Phyton (Horn, Austria)	**Vol. 39**	Fasc. 2	239–249	28. 12. 1999

Histopathology of Floral Organs of *Mangifera indica* L. (*Anacardiaceae*) as Affected by *Fusarium moniliforme* J. Sheld.

By

A. C. M. Koti Babu*) and K. S. Rao*)

With 2 Figures

Received November 11, 1998

Accepted April 13, 1999

Key words: *Fusarium moniliforme, Mangifera indica*, anther and ovary.

Summary

Koti Babu A. C. M. & Rao K. S. 1999. Histopathology of floral organs of *Mangifera indica* L. (*Anacardiaceae*) as affected by *Fusarium moniloforme* J. Sheld. – Phyton (Horn, Austria) 39 (2): 239–249, with 2 figures. – English with German summary.

The hyphal filaments of *Fusarium moniliforme* J. Sheld were found closely associated with the essential organs of flowers from malformed panicles of five different varieties of mango (*Mangifera indica*) viz. Rajapuri, Kesar, Dadamio, Langra and Amrapali. The pathogen association with floral organs was noticed at a very young stages of bud development. The fungus often occurred in the form of a tuft or thick mat of mycelia in mature buds. In the open flowers of malformed panicles thick mycelial mat was often noticed in the basal groove of two anther lobes where the filament connected. In the pistil the mycelial mat was found along the stylar transmitting tissue and in the ovular chamber. The interaction of pathogen with the pistil and anther at initial stages of their development may lead to the development of malformed panicles. The close association of fungus with the reproductive organs appeared to disrupt the pollination and fertilization mechanisms leading to poor fruit-setting in the malformed panicles.

Zusammenfassung

Koti Babu A. C. M. & Rao K. S. 1999. Histopathologie von mit *Fusarium moniliforme* J. Sheld. befallenen Blütenorganen bei *Mangifera indica* L. (*Anacardiaceae*).

*) A. C. M. Koti Babu, K. S. Rao, Department of Biosciences, Sardar Patel University, Vallabh Vidhyanagar, India.

– Phyton (Horn, Austria) 39 (2): 239–249, 2 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Es wurde beobachtet, daß die Hyphen von *Fusarium moniliforme* J. SHELD in enger Verbindung mit den wichtigen Blütenorganen von mißgestalteten Rispen von 5 verschiedenen Variätäten von Mango (*Mangifera indica*) nämlich Rajapuri, Kesar, Dadamio, Langra und Amrapali stehen. Die Vergesellschaftung von Blütenorganen mit dem Pathogen wurde bereits in sehr jungen Stadien der Knospenentwicklung beobachtet. Der Pilz erscheint oft als ein büscheliges oder dichtes Mycelgeflecht in reifen Knospen. In den offenen Blüten der mißgestalteten Rispe wurde oft dickes Mycelgeflecht in der Basalfurche von 2 Antherenlappen, wo die Filamente miteinander verbunden sind, beobachtet. Im Stempel wurde das Mycelgeflecht im Transmissionsgewebe des Griffels und in der Ovarhöhle gefunden. In den Anfangsstadien ihrer Entwicklung kann die Wechselbeziehung zwischen Pathogen und Stempel sowie Antheren zur Bildung mißgestalteter Rispen führen. Die enge Vergesellschaftung von Pilz und reproduzierenden Organen scheint die Pollenbildung und die Befruchtungsmechanismen massiv zu stören, was zu einem dürftigen Fruchtansatz in den mißgestalteten Rispen führt.

Introduction

Malformation is considered to be one of the most serious and widespread diseases of mango (*Mangifera indica* L.) affecting vegetative and floral shoots considerably. The etiology of mango malformation is controversial. The disease may be mediated by various organisms like virus, fungus, mite etc. or caused by a physiological disorder (KAUSER 1959, NARIANA & SETH 1962, SINGH & DHILLON 1989).

