Indian J. Hort. 67(1), March 2010: 21-25

Significance of *Lasiodiplodia theobromae* and *Colletotrichum musae* in causing crown rot in banana and their reaction on some commercial banana cultivars

G. Sangeetha*, S. Usharani and A. Muthukumar
Faculty of Agriculture, Annamalai University, Chidambaram 608 002, Tamil Nadu

ABSTRACT

Crown rot is an important post-harvest disease of banana infecting the pad of cut hands. Among the 108 isolations made from samples of 16 commercial banana cultivars from different regions, 40 isolations yielded *Lasiodiplodia theobromae*, 22 isolations yielded *Colletotrichum musae* 34 isolations yielded both *L. theobromae* and *C. musae*, 6 isolations yielded *L. theobromae C. musae* and *Fusarium* spp. and only 6 isolations yielded *Fusarium* spp. Artificial inoculation with single species and combinations on cv. Robusta revealed, the combination of *L. theobromae* and *C. musae* and *L. theobromae C. musae* and *Fusarium* spp. produced the highest level of rotting on ripe fruits. Cultivar Robusta (AAA) and Dwarf Cavendish (AAA) were highly susceptible and cv. Karpuravalli (ABB), Nendran (AAB) and Virupakshi (AAB) were found to be least susceptible to crown rot disease complex under artificially inoculated conditions. This was confirmed by *in vitro* testing of the fruit leachates of ten banana cultivars on spore germination of the most virulent isolates of *L. theobromae* and *C. musae*.

Key words: Banana, crown rot, *Lasiodiplodia theobromae, Colletotrichum musae,* leachates.

INTRODUCTION

Banana is a highly perishable fruit and suffers severe post-harvest losses due to diseases caused by various pathogenic microorganisms, which needs to be seriously addressed. After harvest, the fruit is exposed to a range of fungal pathogens, among them anthracnose caused by *Colletotrichum musae* (Berk and Curtis) Arx and crown rot caused by complex of *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl and *C. musae* are the two important post-harvest diseases that infect banana (Ploetz *et al*., 14). Not only in India, but also in other banana producing countries like Sri Lanka (Anthony *et al*., 2), Pakistan (Haque *et al*., 8) and Bangladesh (Alam *et al*., 1), both pathogens were found to be associated on crown rot infected banana fruits. Many fungi and other microorganisms have been isolated from decaying crowns. Different organisms predominate according to locality, season and other environmental factors. Two or more fungi may attack the crown simultaneously or successively and cause tissue rotting (Jones, 9). The fungal spectrum found on rotten crowns from various countries has been reviewed by Meredith (12). Pathogenicity test carried out by Griffee (7) indicated that *C. musae*, *Botryodiplodia theobromae* and *Ceratocystis paradoxa* were primary pathogens and that of *Fusarium pallidoroseum* and *F. graminearum* were secondary invaders. Knight *et al*. (10) suggested that *F. semitectum* was also a primary wound pathogen and was as pathogenic as that of *C. musae*. The objective of the present study was to know the significance of individual crown rot pathogen *viz*., *L. theobromae*, *C. musae*, *Fusarium* spp. as well as their combination in causing crown rot disease in cv. Robusta. Another study was conducted to note the effect of artificial inoculation of crown rot pathogens *viz*., *L. theobromae*, *C. musae* on ten commercial banana cultivars.

*Corresponding author's E-mail : sangeethaau@hotmail.com

MATERIALS AND METHODS

The pathogens causing post-harvest rotting in banana were isolated from the crown rot infected fruit samples. The varieties namely, Poovan (AAB), Rasthali (AAB), Robusta (AAA), Monthan (ABB), Virupakshi (AAB), Red banana (AAA), Karpuravalli (ABB), Nendran (AAB), Pachanadan (AAB), Dwarf Cavendish (AAA), Matti (AA), Singhan (AAB), Palayangottan (AAB), Peyan (AAB), Moris (AAA) and Vellaithuluvan (AAB) were collected from different banana growing regions and markets of Tamil Nadu for the isolation of the pathogen(s). The pathogenicity test was conducted for all the *L. theobromae* and *C. musae* isolates and Koch's postulates were successfully proved in cv. Robusta; Isolates of *L. theobromae* and *C. musae* obtained from cv. Robusta, was used thoroughout the study unless otherwise specified. The identity of all isolates of *L. theobromae* and *C. musae* was confirmed by microscopical observation. All isolates of *L. theobromae* examined during this study agreed with the description given by Goos *et al*. (6) and Punithalingam (16). All isolates of *C. musae* examined agreed with the descriptions given by Sutton and Waterson (19).

