CHAKRABARTI, D.K.

Department of Horticulture, N.D. University of Agriculture and Technology, Kumarganj, Faizabad - 224229, Uttar Pradesh, India

Websites : http://www.drdkchakrabarti.com ,
http://www.geocities.com/dkcnduat

E-mail : dkcnduat@yahoo.com

Comprehensive Account of Research Findings Reported in Papers in the Website

The salient research findings and their implication to have better insight and management of the disease are enumerated.

Etiology. The lack of an appropriate inoculation technique vis-à-vis knowledge in physiology of pathogenesis was the main impediment to remove the ambiguity in the identity of the causal organism of the disease. It was envisaged on the basis of the results of several experiments conducted by the present investigator on the mechanism of the disease development that during initial stage of inoculation i.e. during penetration by the fungus and early stage of its colonization, the hypersensitive system of the host should be arrested for successful manifestation of the disease symptoms. This hypothesis was later adopted successfully to reproduce both vegetative and floral malformation consistently and in very large proportion. A scientist at Central Institute of Sub-tropical Horticulture, Lucknow even succeeded to reproduce hundred per cent vegetative malformation following this hypothesis (ICAR News, July-September, 2003). Attempts were also made to correlate effects of *Fusarium moniliforme* Sheld. var. *subglutinans* on biochemical constituents and physiological processes of the infected host with malformation disease syndrome. In 1979, it was demonstrated for the first time by the present investigator that the *Fusarium* secreted a number of toxins inside the host that played important role in producing some of the disease symptoms. Later it was confirmed by Ram and Bist (1998) and Kumar *et al* (1993) who described it as toxic principles (TP). But most fascinating aspect of the study was to reveal the role of aberrant host metabolites (phenol and steroids) produced in response to the infection. Each of the morphological and biochemical changes in malformed plants was linked with these metabolites. Similar observations were also made by other workers (Kumar *et al*. 1993) who termed it as malformation inducing principles (MIP). The study also provided the only report on cyto-genetical anomalies in malformed cells. The correlation between the disease symptoms and chromosomal degeneration in the host was established. The identity of the pathogen was further investigated. Biochemical and molecular evidence for the first time in 1992 (Kumar and Chakrabarti) established that the wild strain of *F. moniliforme* var. *subglutinans* after long association with the host was transformed into a physiological strain, *F. moniliforme* var. *subglutinans* f. *sp. magnifera* by the host metabolite, mangiferin. Recently Britz *et al.* (2002) recognized the isolate of *F. moniliforme* from malformed mango as a new taxa in the section *Liseola* and described it as *F. mangifera*. Thus, substantial information was generated that dispelled the earlier doubt for the *Fusarium* as causal agent of the disease.

Epidemiology. The patho-system and dynamic interaction among the pathogen, vector, physical environment and host physiology in temporal as well as spatial progress of the disease were analyzed and modeled mathematically. The disease cycle was depicted. The physiological time scale for the disease development was brought into focus and quantified. The observations were used to predict the outcome of other still unknown situation i.e. in forecasting the epidemic and in selection of epidemiology oriented control principles.

Assessment of loss in yield. An accurate system for measurement of the disease was developed. Earlier loss in yield was assessed only on the basis of loss in direct yield i.e. loss due to transformation of healthy panicles into malformed one. The disease also inflicted indirect loss by reducing number of total panicles. Up to 50% level of disease severity, plants yielded more flowers to compensate the loss of panicles rendered malformed, but with further increase in severity, flowering declined sharply A linear regression equation was developed with which the extent of loss in yield could be predicted accurately before hand by counting number of malformed panicle. Incidence of floral malformation was more in on year but considerably low in off year.

