hanging drop method after Brewbaker and Kwack[6]. *In vivo* pollen germination was checked by aniline blue fluorescence microscopic method as described by Shivanna and Rangaswamy[3]. Breeding behaviour by autogamy, geitonogamy and xenogamy was tested using controlled pollination studies. In order to observe the rate of natural fruit-set, two hundred inflorescences on different trees were tagged and were followed until fruit development. Foraging behaviour of insects and birds was recorded. They were observed with binoculars and photographed. Foraging activity during night was observed using a torchlight. Pollination efficiency of different bees was checked by observing the pollen load on different body parts under a microscope, according to the procedure given by Kearns and Inouye[7].

C. religiosa sheds all its leaves before the initiation of flowering (Figure 1 *a*). Flowering starts from 26 March and continues till the last week of May, with maximum bloom in April. A limited number of flowers continues to appear even during June and July. A flush of new trifoliate leaves appears with the cessation of flowering by the end of May. The inflorescence is a corymbose–raceme and consists of 25 ± 3.0 flowers (*R* 19–30; Table 1, Figure 1 *b*). Flowers are large, hermaphrodite, actinomorphic, hypogynous and complete (Table 1, Figure 1 *c*). The calyx is gamosepalous with four sepals, and corolla is polypetalous with the same number of petals that are arranged alternate to sepals (Table 1). Stamens are numerous, as many as 25 ± 1.5 (*R* 18–31) (Table 1). They are polyandrous, adnate to the base of the gynophore, longer than the petals, bicelled and introrse. Single pistil is differentiated into stigma and ovary. Stigma is capitate, dry and non-papillate (Table 1). The ovary is bicarpellary, syncarpous (Table 1) and elevated on a long stalk called gynophore. The number of ovules/ovary is 90 ± 9.7 (*R* 76–106), lying on two parietal placentae (Table 1).

The flowers open between 1900 and 2030 h (Table 1). Opening of the flowers is confined to the hours of darkness. Nectary is present at the base of the gynophore. Anthers dehisce by longitudinal slit around 1930–2100 h. Pollen grains are spherical, tricolpate with reticulate exine and 23.5 μm in diameter. The mean number of pollen grains per anther is 3510 and per flower is 87,750. The ratio of pollen grains to ovule number is 975 : 1. The stigma becomes receptive after anther dehiscence around 2200 h and remains so until the late morning (0800–0930 h) of the next day. *In vitro* pollen germination studies indicate that the pollen grains remain viable for 16 h after anther dehiscence. At the time of anther dehiscence, 94% of the pollen grains are viable. However, there is substantial reduction in pollen viability after 10 h (50%) up to 16 h (3%) (Table 2). *In vivo* pollen germination studies show 58% pollen germination after 10 h (Figure 1 *f*) and germination percentage is only 7% after 16 h on the stigmatic surface (Table 2).

Each flower lasts for two days. At initial bud stage petals are green in colour, but acquire white colour at late bud

Table 1. Floral characters of *Crataeva religiosa*

Floral character	Observation
Inflorescence	Corymbose-raceme
No. of flowers/inflorescence	25 ± 3.0
Flower	Hermaphrodite, actinomorphic, complete
No. of stamens	25 ± 1.5
Calyx	Gamosepalous with four sepals
Corolla	Polypetalous with four petals
Time of flower opening	1900–2030 h
Time of anther dehiscence	1930–2100 h
Mode of anther dehiscence	Longitudinal slit
Pollen grains/anther	3510
Pollen grains/flower	87,750
Pollen size	23.5 μm
Stigma type	Capitate, dry and non-papillate
Ovary type	Bicarpellary and syncarpous
No. of ovules/ovary	90 ± 9.7

Table 2. Pollen germination at different time intervals after anther dehiscence

Hours after anther dehiscence	*In vitro* pollen germination (%)	*In vivo* pollen germination (%)
10	50 ± 0.5	58 ± 0.3
12	30 ± 1.4	35 ± 1.2
14	12 ± 2.62	18 ± 2.50
16	3 ± 0.92	7 ± 0.76

±, Standard deviation.