SUMMANWAR & al. 1966 were the first to report *Fusarium moniliforme* sheld as the causal agent of the disease and now there is a general agreement that fungus, *Fusarium moniliforme* sheld var subgluatinans is the causal agent of the disease (MANICOM 1989, VARMA & al. 1972, 1974). On the other hand, very little is known about the epidemiology of mango malformation (MANICOM 1989, KUMAR & al. 1993). Floral malformation can be induced when apical buds are wounded and artificially inoculated with *F. subglutinans* but symptoms may take six months to develop and disease incidence is often low. It is not clear whether these results reflect inefficient or non-aggressive behaviour of the fungus (PLOETZ 1994). Our knowledge on functional nature of essential organs of flowers is meagre (MALLIK 1963, SHAWKY & al. 1980). This is due to the lack of detailed studies on the histology of the diseased organs. This paper, therefore, reports the occurrence of mycelial aggregates (mat) with essential organs and the interaction of causal agent on the functional status of the anther and pistil of malformed flowers of five varieties of *Mangifera indica*.

Materials and Methods

Young floral buds at various stages of their development and open flowers were collected from malformed and healthy panicles of *Mangifera indica* L. for a period of three floral seasons (1995–97). The samples were collected from var. *Rajapuri, Am-*

rapali, Kesar, Dadamio and *Langra* growing in central fruit Nursery, Baroda. The collection was made from December to February (floral season) during morning hours. The excised samples were immediately fixed in FPA (Formaldehyde Propionic acid – Alcohol) (DRING 1971). The anthers and ovaries separated from open flowers and floral buds were processed for routine paraffin embedding (BERLYN & MIKSCHE 1976). Longitudinal serial sections of 8–10 µm thickness were taken using a Leica 2035 rotary microtome. The sections were then stained with periodic schiff=s reagent combination (DRING 1971). Dimensions of buds, flowers, anthers and ovary were measured under microscope with the help of a ocular micrometer. For each parameter hundred readings were taken and standard deviation was calculated from the average. The fungus isolated from buds, anthers and ovary was identified as *Fusarium moniliforme* by IARI, New Delhi (Anthers – 2274.96 to 2277.96 and ovary 2278.96).

Results

The apical floral buds giving rise to malformed panicles appear distinct from those producing healthy panicles. These buds are larger in size and often covered with dark-brown resinous substances. Even during the early stages of their development, malformed panicles could be easily identified by their thick inflorescence axis surrounded by a large number of buds. The malformation symptoms appear more distinct with further development of the inflorescence with buds appearing larger in size and becoming crowded around the hypertrophied axis of the panicle. The size of the buds and flowers is larger as a result of enlargement of sepals, petals, anthers, ovary and gland (Table 1).

Table 1

Dimensional size changes of buds, flowers and floral parts between malformed and healthy panicles of *M. Indica* var. Rajapuri

	Malformed	Healthy
1. Young buds	0.72 mm ± 0.02	0.60 mm ± 0.02
2. Mature buds	3 mm ± 0.23	1.55 mm ± 0.18
3. Open flowers	4.25 mm ± 0.46	3.57 mm ± 0.25
4. Anthers	2.25 mm ± 0.35	2.00 mm ± 0.17
5. Anther cuticle thickness	11.36 µm ± 3.52	3.52 µm ± 1.28
6. Ovary	2.1 mm ± 0.35	1.1 mm ± 0.20

The sections of samples collected from malformed panicles reveal the close association of fungus with the organs of young and mature floral buds and with the essential organs of open flowers.

Young buds : Longitudinal sections of young buds measuring about 0.7 mm in diameter (Table 1) from malformed inflorescence have revealed association of hyphal filaments with all the floral organs. Mycelial fragments are often found in the narrow space between the overlapping sepals and petals. In resin embedded semithin sections mycelial mat appears as a pile or row of hyphae or microconidia of *Fusarium moniliforme*. Hyphal filaments of the pathogen have also been encountered in the ovary and anther wall (Fig. 1A). Mycelia often appear in the anther locule at microspore mother cell phase. The entry of the pathogen into these buds appears to be followed by necrosis of sepals and petals. The hyphae are also noticed attached to the ovary wall and ovular chamber at the stage.