Pattern of the epidemic. This was the first analytical approach to describe the structure of the epidemic and pattern of the disease spread. From the results it was concluded that the epidemic progressed in regular bearing cultivar fast and steadily forming sigmoid curve. But in alternate bearing cultivar the progress was slower and took longer period to reach the peak. The increase and decline of the epidemic in alternate bearing cultivar were sharp forming bimodal curve. The epidemic stage (logarithmic growth phase) may be initiated with small amount of initial inoculum and once the tissues are infected they remained infectious throughout. The epidemic after 4-5 years entered into the endemic stage. At this stage a balance between the host and the pathogen was reached and production of malformed panicles became less. However, the constant presence of the pathogen over the host makes the plant weak and thus overall yield suffered. The spatial patterns of spread of the disease among genetically diverse cultivar of mango were investigated. The disease gradient curves in all cultivar at early stage were hyperbolic and decreased steeply within short distance indicating that the disease spread in a stepwise progression and plants received inoculum from immediate neighbor. Hence, the disease spreads over short distance only. With the establishment of the secondary source of inoculum within the perennial plants the hyperbolic disease curve became flatten near the source. Faster the rate of infection the gradient curve would be more flat. The wide and erratic distribution of the disease was the inadvertent propagation and distribution of the malformed plants.

Vector: mites. Role of a different group of mite other than eriophyes i.e. *Tyrolichus case* Oudemans, a mycophagus mite, was brought into notice. Earlier role of mite was described as a passive carrier of the fungus. But for the first time it was revealed that the mite and the pathogen, *F. moniliforme* var. *subglutinans* had a unique interdependent mutually beneficial relationship. *T. casei* became attracted by the *Fusarium* copiously growing over dead necrotic malformed panicles in hot humid climate after rains which served as feed over of *T. casei* and increased its multiplication. The mite in turn carried the conidia and mycelia to healthy buds and facilitated the ingress of the pathogen into the host cells.

Disease cycle. On the basis of the above information a disease cycle was envisaged. Dead necrotic malformed panicles served as primary inoculum and site of multiplication of the fungus from where mites disseminated the conidia and mycelia to emerging buds. The *Fusarium* entered into the host cells through wounds either natural or inflicted by the visiting mites. The hyphae of the *Fusarium* moved through intercellular space and remained restricted to the epidermal and cortical layers of cells. It did not move systemically; thus all the infection was localized. The symptoms appeared 5-6 weeks after infection. Production of conidia over necrotic panicles and their germination on young buds were greatly influenced by the environmental factors but the process of invasion and subsequent colonization and symptom production were largely governed by the physiological condition of the host cells. The infected buds did not produce any malformed shootlets and panicles after flushing was over. The host in response to infection produced a phenol compound, mangiferin, in large amount which was accumulated at the site of infection. Mangiferin acted antagonistically against the pathogen as well the vector. In emerging tender buds mangiferin remained in low concentration; hence the fungus easily penetrated into the host cells. In and around infected cells mangiferin was increased up to cytotoxic level inhibiting further ingress of the pathogen and its conidia formation in the host cells. Later mangiferin was oxidized and became ineffective. At this stage the surviving propagules grow over the dead necrotic panicles and produced conidia. Mangiferin content during April-May and December-January was increased to a high level with concomitant decline in the fungal population. But the same was just reverse during July to September and February. The disease to attain the form of an epidemic (logarithmic phase), a balance among host metabolite (mangiferin), pathogen and the vector was required. Thus, the study underlined the importance of physiological time scale for the disease development vis-à-vis seasonal variation of the disease incidence. The proposed disease cycle generated lot of interest among the malformation workers (Kumar *et al*. 1992).

Climatic factors. The useful climatic parameters that exerted the greatest influence on the rate of transformation from one state to another of the

epidemic were identified in growth chamber study and the values of the parameters were estimated by field experiments. The maximum conidia production was recorded in July (temp. 25-30°C, RH 78-92%). Conidia showed maximum viability in the morning hours and germinated within 5-6 h at 30°C. The intensity of vegetative malformation progressed steadily from mid June to end of July (temp. 25-30° C, RH > 85%) while floral malformation in February-March (temp. 9.8-19°C, RH>87%). Development of floral malformation compared with vegetative one was more sensitive to higher temperature. The highest percentage of infected buds and development of malformed shoots were recorded during spring flush and minimum in summer. Mild temperature (8-19°C) and high humidity (87%) were conducive both for invasion and symptom manifestation. The above observations were later confirmed by Kumar *et al* (1993) and Noriega-Cantu *et al.* (1999).