Spore suspension of two test pathogens were obtained by flooding the petri plates containing 10 days old culture of respective pathogen with sterile distilled water and crushing them to release the spores, filtered through Whatman No. 2 filter paper to remove mycelial fragments. A spore concentration of 10^5 spores per ml was used in all the studies unless otherwise specified. Required spore concentration was adjusted using haemocytometer.

Hands of banana cv. Robusta with units of 5-10 'fingers' attached to the cushion were selected for inoculation. Selection was done in such a way that all the fruits were of similar size and taken from bunches at harvesting maturity. The dehanded stalk was kept for sometime until the latex stopped flowing from the cut end. After that, the hands were washed in running tap water, surface sterilized with 70 per cent alcohol and allowed to dry. At the centre of the crown region, 5 to 10 mm deep cavity was formed using a sterilized needle and inoculated with *L. theobromae*, *C. musae* and *Fusarium* spp. singly and in all two-way and three-way combinations at 100 ml (containing 10^5 conidia/ml of spore suspension*)* of using a sterile micropipette. Sterile distilled water served as control.

The crown region was covered with moist cotton (to ensure the initial spore germination) and fruits were placed inside perforated polythene bags to maintain high humidity and incubated at room temperature (30 ± 2°C) for the development of crown rot symptoms. Observations on the development of disease were taken from the day of disease appearance on any one of the treatments. In each case, four hands were inoculated to maintain the required number of replication. The experiment was conducted based on completely randomized block design (CRD). Disease development was observed and recorded following standard procedure developed by Finlay and Brown (4) based on crown rot severity (0-5 scale, where 0 - no disease. 1, 2, 3, 4 was for rot progression of 25, 50, 75 and 100% respectively and 5-where rot extended up to the pedicel), crown colour (1-7 scale where 1 = Green; 2 = Green / Yellow; 3 = Yellow / Green, 4 = Yellow; 5 = Yellow/black; 6 = black/Yellow; 7 = black) and crown texture (0-4 scale, where 0 = Hard, 1 = 0 to 25%, 2 = 25 to 50%, 3 = 50 to 75%, 4 = 75 to100% of the crown soft).

Varietal reaction was studied in selected ten commercial banana cultivars *viz.*, Poovan, Rasthali, Robusta, Monthan, Virupakshi, Red Banana, Karpuravalli, Nendran, Pachanadan and Dwarf Cavendish by artificial inoculation of crown rot pathogens *viz.*, *L. theobromae* and *C. musae* each alone and in combination. Similar inoculation and scoring procedure was followed as mentioned above. Based on the rot severity score (0-5) the cultivars were assessed for their susceptibility to crown rot disease for combined inoculation of crown rot pathogen {> 4-highly susceptible (HS), 3-4 susceptible (S), 2-3 moderately susceptible (MS) and 1-2 moderately resistant (MR) and <1 resistant (R).

The above selected ten commercial cultivars of banana were used to know the effect of their ripe fruit leachates on spore germination of crown rot pathogens. Hands were washed thoroughly with sterile, distilled water and made to ripen artificially. Then 7-10 drops of sterile, distilled water were placed onto the surface of each fruit of individual cultivars. Fruits were then incubated in a humidity chamber (30 ± 2°C, 90% RH) for 18 h. After incubation, the droplets were collected (with minimal disturbance), pooled together for each treatment and filtered through a 0.2 µm Millipore® filter. The filtrate containing the banana leachates was then used for the spore germination study (Vijaya *et al.,* 20). For the spore germination study, a spore suspension of pathogen isolates of *L. theobromae* and *C. musae* were prepared and final spore concentration was adjusted to 10^5 spores/ml using a haemocytometer. The spore suspension of two pathogen each alone and the leachates of individual varieties were mixed separately (in equal volumes of 100/µl) in a clean cavity glass slide and incubated in a humidity chamber (12 h, 28 ± 2°C, 90% RH). A control treatment was also set up using sterile, distilled water. The percent germination of spores was calculated by scoring 100 conidia for germination and replicated three times to obtain an average percent germination.