Young buds of healthy panicles measure comparatively less in size (Table 1) than those of malformed ones and are free of pathogen association.

Mature Buds : The buds of Rajapuri cultivar collected from fully developed malformed panicles measure nearly twice in size compared to those of healthy panicles (Table 1). The mycelial filaments are prominent and often appear in between sepals and petals. Hyphae are also noticed in close association with the surface of the ovary and gland. Anthers are tetralocular and dorsifixed with a filament that narrows at the point of attachment with the anther. The cuticle covering the epidermal layer of anther is thicker and grooved.

A thick mat (network) of *Fusarium* hyphae is consistently noticed in the basal groove of anther lobe where the filament is connected (Fig. 1B, C). In a few instances fusarial mat appears close to the disorganised filament (Fig. 1D). The fungus seems to enter into the anther locule from the base of the anther lobes through the epidermal cells (Fig. 1E). The cells at

---

Fig. 1. (A–H) Longitudinal sections of mango flowers of var. Rajapuri (A–E) and Dadamio (F–H) from malformed panicles.

A. Young monoecious bud showing close assocaition of hyphal filaments of *Fusarium* with anthers (arrow) and ovary (arrow head).

B. Mature anthers showing attachment of thick darkly stained mycelial mat of *Fusarium* in the basal groove of anther lobes (arrows).

C. A thick mat of *Fusarium* (arrow) in close association with basal portion of anther lobes.

D. Basal portion of mature anther showing disorganised portion of filament (arrow head) and mycelial mat attached in the anther groove.

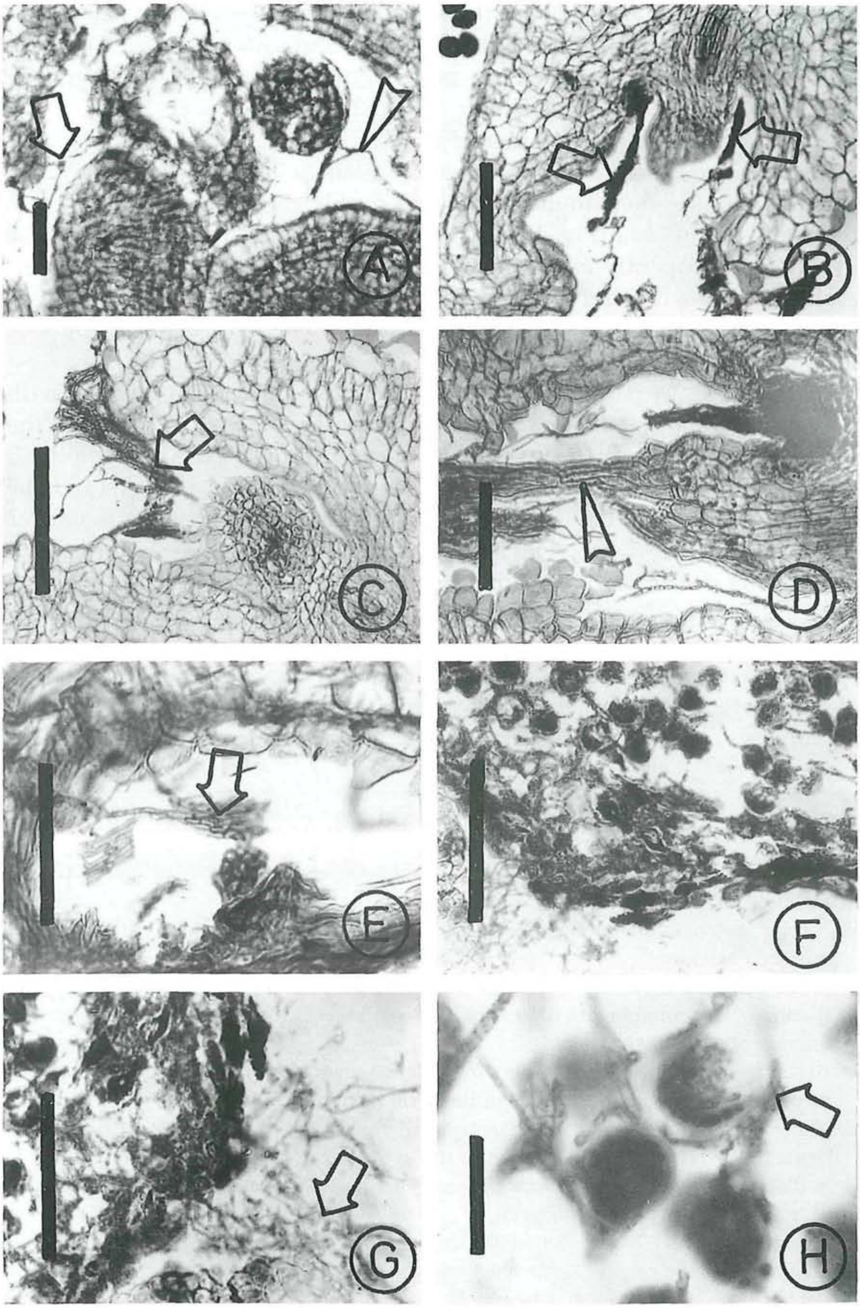
E. An anther locule showing filaments and mat of *Fusarium moniliforme* (arrow).