Host factors. Optimum age for maximum incidence of floral and vegetative malformation in cv Dashehari were 10 and 5 years respectively. Floral malformation in regular bearing cultivars compared with alternate bearers was more. But early flowering varieties irrespective of regular and alternate bearing habit suffered more than those flowered later in warmer period. In alternate bearing cv smaller number of malformed panicles (initial inoculum) in off year and subsequent galloping increment in total flush in on year resulted into lesser disease percentage in on year. But in regular bearing cultivars there was no much difference in total as well as malformed panicles in different years.

Adaptability of the pathogen. There was popular perception that the disease could not occur in the states situated at the coastal region of India. This might be due to constant high temperature in the region and unlike the northern part of the country fluctuation in temperature between winter and summer months was not extreme. In the present study, on the contrary, it was observed that the pathogen on being introduced into the coastal state West Bengal succeeded to survive and initiate fresh infection. From the source plats the disease was found to spread to the local cultivars already growing in the orchard. However, the pathogen in the new agro-climatic condition lost some of its virulence which was reflected in the less severe manifestation of the disease symptoms. Thus it was apprehended in 1997 that malformation may soon pose threat, as in north India, to the mango industry in West Bengal. Unfortunately the apprehension has been proved correct. The disease in the mean while has assumed a serious proportion. The ICAR send a team this year (2004) headed by the scientist of Central Institute of Sub-tropical Horticulture, Lucknow to assess the disease situation in the mango belt of the state.

Disease forecasting. To develop an accurate forecasting system we adopted analytical approach (Shrun, 1978). In analytical approach every phase of the disease cycle was modeled starting from formation of infection unit to development of the symptoms (Fig.1). The dead necrotic malformed panicles

which served as the site of proliferation and source of dissemination of the pathogen play the most important role in perpetuation of malformation. But the success of the dead panicles to serve as primary source of inoculum in turn depends on the favorable climatic parameters particularly during July-August. There should also be sufficient mites to carry the conidia to the infection site. Then for successful infection germination of conidia and favorable climatic condition for their germination are needed. However, production and germination of conidia have similar weather requirement. It was also recorded in the present investigation that high concentration of conidia affected each others germination. From mango buds infected during July-August only 25% malformed shoots were developed up to first week of following October. In between October-February about 43% infected buds were developed into malformed panicles or shoots depending on weather condition and alternate bearing phenomenon. Thus number of malformed shoots during October may well indicate for malformed panicles to be developed in the forthcoming crop season.