RESULTS AND DISCUSSION

In the present study, crown rot was the major post-harvest disease identified on ripening bananas next to anthracnose in various districts of Tamil Nadu on most of the commercial cultivars of banana. Among the 108 isolations made from samples of 16 commercial cultivars of banana from different regions, 40 isolations yielded *Lasiodiplodia theobromae*, 22 isolations yielded *Colletotrichum musae,* 34 isolations yielded both *L. theobromae* and *C. musae*, 6 isolations yielded both *L. theobromae*, *C. musae* and *Fusarium* spp. and only 6 isolations yielded *Fusarium* spp. (data not shown). Hence, the two major crown rot pathogens *viz.*, *L. theobromae* and *C. musae* were inoculated singly or in combination (simultaneously) to record the nature of associative effect between some pairs of pathogens, which were observed as mixed infection in nature. The rot initiated by *L. theobromae* and *C. musae*; and *L. theobromae, C. musae* and *Fusarium* spp. appeared obviously on the crowns in 6 days of inoculation, whereas very little rot was visible on fruits inoculated with any one of the fungus. This difference was highly significant and more obvious on fruits ripened to stage

Table 1. Effect of combined inoculation of *L. theobromae C. musae* and *Fusarium* spp. on banana cv. Robusta.

Pathogen	Days taken for initiation of rotting*	Categorical score on 10th C* at the 30 ± 2°C			Total shelf-life* (days)
		Rot (0-5)	Colour (1-7)	Texture (0-4)	
Lasiodiplodia theobromae	7.0bc	2.7cde	5.3cd	2.3c	12.3b
Colletotrichum musae	8.0e	2.3de	5.3cd	2.0c	13.0c
Fusarium spp.	7.7de	3.7bc	6.7ab	3.0b	12.0b
L. theobromae + *C. musae*	6.0a	4.7ab	6.7ab	3.7a	10.0a
L. theobromae + *Fusarium* spp.	6.8b	2.7cd	6.0bc	2.3c	12.0b
C. musae + *Fusarium* spp.	7.3cd	3.3cd	5.7c	4.0a	12.3b
L. theobromae + *C. musae* + *Fusarium* spp.	6.0a	5.0a	7.0a	4.0a	10.0a
Control (uninoculated)	10.0f	2.0e	4.7d	2.0c	

*Mean of four replications; DAI = Days after inoculation.

In a column means followed by a common letters are not significantly different at 5 per cent level by DMRT.

Table 2. Effect of artificial inoculation of crown rot pathogens on different banana cultivars.

Cultivar	Rot grade (0-5)*			Mean rot	Cultivar reaction to combined inoculation of pathogens#
	L. theobromae	*C. musae*	*L. theobromae* and *C. musae*#		
Poovan	2.0bcd	2.3bc	3.7ab	2.7	S
Rasthali	2.0bcd	2.0c	2.7c	2.2	MS
Robusta	3.0a	3.3a	4.3a	3.5	HS
Monthan	1.7cd	1.7cd	2.7c	2.0	MS
Virupakshi	1.3d	1.7cd	1.7d	1.6	MR
Red Banana	1.7cd	2.0c	3.0bc	2.2	S
Karpuravalli	1.3d	1.0d	1.7d	1.3	MR
Nendran	2.0bcd	1.7cd	1.3d	1.5	MR
Pachanadan	2.7ab	3.0ab	3.7ab	3.1	S
Dwarf Cavendish	3.0a	3.3a	4.3a	3.5	HS

*Mean of four replications.

In a column means followed by a common letters are not significantly different at 5 per cent level by DMRT.

#Rot severity scale (0-5): > 4 - highly susceptible (HS), 3-4 susceptible (S), 2-3 moderately susceptible (MS), 1-2 moderately resistant (MR) and <1 resistant (R)}.

5 based on colour. The *L. theobromae* and *C. musae,* and *L. theobromae, C. musae* and *Fusarium* spp. combination produced the highest level of rotting (4.5 and 5.0, grade respectively) on ripe fruits with complete blackening of crown region (colour score of 6.7 and 7.0 respectively) (Table 1). Therefore, it is evident that these three fungi act in association to increase the severity of crown rot. In spite of this fact, surprisingly in majority of the reisolation studies, *L. theobromae* and *C. musae* were frequently recovered (data not shown). These results thus indicated that *L. theobromae* and *C. musae* are the primary pathogens involved in the crown rot complex and *Fusarium* spp. was considered as the secondary invader or minor pathogen. It reveals that a number of well-defined pathogenic fungi, which sometimes act alone, but more usually in combination, cause crown rot with high rot severity. Similar kind of documentation was made by Lukezic *et al*. (11), Shillingford (17), and Wardlaw (21). Mixed infection by a number of pathogens has been clearly reviewed by Eckert (3). The results of combined inoculation might be due to aggravation, suppression,

Table 3. Effect of fruit leachates of different banana cultivars on the spore germination of *L. theobromae* and *C. musae.*