F. One of the anther lobes showing association of fungal hyphae with the pollen grains.

G. Part of the anther locule with fungus in association with pollen grains. Note the tuft of mycelium at anther-filament attachment (arrow head).

H. Pollen grains with attached fungal hyphae (arrow) in the anther locule.

Fig. 1 A–G Scale bar = 100 µm, H Scale bar = 30 µm


A
B
C
D
E
F
G
H

the base of anther lobes appear thick walled and filled with phenolic contents. The pollen grains are usually round and exhibit thin cell walls. However, in the variety Langra, pollen grains appear oval-eliptical in shape.

The severely infected anther of the varieties studied show loss of cell identity in the tapetal layer followed by phenolic accumulation. Such infected anther locule is found filled with darkly stained pollen grains and fragments of mycelia (Fig. 1F and G). Pollen grains with attached fungal hyphae are frequently found in the locule (Fig. 1H). In some cases pollen grains appear with thin wavy walls and empty due to plasmolysed cytoplasm (Fig. 2A) in comparison to healthy pollen with dense cytoplasm (Fig. 2B).

In the healthy panicles anthers are free from fusarial association with no lesions or trace of phenolic accumulations. The tapetal cells around the anther locule appear distinct and the pollen grains are usually round in all the varieties. The ovary with a laterally attached style is sessile and the anatropous ovule is arranged with basal placentation. The style is slender and short with a bifid stigma. Stigma consists of a narrow opening which is 4–5 cells deep. The longitudinal sections passing through style reveal that it is continuous with the ovary wall to the ovular chamber with a few resin ducts along the transmitting tissue. Moreover, it is also noticed that lesions occur along the transmitting tissue of the style (Fig. 2C) leading to the disruption of cells. This leads to the formation of a canal in the style opening into the ovular chamber. The fungus in the form of filaments and

---

Fig. 2. (A–H) Longitudinal sections of flowers of healthy (B) and malformed (A and C to H) panicles of mango varieties.

A. Anther locule of var. Dadamio with aborted pollen grains (arrow) and necrotic cell walls.

B. *Fusarium* free anther locule showing pollen grains with dense cytoplasm in the variety Dadamio.

C. A distinct lesion (arrow) in the transmitting tissue of the style of var. Kesar indicating the probable site of entry of the pathogen. Note the bifid stigma with a narrow opening (arrow head).

D. Stylar tissue of var. Rajapuri showing fusarial mat (arrow). Note the accumulation of phenolic contents in the cells of the style (arrow head).