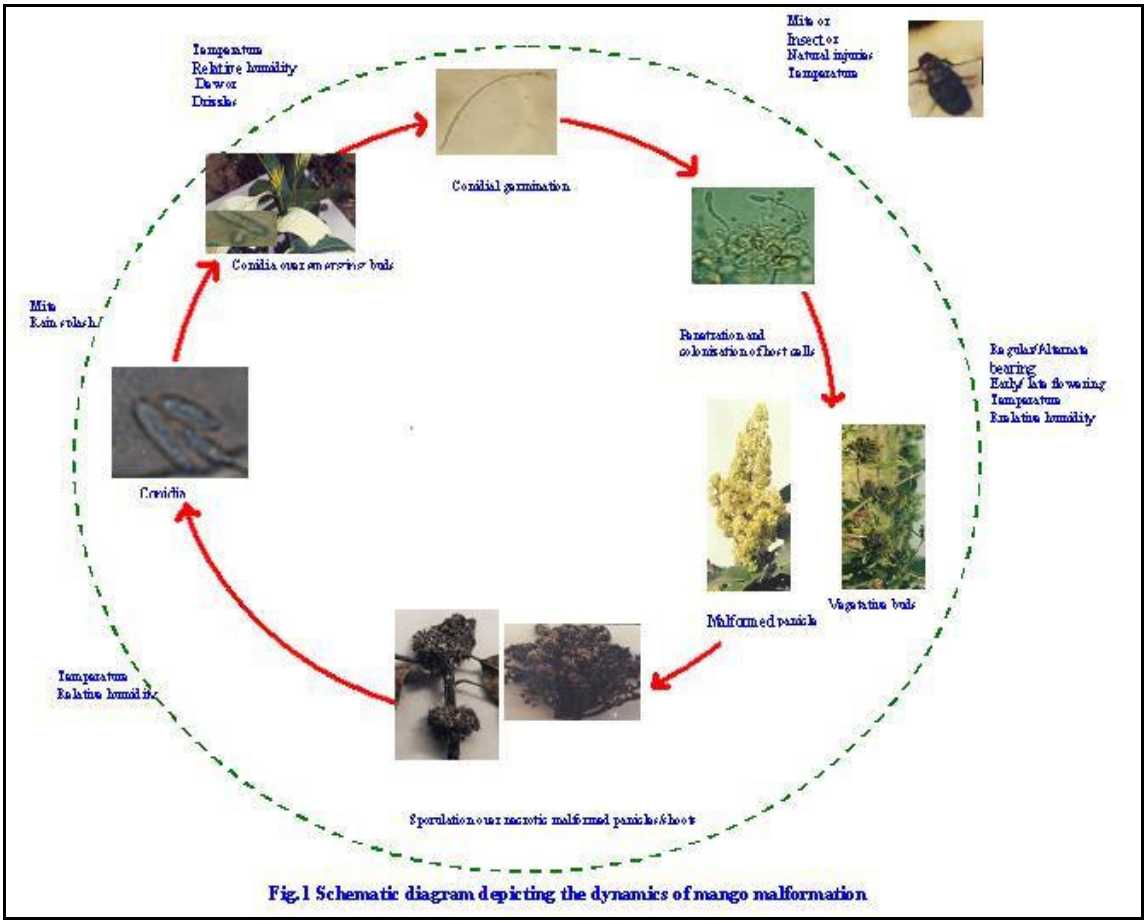

Fig.1 Schematic diagram depicting the dynamics of mango malformation

Fig.1. In analytical approach every phase of the disease cycle was modeled starting from formation of infection unit to development of the symptoms

Predicting equation for formation of conidia on dead necrotic malformed panicles in July was as follows: $Y=293430.6 - 10061.9X_1 + 1775.44X_2$ where Y X_1 and X_2 were number of conidia/g plant material, average temperature and relative humidity respectively. Linear regression equation between *T.casei* and floral malformation: $Y=16.8430 - 7.2094$ X, Y and X were malformed panicles and number of *T. case*i /g bud.Prediction for development of vegetative malformation during July-October: $Y=10.234 + 4.336X_1 - 0.243X_2 - 0.141X_3$, where Y = number of malformed shoots, X_1 + number of days with optimum temperature (25-30^{o}C) and RH >85%, X_2 = number of rainy days and X_3 = total rainfall. Predicting equation for number of malformed panicles to be developed during flowering season: $Y=2.1364 + 0.6968X$ where Y and X were number of malformed panicles in April and X number of malformed shoots developed July – October. Thus, simple counting of number of malformed shoots present prior to the onset of flowering process in October made it possible with the help of the predicting equation to predict the number of malformed panicles to be produced in the flowering season (February -March) in cv Chausa under agro-climatic condition of Rudauli - Sohawal mango belt of Uttar Pradesh. But floral malformation of April developed 2nd order polynomial equation with dead necrotic malformed panicles of the preceding year's July i.e. incidence of floral malformation in April initially increased with increment in number of dead malformed panicles in July; but with further increase in number of dead panicles (mother inoculum) subsequent production of malformed panicles (progeny) was reduced. Prediction equation for severity of floral malformation i.e. in terms of weight of the panicle:$Y= - 203.743 - 5.341X_1 + 10.023X_2 - 0.268 X_3$ where Y= weight of panicle (g), X_1 = min. temp. (^{o}C), X_2 = max. Temp. (^{o}C), X_3 = average RH (%). We also tested some other independent variables that had been reported to effect development of the disease viz. mangiferin, C:N ratio, micronutrient (zinc, copper, iron and manganese) contents of leaves attached to the apical buds amount of auxin, population density of *Fusarium moniliforme* var. *subglutinans* before flowering, average number total flowers and malformed panicles in May to select forecaster. None of these variables showed consistent significant correlation with floral malformation. The fungus showed negative correlation i.e. reduced the infection in the year when its population became very high. Attempt was also made recently (2003) to predict the development of both vegetative and floral malformation using age of the diseased plants as independent variable in an orchard where the disease had already been established. The data of incidence of vegetative and floral malformation and age of the malformed plants were subjected to regression analysis and the host age appeared as a good predicting independent variable. Thus, in the 7-year-old plants of cv Chausa after treatment average number of total number of panicles per plant produced were 222 whereas in untreated plants the same was only 85. Each treated plant yielded on an average 109 fruits. But there were only 22 fruits in untreated plants (Kumar and Chakrabarti, 1998). Similarly in 7-year-old cv Dashehari, the treatment resulted