Cultivar	Spore germination (%) in fruit leachates*			
	L. theobromae		*C. musae*	
Poovan	88.3i	(70.0)	82.3g	(65.1)
Rasthali	70.0e	(56.8)	78.3e	(62.3)
Robusta	92.0k	(73.6)	90.0i	(71.6)
Monthan	72.7f	(58.5)	73.0d	(58.7)
Virupakshi	59.0b	(50.2)	67.3c	(55.1)
Red Banana	74.0g	(59.3)	80.3f	(63.7)
Karpuravalli	67.7d	(55.3)	68.3c	(55.8)
Nendran	63.0c	(52.5)	60.0b	(50.8)
Pachanadan	84.7h	(67.0)	85.3h	(67.5)
Dwarf Cavendish	91.0j	(72.5)	92.7j	(74.3)
Control (sterile water)	40.3a	(39.4)	45.3a	(42.3)

*Mean of four replications.

In a column, means followed by a common letters are not significantly different at 5 per cent level by DMRT. Values in the parenthesis are Arcsine $\sqrt{\%}$ transformed values.

predominance *etc.* as indicated by Gangopadhyay and Sharma (5).

To assess the varietal reaction of banana cultivars to crown rot pathogens *viz*., *L. theobromae* and *C. musae* alone and in combination were artificially inoculated on fruits with their respective varietal isolates and their preferred hosts. All the varieties tested were found to be susceptible to crown rot disease with varying degrees of susceptibility (Table 2). The cultivars Robusta and Dwarf Cavendish were found to be similar in their susceptibility to crown rot pathogens respectively as highly susceptible. Cultivars Poovan and Pachanadan were susceptible when inoculated with combination of both pathogens than with individual inoculation of both pathogens. The cvs. Rasthali, Monthan and Red banana were found to be moderately susceptible. Karpuravalli, Nendarn and Virupakshi were least susceptible. Some of the cvs. like Karpuravalli, Nendarn and Virupakshi were not severely affected by crown rot disease under natural condition, but when artificially inoculated on cut crown surface, infection had occurred. It is probable that the biochemical constituents of the peel might have been responsible for the resistance under natural conditions and when this barrier was broken through artificial inoculation, the fruits became susceptible. On control hands (without pathogen inoculation), also some amount of infection and rotting was noticed indicating the latent infection by the crown rot pathogens.

In case of cvs. Robusta and Monthan, yellowing of the fruits was noticed a week after inoculation. It might be due to the biochemical changes brought out by the pathogen resulting in prematured ripening. This kind of reaction was not observed in other varieties tested. Forced ripening by the invading pathogen to its advantage could be visualized from the reports of Palaniswami (13) who had observed prematured ripening because of *B. theobromae* infection on banana.

Results indicated that leachates of ripe fruit of all the cultivars had significantly increased the spore germination of both pathogens compared to control (sterile water) (Table 3). The highest percentage of spore germination of *L. theobromae* was recorded in the leachates of cv. Robusta (92.0%) followed by leachates of Dwarf Cavendish (91.0%), Poovan (88.3%) and Pachanadan (84.7%). The lowest germination percentage of *L. theobromae* was observed in leachates of cvs. Virupakshi (59.0), Nendarn (63.0) and Karpuravalli (67.7). Similarly, the highest percentage of spore germination of *C. musae* was recorded in the leachates of cvs. Dwarf Cavendish (92.7), Robusta (90.0) followed by in the leachates of cv. Pachanadan (85.3) and Poovan (82.3). The lowest germination percentage was observed for leachates of cv. Nendarn (60.0), Virupakshi (67.3) and Karpuravalli (68.3). Therefore, it is possible that the difference in virulence may actually be attributed to the production of leachates by different varieties. Further work could investigate the varietal leachates by finding looking at the compounds present. Earlier, Preece and Dickinson (15) opined that the presence of chemicals on the surface of banana fruit could influence the germination and appressorial formation of post-harvest pathogens. From the present study it was confirmed that the cvs. Robusta and Dwarf Cavendish (AAA) are highly susceptible to crown rot complex, whereas cvs. Karpuravalli (ABB), Virupakshi (AAB) and Nendran (AAB) are moderately resistant susceptible to crown rot disease caused by *L. theobromae* and *C. musae*. These results are in

agreement with that of Stover (18) who had reported that all commercial dessert bananas cultivars of AAA genome were susceptible to crown rot disease and the problem was rare in cultivars belonging to the sub-group AAB. Hence, the study on varietal reaction to a particular disease is expected to give a clear picture about the distribution, development and seriousness of that disease over a group of population and cultivars. It is also very important to understand the dominant pathogens involved in a disease caused by complex organisms for planning a perfect management strategy.

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Received: September, 2008; Revised: November, 2009; Accepted : December, 2009