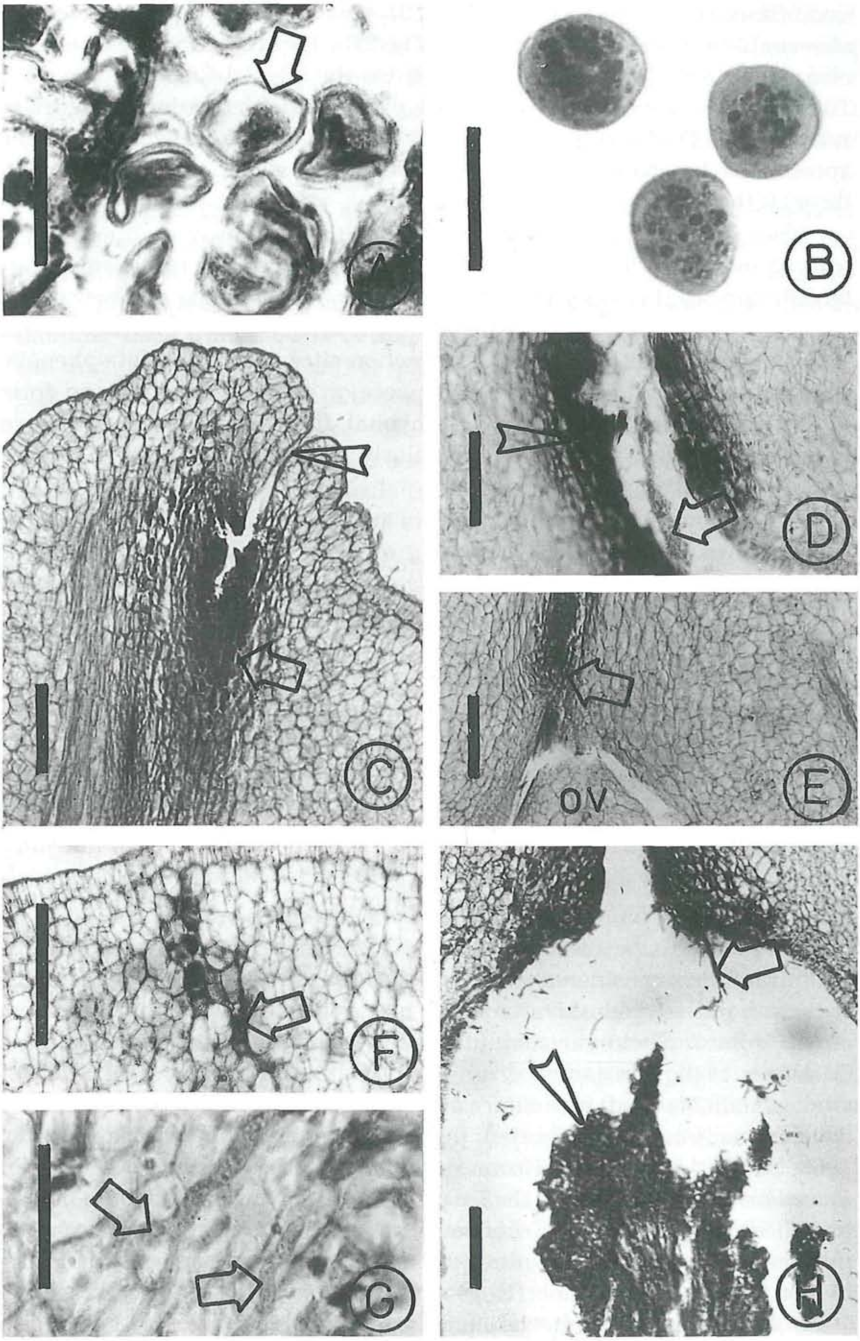
E. The continuation of style in the ovary wall close to ovular chamber with a part of ovule (OV) showing lesion (arrow) in the ovary of variety Kesar.

F. A distinct lesion (arrow) across the ovary wall of var. Kesar indicating the probable site of entry of the pathogen.

G. Ovary wall of var. Kesar showing intracellular hyphae (arrows).

H. Ovular chamber of var. Kesar showing an aborted ovule (arrow head) with dark contents. Note the mycelial filaments emerging from the inner ovary wall layers (arrow) surrounded by cells containing phenolic contents.