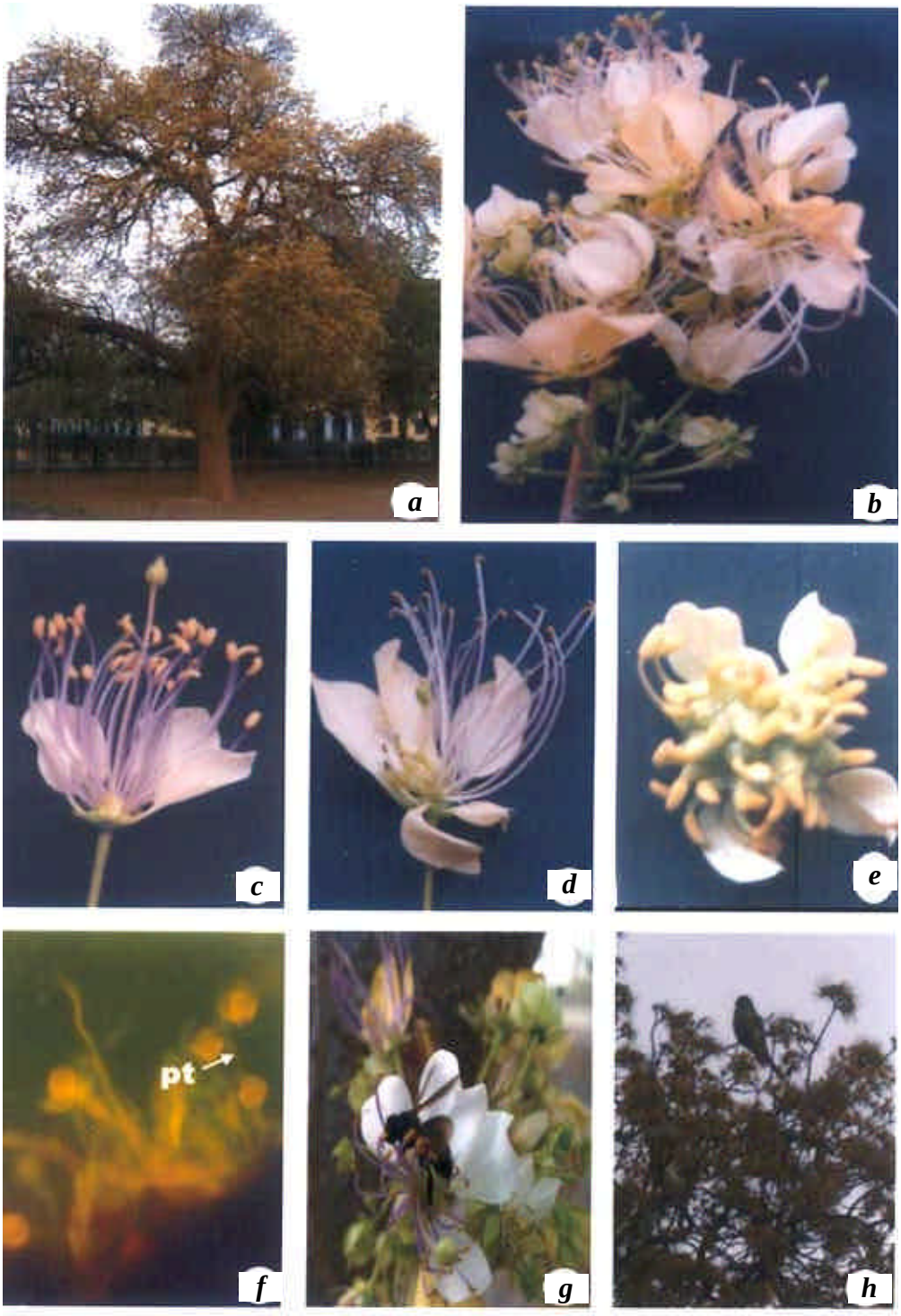

Figure 1. *Crataeva religiosa*. ***a***, Tree in flowering; ***b***, Flowering branch; ***c***, Normal flower; ***d***, Partially infested flower; ***e***, Fully infested flower; ***f***, *In vivo* pollen germination on stigmatic surface showing pollen tubes (pt); ***g***, *Apis dorsata*; ***h***, *Psittacula krameri*.