into prolific flowering (1030/plant). But in untreated plant numbers of panicles were meager 486. Number of fruits set per treated and control plants were 490 and 22 respectively (Chakrabarti *et al*., 2001). Thus in a severely infected orchard, 80-90 more fruits per plant could be obtained only after 1-year treatment. But if the initial infection was low the fruit yield could be increased by a staggering 400 per plant in an on year. However, increment in yield was greatly affected by various factors like 'on' and 'off' year, initial inoculum load, host age and genetic potentiality of the cultivar.

Management. The results of the present experiment showed that initial inoculum level played the crucial role to break out malformation epidemic. Hence attempt was made to manage the epidemic by minimizing primary inoculum i.e. increasing Δt (delaying on set of disease development) by destroying the propagules through sanitation or spraying fungicides (chelated Cu^{++}) or by employing an antagonist (*Aspergillus niger* van Tiegh). In *in vitro* test mangiferin Cu^{++} chelate killed the conidia and mycelia, *A. niger* parasitized the *Fusarium* while carbendazim arrested germ tube growth and reduced conidia production thus affected infection rate (r) of *F. moniliforme* var. *subglutinans.* As natural sequel of the *in vitro* experiment we tested the above treatments in various combinations over 100 infected plants (7-year-old) cv Dashehari. The biochemical changes associated with the treatments in the host were also investigated. Total eradication of mother (initial) malformed panicles reduced the development of progeny malformed panicles in the next flowering season and also increased number of total panicles. But lowering of initial inoculum increased the rate of progress of the disease. Thus, effects of pruning did not sustain long. Spraying of mangiferin Cu^{++} chelate before flowering induction reduced the floral malformation while application of *A. niger* reduced the number of propagules over necrotic malformed panicles. Spraying of carbendazim before panicle emergence and panicle development reduced the disease incidence. But carbendazim required longer period to bring down the disease level. Its effects were more apparent in the second year of application. It may be mentioned here that carbendazim affected the infection rate of he disease. But in mango malformation infection rate was very low and not an important factor to break the epidemic. Of all the treatments tested, a combination of eradication of malformed panicles and shoots and spraying with Cu^{++} and Zn^{++} appeared to be the best. The treatment yielded maximum flowers and fruits. But it considerably reduced the number of malformed panicles and population of the pathogen. It is worth mentioning that the treatment reduced the Fe^{++} content of the cells the maximum. Earlier we recorded that Fe^{++} had a positive correlation with development of malformation (Chakrabarti *et al*., 2003). It also increases the nitrogen level of the treated cells; thus, maintained a proper C: N ratio. It is well known that imbalance in C: N ratio (more carbohydrate and low nitrogen content) aggravated the disease expression. The treatment having combination of