Fig. 2 A, B, G Scale bar = 30 μm, C–F, H Scale bar = 100 μm.


A
B
C
D
E
OV
F
G
H

mat often occur along the canal (Fig. 2D) surrounded by lesions which are also continuous till ovular chamber (Fig. 2E). The ovary wall besides style also consists of lesions characterized by the phenolic containing cells. These lesions often appear across the ovary wall from outer to the inner surface (Fig. 2F). Thus the invasion of *Fusarium moniliforme* into the ovule appears to be mainly occurring through the stylar tissue or directly through the ovary wall.

The ovule is invaded by the pathogen either via micropylar end or the wall of the ovule. The mycelium of *Fusarium* is noticed proliferating intercellularly in the ovary wall (Fig. 2G). Hyphal filaments and mat of the pathogen are also found free in the ovular chamber and attached to the wall surrounding it (Fig. 2H). The infection sites too accumulate phenolic contents either in the intercellular spaces or entire cell lumen and form lesions which are associated with hyphal filaments. Severely infected flowers are noticed containing irregularly shaped ovule filled with dark contents (Fig. 2H).

Open Flowers : Malformed flowers are usually appear larger than the healthy ones (Table 1). The blooming of buds of malformed panicles is much delayed and only a few flowers open even in the mature panicles. The open flowers also show similar mode of distribution of fungus in the essential organs as described in mature buds. However, open flowers from malformed as well as healthy panicles generally show dehisced anthers with a few pollen grains. The anther locules in the flowers of malformed inflorescence are filled with necrotic cells and extensive deposits of phenolic contents and fusarial mat. Heavy deposition of phenolic contents along with fusarial mat, conidiophores and microconidia are also found associated with the stylar tissue, ovary wall and ovule.

Discussion

Compared to various other diseases infecting *Magifera indica*, a floral malformation is considered to be a serious disease as it directly leads to the reduction in the yield of fruits. It has now been considered that *Fusarium moniliforme* J. Sheld var subglutinans is the causal agent of the disease (MANICOM 1989, SUMMANVAR & al. 1966, VARMA & al. 1972, 1974). Earlier studies indicate that in malformed panicles the hermaphrodite flowers develop nonfunctional aborted pistil and viable pollengrains (MALLIK 1963, SHAWKY & al. 1980). However, detailed studies on the association of *Fusarium moniliforme* with the essential floral organs of *Mangifera indica* have been not made. Our earlier results suggest that entry of the pathogen into the host terminal buds occur as early as in November i.e. few months before floral bud inception (KOTIBABU & RAO 1998). Accordingly present study reveals the close association of *Fusarium moniliforme* with youngest unopen buds.

The infection sites of the host such as basal portion of anthers, the tip of anthers filament, anther wall and the stylar tissue of the ovary are accumulated with phenolic contents. Such accumulation is a result of host species hypersensitive reaction producing antagonistic substances (e.g. phytoalexins) to delimit the growth of the pathogen (GHOSAL & CHAKRABARTI 1988).

The phenolic contents accumulated at these infection sites may represent mangiferin, a phenolic metabolite, which accumulates at high concentrations in the malformed shoots of *M. indica*. Mangiferin is known to inhibit the ingress of *Fusarium moniliforme* into the host and also immobilize itself from the site of infection (CHAKRABARTI & al. 1990). Further the thick cell wall of the anther might certainly offer itself a physical barrier against the attacking pathogen. But the thick mycelial mat of *Fusarium* perhaps would produce sufficient concentration of enzymes which would dissolve the thick anther walls at the basal groove. Thus the fungus seems to enter into anther locule from base of the anther lobes where the filament is attached.

Moreover, the phenolics compounds accumulated in the malformed essential organs might be insufficient to combat the invading pathogen and the necrotic cells in these organs enhance the immobilization potential of mangiferin. Thus mangiferin mediated transport of micronutrients to developing organs becomes disrupted and affects the normal growth of the malformed panicles (CHAKRABARTI & al. 1990). Interestingly the infection sites of malformed flowers, anthers and style chosen by the pathogen are anatomically weak barriers enabling its luxuriant growth and establishment. The basal groove formed by the anther lobes and the stylar transmitting tissue might be the probable entry zones of the pathogen into the essential organs of *M. indica*.