stages. At the time of anthesis petals remain white, but in the morning of the next day they gradually turn light yellow and finally become dark yellow by the evening. The next morning, the petals begin to wither and by the evening petals and anthers from pollinated flowers drop-off, leaving only the sepals and pistil. Change in floral colour in *Helicteres isora* is well known, as the colour changes from bluish-grey to dark red[8].

The beauty of flowers as well as fruit production is adversely affected by the formation of floral galls induced by

the insect, *Neolasioptera crataevae* Mani, order Diptera. Galls start developing a few days after the initiation of flowering. Their number increases with increase in floral density and then declines with rise in temperature. Percentage of gall formation is low in young plants, but higher in older plants. Galls usually develop from meristematic and young tissues of the thalamus and grow towards the base of the anther filament and gynophore, which become bulbous. Infestation of the insect on a single flower is usually not uniform as is evident by the presence of normal, semi-infested and fully infested floral parts in the same flower. Quite often, the whole flower gives rise to an irregular mass somewhat depressed above and funnel-shaped at the base. During gall formation, floral components are highly modified. Those near the galls are severely affected, whereas the ones away from the zone of infection tend to be normal. Sokhi and Kapil[9,10] have also reported morphological changes in the flowers of *Terminalia arjuna* induced by the insect *Trioza*.

Fruiting starts in the second week of April and fruits begin to mature in the last week of May. Mature fruits start to disperse their seeds during the second week of June. Natural fruit-set is 22%. A sample of 200 inflorescences consisting of 5024 flowers, selected at random on different trees at flower stages was used for estimating fruit and seed-set rate. An inflorescence consists of 25 ± 0.5 flowers; among them 18 ± 1.2 are normal and 7 ± 0.5 are infested. Thus, among 5024 flowers, 3641 are normal and 1383 are infested. Among normal flowers, 897 flowers with 80,730 ovules set fruits with 50,456 seeds. Seed-set is 62%.

Among the infested flowers, 40% is partially infested (Figure 1 *d*) and 60% fully infested (Figure 1 *e*). None of these produces fruits. Thus, among all the flowers on a tree, 75% is normal and 25% infested. Among the 25%, 10% is partially infested and 15% fully infested. They are not visited by pollinators. Considering the total flower investment as 100%, the species loses 25% to infestation and another 53% to flower abortion, leaving just 22% for natural fruit-set.

Flowers are cross-pollinated as confirmed by various hand-pollination experiments. The fruit-set percentage through xenogamy is 92 and there is no fruit-set by other modes of pollination (Table 3).

The flowers are visited by honey bees (*Apis dorsata*; Figure 1 *g*, *A. indica* and *A. florae*), moths (*Achoria grisella*), butterflies (*Pieris brassicae* and *Danaus plexippus*), wasps (*Polistes hebraeus* and *Vespa* sp.), bugs (*Bagrada cruciferarum*) and birds, including sun birds (*Nectarinia asiatica*), parrot (*Psittacula krameri*; Figure 1 *h*), koel (*Eudynamys scolopacea*) and pigeon (*Columba livia*). They visit the flowers between 1900 and 0900 h for nectar and/or pollen. Of the total visits, honey bees made 61%, moths 15%, butterflies 8%, birds 7%, wasps 6% and bugs 3%. Among honey bees, *A. dorsata* forage during 1900–0900 h, whereas the other two species during 0500–0900 h. Honey bees forage for both nectar and pollen. Moths forage after anthesis. They are attracted by the white colour and heavy fragrance of the flowers. They forage during 1900–0100 h for pollen and nectar. Butterflies, wasps and bugs forage during 0500–0900 h for nectar only. All insects are pollen carriers and their frequent inter-plant movement facilitates cross-pollination. Birds forage during 0500–0900 h for nectar and for eating floral parts and fruits. Sun birds forage largely for nectar, whereas other birds visit the flowers for nectar and for eating floral parts and fruits. While collecting nectar, all these birds contact the stamens and stigma with their bill and forehead, both ventrally and dorsally and their frequent movements among the conspecific trees facilitate cross-pollination.