pruning and Cu^{++} chelate showed the minimum disease incidence. But in this treatment total yield was low; hence unacceptable to the farmers. In 1998 (Kumar and Chakrabarti) we developed an integrated management strategy. Although our primary objective was to manage the malformation below economic threshold level but to make it acceptable to the farmers we also endeavored to increase the ultimate yield simultaneously. The **IPM strategy** included the following treatments: eradication of malformed shoots and panicles after spring and autumn flushes (April and October), spraying with acaracide (phosphomidon 0.05%) immediately after 3 flushing (February, May and October), spraying with chelated copper (40 ppm) (mangiferin chelate or amino acid based chelate or copper fungicide) twice (August-September and December –January) before advent of the peak period of the fungal population, spraying with chelated Zn^{++} twice (40 ppm) (December and February) to replenish the deficiency in the plants suffering long from the disease. To check the powdery mildew and hopper damage sulfur fungicide and insecticide (monocrotophos) were also sprayed. The treatment increased the fruit yield by 23.9% in cv Chausa which was severely infected (22-34% malformed panicles) in the crop season preceding to the treatment (Kumar and Chakrabarti, 1998). In cv Dashehari where infection was in the range of 1-5% only, the increment in fruit yield was 31.14% (Chakrabarti *et al.*,2001). The treatment reduced floral malformation by 7-9% considered as direct loss. However, its effect against indirect loss was more pronounced which manifested in the form of increased flowering and fruit setting.

Effect of the IPM technique on productivity, profitability and sustainability. In 7-year-old plants of cv Chausa after treatment average number of total number of panicles per plant produced were 222 whereas in untreated plants the same was only 85. Each treated plant yielded on an average 109 fruits. But there were only 22 fruits in untreated plants (Kumar and Chakrabarti, 1998). Similarly in 7-year-old cv Dashehari, the treatment resulted into prolific flowering (1030/plant). But in untreated plant number of panicles was meager 486. Number of fruits set per treated and control plants were 490 and 22 respectively (Chakrabarti *et al.*, 2001). Thus in a severely infected orchard, 80-90 more fruits per plant could be obtained only after 1-year treatment. But if the initial infection was low the fruit yield could be increased by a staggering 400 per plant in an on year. However, increment in yield was greatly affected by various factors like 'on 'and 'off' year, initial inoculum's load, host age and genetic potentiality of the cultivar.

Profitability. Operational cost (including cost of chemical and wages of labours) per plant was about Rs. 100/- per annum. By the treatment on an average 100-300 i.e. 20-50 kg more fruits per plant could be obtained depending upon the factors mentioned above. Thus a profit of Rs. 250-700 per plant might be accrued by using this technique. This could be verified from an orchard owner, Mr. Javed Khan, Advocate, Village Jaganpura, Sohawal, District

Faizabad, Uttar Pradesh. His orchard of 250 mango plants of 20-year-old was rendered totally abortive. After adopting this technique he has now been earning about 1.5 lacs per annum. Similarly, Mr. Rana Pratap Singh of village Lohati Sarai, Rudauli, Faizabad, Mr. Amin, of the village Amroha, Rudauli, Faizabad and others who adapted the IPM strategy earning handsome revenue from their orchards.

Sustainability. The IPM technique developed in the project helps to identify appropriate conditions of host and climate to apply control measures thus, has minimized use of pesticides and chances of developing of iatrogenic disorder like fruit cluster (*Jhumka*) etc. Emphasis has been given to enhance immunity of the host and to restore normal health by bringing back biochemical balance of the diseased plants. The technique functions by manipulating propagules and infection rate mainly by physical elimination and tapping host potentiality of disease resistance. The Uttar Pradesh Directorate of Horticulture and Food Processing, Lucknow accepted the above recommendations, published and distributed among its field staffs for necessary action.