Invasion of the pathogen into essential organs and its feasible growth in them is not reported earlier. The mode of infection of *F. moniliforme* in panicles of *M. indica* via terminal buds (meristems) – young floral buds – anthers and ovary as suggested in the present study gives a possible evidence of the epidemiology and etiology of the mango malformation disease. *F. moniliforme* entry directly into the host crossing the physical and chemical barriers such as anther and ovary wall and the phenolic accumulation respectively and gaining intimate association with pollen and pistil suggests the virulent activity of the fungus. Such virulent nature of this strain of fungus is due to the toxic substances like carotenoid entities (Violaxanthin and Zeaxanthin) produced by it (GHOSAL & al. 1979). Similarly the thick mat of mycelium in the anthers as well as ovary might release such toxic substances which result in non-functional or abortive nature of pollen and pistil in the affected flowers. The close and direct association of fungus with the male and female reproductive organs of flowers might lead to the development of non-viable gametes, thus disturbing the fertilization process whereby fruit setting is hindered in malformed panicles.

Acknowledgement

This work was carried out under the Indian Council of Agricultural Research (ICAR), New Delhi funded research project "Histological, histochemical and pathological studies on mango malformation" sanctioned to one of us (KSR).

References

BERLYN G. P. & MIKSCHE J. P. 1976. Botanical microtechnique and cytochemistry. – Iowa State University Press, Ames, Iowa.

CHAKRABARTI D. K., SINGH A. & SINGH K. 1990. Physiological and biochemical changes induced by accumulated mangiferin in *Mangifera indica*. – J. Hort. Sci. 65: 731–737.

DRING D. M. 1971. Techniques for microscopic preparation. – In: Methods in Microbiology; Vol. 4 pp. 95–111 ed. C. BOOTH (Academic Press) London, U.K.

GHOSAL S. & CHAKRABARTI D. K. 1988. Differences in phenolic and steroidal constituents between healthy and infected florets of *Mangifera indica*. – Phytochemistry 27: 1339–1343.

— , — , BISWAS K. & KUMAR Y. 1979. Toxic substances produced by *Fusarium*. X. concerning the malformation disease of mango. – Experientia 35: 1633–1634.

KAUSER A. G. 1959. Malformation of inflorescences in mango. – Punjab Fruit J. 22: 19–21.

KOTIBABU A. C. M. & RAO K. S. 1998. Mango malformation: Histopathological and histochemical studies on shoot and floral apices. – Indian phytopathol. 51: 349–352.

KUMAR J., SINGH U. S. & BENIWAL S. P. S. 1993. Mango malformation: One hundred years of research. – Annu. Rev. Phytopathol. 31: 217–232.

MALLIK P. C. 1963. Mango malformation. Symptoms, causes and cure. – Punjab Hort. J. 3: 292–299.

MANICOM B. Q. 1989. Blossom malformation of mango. – S. Afr. Mango Growers Assoc. Yearb. 10: 11–12.

NARIANA T. K. & SETH M. L. 1962. Role of eriophyes mites in causing malformation disease in mango. – Indian phytopathol. 15: 231–233.

PLOETZ R. C. 1994. Distribution and prevalence of *Fusarium subglutinans* in mango trees affected by malformation. – Can. J. Bot. 72: 7–9.

SHAWKY I., ZIDAN Z., EL-TONI A. & DAHSHAN D. I. 1980. Flowering malformation in relation to vegetative growth of Taimour mangoes. – Egypt. J. Hort. 7: 1–8.

SINGH Z. & DHILLON B. S. 1989. Hormonal changes associated with vegetative malformation of mango (*Mangifera indica*). – J. Phytopathol. 125: 193–197.