Observations of different body parts indicate that *A. dorsata* specimens have a higher amount of pollen grains on their body than other species (Table 4). It is the most dominant in number and visits the flowers soon after anthesis at night, showing brisk activity up to 0730 h. Later its activity declines and disappears at around 0900 h. This suggests that *A. dorsata* is capable of collecting forage during moon night hours. This bee is intolerant to excessively high temperatures and has thus developed adaptations to forage during night under moonlight. Rao and Raju[11] have reported *A. dorsata* activity during night under moonlight on the flowers of *Pterocarpus santalinus* during March–May, to avoid excessively high temperature during daytime. The other honey bees too restrict their foraging schedules to the early morning time. Therefore, activities of pollinators are related to the ambient temperatures. Their activities are being limited by low temperatures in temperate climates and high temperatures in tropical climates.

Obligate outbreeding is the predominant reproductive strategy in tropical ecosystems, either through self-incompatibility mechanisms or various forms of functional dioecy.

Table 3. Results of breeding systems in *C. religiosa*

Treatments	No. of flowers pollinated	No. of fruits	Fruit-set percentage
Autogamy	50	0	0
Geitonogamy	50	0	0
Xenogamy	50	46	92

Table 4. Pollen pick-up by bees on *C. religiosa* as revealed by observing pollen on different body parts

Visitor species	Sample size	Pollen grains	
		Range	Mean
Apis dorsata	10	420–510	448 ± 345
A. indica	10	350–380	367 ± 310
A. florea	10	200–240	214 ± 186

±, Standard deviation.

Most of the *Acacia* species in the tropical ecosystems are found to be self-incompatible and out-crossing. *Acacia sinulata* exhibits self-incompatibility, as indicated by Raju and Rao[12]. Hand pollination experiments show that *C. religiosa* is a self-incompatible, obligate out-crosser. This breeding system is functional only when conspecifics bloom simultaneously and occur nearby, and requires vectors to mediate pollen among conspecific plants.

In *C. religiosa*, the natural fruit-set is low when compared to the high flower production. Different factors could affect fruit-set. First is the floral galls formation, which leads to loss of productivity. Secondly, the intensive pollen collecting behaviour of attending bees and their tendency to confine to the same plant that they first forage may result in more wasteful self-pollen transfer.

C. religiosa is of ornamental as well as medicinal importance and is an obligate out-crosser. For higher fruit-set, conspecific trees should be planted nearby and remedial measures should be adopted to combat infection of floral parts.