SUMMANWAR A. S., RAYCHAUDHURI S. P. & PHATAK S. C. 1966. Association of the fungus *Fusarium moniliforme* sheld with the malformation in mango (*Mangifera indica* L.). – Indian Phytopathol. 19: 227–228.

VARMA A., RAYCHAUDHURI S. P., LELE V. C. & RAM A. 1972. Towards the understanding of the problem of mango malformation. – Proc. Indian Natl. Sci. Acad. 37: 291–300.

— , LELE V. C., RAYCHAUDHURI S. P., RAM A. & SANG A. 1974. Mango malformation: A fungal disease. – Phytopathol. Z. 79: 254–257.

Phyton (Horn, Austria) 39 (2): 249–250 (1999)

Recensio

WAGENITZ Gerhard 1996. Wörterbuch der Botanik. Fortsetzung von p. 238.

Enantiostylie: G: ältester mir bekannter Gebrauch bei TAUBERT 1894 in ENGLER & PRANTL, Natürl. Pflanzenfam. 3(3):93 ! Die Arbeiten von H. MÜLLER 1883 (Arbeitstheilung bei Staubgefässen von Pollenblumen in Kosmos. Z. einheitl. Weltansch. Entwicklungsl., 7. Jahrg., Bd. 13: 241–259) und ROBERTSON 1890 (Flowers and insects in Bot. Gaz. 15(4): 79–204 [darin zitiert: TODD 1882, On the flowers of *Solanum rostratum* and *Cassia chamaecrista*, American Naturalist 16(4): 281–287]) sind hier – sowie in C. K. SCHNEIDER & O. PORSCH 1905 (Illustr. Handwörterb. d. Botanik p. 194) TODD – so zitiert, daß der Eindruck entstehen könnte, diese Autoren hätten den Terminus geprägt. In den genannten drei Arbeiten kommt der Terminus jedoch n i c h t vor. H. MÜLLER verwendet konsequent „Rechts- und Linksgriffeligkeit", was dem Wort Enantiostylie als deutschsprachiges Äquivalent angefügt werden kann. In diesen frühen Beschreibungen enantiostyler Phänomene ist es zunächst verwirrend, daß rechts und links im Gegensatz zu EICHLER 1875, Blüthendiagramme 1 : 6, verwendet werden, sodaß bei diesen Autoren in „rechtsgriffeligen Blüten" der Griffel im Sinne der EICHLERschen Definition nach links gebogen ist (vgl. TEPPNER in Phyton 30(2): 335). Weiters muß man sich dessen bewußt sein, daß im Falle von Enantiostylie nicht nur das Gynözeum (Abb. 5), sondern vielfach auch das Andrözeum und z.T. selbst die Blütenhülle, vor allem die Krone (Abb. 6), von der Asymmetrie betroffen sind.

Enantiomorphie: In *Solanum*-Literatur fand ich diesen Ausdruck für die Erscheinung, daß in cymösen Blütenständen bei schräg zygomorphen (!) Blüten die Neigung der Symmetrieebene aufeinanderfolgender Blüten wechselt. Es spricht für die Qualität des Wörterbuches, daß hier eine Definition von Enantiomorphie enthalten ist: ORNDUFF & DULBERGER 1978 behandeln asymmetrische Blüten, also steht der Terminus nicht für den eben angesprochenen Fall zur Verfügung. Die beiden Autoren schildern zwar in *Wachendorfia* (*Haemodoraceae*) ein Beispiel, in dem die beiden spiegelbildlich verschiedenen Blütentypen auf verschiedene Individuen einer Population verteilt sind, doch die Lektüre der ganzen Arbeit, insbesondere der Anfang der Diskussion auf p. 432, zeigt klar, daß sie Enantiomorphie schlicht und einfach an Stelle von Enantiostylie verwenden – kein schlechtes Vorgehen, da ja, wie oben angeführt, nicht nur das Gynözeum Ursache der Asymmetrie von Blüten ist; in diesem Sinne ist Enantiomorphie auch bei ENDRESS 1994, Diversity evol. biol. trop. flowers, p. 467 definiert. Es empfiehlt sich daher, Enantiomorphie als Überbegriff für