1. Parker, R. N., *Forty Common Indian Trees and How to Know Them*, A.A. Vishhkar Publ., Jaipur, 1986.
2. Anon., *The Wealth of India*, 1959, vol. 5, pp. 318–319.
3. Shivanna, K. R. and Rangaswamy, N. S., *Pollen Biology: A Laboratory Manual*, Narosa Publishing House, New Delhi, 1992.
4. Cruden, R. W., Pollen–ovule ratios, a conservative indicator of breeding systems in flowering plants. *Evolution*, 1977, **31**, 32–46.
5. McKone, M. J. and Webb, C. J., A difference in pollen size between the male and hermaphrodite flower of two species of *Apiaceae*. *Aust. J. Bot.*, 1988, **36**, 331–337.
6. Brewbaker, J. L. and Kwack, B. H., The essential role of calcium ion in pollen germination and pollen tube growth. *Am. J. Bot.*, 1963, **50**, 859–865.
7. Kearns, C. A. and Inouye, D. W., *Techniques for Pollination Biologist*, University Press of Colorado, Niwot, Colorado, USA, 1993.
8. Atluri, J. B., Rao, S. P. and Reddi, C. S., Pollination ecology of *Helicteres isora*. *Curr. Sci*., 2000, **78**, 713–718.
9. Sokhi, J. and Kapil, R. N., Morphogenetic changes induced by *Trioza* in the flowers of *Terminalia arjuna* – I, Androecium. *Phytomorphology*, 1984, **34**, 117–128.
10. Sokhi, J. and Kapil, R. N., Morphogenetic changes induced by *Trioza* in the flowers of *Terminalia arjuna* – II, Gynoecium. *Phytomorphology*, 1985, **35**, 69–82.
11. Rao, S. P. and Raju, A. J. S., Pollination ecology of the Red Sanders *Pterocarpus santalinus* (Fabaceae), an endemic and endangered tree species. *Curr. Sci*., 2002, **83**, 1144–1148.
12. Raju, A. J. S. and Rao, S. P., Pollination ecology and fruiting behaviour in *Acacia sinulata* (Mimosaceae), a valuable non-timber forest plant species. *Curr. Sci.*, 2002, **82**, 1466–1471.

Received 22 July 2005; revised accepted 22 November 2005

Histological observations on the scleractinian coral *Porites lutea* affected by pink-line syndrome

J. Ravindran[1,*] and Chandralatha Raghukumar[2]

[1]National Institute of Oceanography, Regional Centre, Cochin 682 018, India
[2]National Institute of Oceanography, Dona Paula, Goa 403 004, India

A pink-line syndrome (PLS) was reported in the reef building coral *Porites lutea* in Kavaratti island of the Lakshadweep archipelago. The affected corals had dead patches colonized by a cyanobacterium *Phormidium valderianum* and the bordering coral tissue was pink. We examined the histological changes associated with the PLS-affected tissue. Results showed that the zooxanthellae were released from the gastrodermal cells into the coelenteron. Gastrodermal cells undergo necrosis and detachment from the basal membrane. The basic staining of the cytoplasm in the gastrodermal cells bordering the calicoblastic layer suggests accumulation of calcium ions. The ectodermal epithelium and calicoblastic cells showed destruction through 'apoptosis-like' processes. Cell swelling and vacuolation were observed in the gastrodermal and ectodermal cells. In this communication we discuss how the presence of the cyanobacterium adjacent to the PLS-affected tissue could cause the observed damage, bring about imbalance and a shift in the coral-zooxanthellae symbiosis.

Keywords: Cellular changes, pink-line syndrome, *Porites lutea*, symbiosis, zooxanthellae.

EXISTENCE of corals is threatened by environmental changes, pollution and direct human interference in many coral reefs around the world. Diseases bring about destruction of corals to a greater extent than other factors. Investigations on diseases that help identify the factors which cause and manifest the disease will help in the management of diseases[1]. We earlier reported partial mortality in the massive coral *Porites lutea* affected by pink-line syndrome (PLS)[2,3]. Fungi and a cyanobacterium *Phormidium valderianum* were isolated from the PLS-affected specimens. The cyanobacterium was identified as the etiological agent in the PLS through Koch's postulate experiments[3,4]. This communication reports on the cellular changes associated with the PLS-affected corals and the interaction between the host tissue and associated organism.

Coral specimens of *P. lutea* affected by PLS and healthy ones were collected from a lagoon in Kavaratti island and were transported in sea water to the field laboratory. These specimens were fixed and preserved following the standardized method[4]. Healthy and PLS-affected specimens preserved in 70% ethanol were cut into small pieces of

*For correspondence. (e-mail: jravi@nio.org)