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OCCURRENCE OF STEM SPOTS AND ANTHRACNOSE ON DRAGON FRUIT (HYLOCERUS *SPP*) IN SRI LANKA

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ABSTRACT

Dragon fruit (*Hylocerus* spp.) is a newly introduced fruit crop and has become a popular cash crop in Sri Lanka. In November 2010, a new destructive disease with stem spots was reported from many regions of Sri Lanka. *Botryosphaeria dothidea* (Mong. Fr.) Ces and De Not. (Anamorph : *Fusicoccum aesculi*) was identified as the fungal pathogen of stem spots on dragon fruit. A very high disease incidence (100%) and severity (60- 80%) were observed in the highly susceptible red-fleshed species while the white-fleshed species was tolerant to stem spot disease. Lower incidence (5%) of anthracnose caused by *Colletotrichum gloeosporioides* (Renz) Sacc. was also observed in both species. *Botryosphaeria dothidea* is a quarantine pathogen and has the potential to cause serious economic losses in dragon fruit and in other local fruit crops in the future. Both fungal pathogens can spread through planting materials. Lack of pathogen-free planting materials and poor knowledge on proper cultural and disease management practices were identified as the key constraints to dragon fruit cultivation.

KEYWORDS: Anthracnose, dragon fruit, stem spots

INTRODUCTION

The Dragon fruit (*Hylocereus* spp.), also known as Pithaya (Latin America) or strawberry pear (English), is an edible vine cactus native to Mexico, Central and South American countries (Valencia *et al.*, 2003a). Presently, it is grown commercially in more than 20 countries in the tropics and subtropics (Mizrahi and Nerd, 1999) due to its edible qualities such as delicious taste and nutritional value (rich in vitamin C, Phosphorus and Calcium). The fruit contains medicinal properties such as antioxidants, which help to prevent the development of colon cancer and diabetes. It can be consumed as a fresh fruit or used to prepare salads, juice, jam, syrup, ice cream, yoghurt, jelly and candy.

The edible vine cacti species belongs to two different genera *Hylocerus* and *Selenicereus*, based on the nature of stem habit, skin and pulp color. Three species of *Hylocerus undatus* (white flesh with red skin) *H. polyrhizus* (red flesh with red skin) *and Selenicereus megalanthus* (white flesh with yellow skin) are cultivated in the world (Gunasena and Pushpakumara, 2006). Dragon fruit was first introduced to Sri Lanka in 1997 (Pushpakumara *et al.*, 2007) and now is a popular fruit crop grown by many farmers for the export market. The cultivated extent of dragon fruit in Sri Lanka at present is about 1200 ha, which includes commercial cultivations and small scale home gardens distributed in low country wet, intermediate and dry zones (*personal*

communication with planting material producers and fruit exporters). The white-fleshed dragon fruit species were introduced earlier to Sri Lanka for cultivation and later the red-fleshed species were imported from Thailand for commercial cultivation. There is a potential for introduction of new pathogens to the country with imported planting materials of dragon fruit.

Recently, a new destructive disease with stem spots followed by yellowing and rotting of affected stems, was reported from red-fleshed dragon fruit fields grown in Matale, Minuwangoda, Kurunegala, Kirindiwela and Wadduwa areas in Sri Lanka. Several diseases of dragon fruit caused by fungi, bacteria and viruses have been recorded in the world (Masanto, 2009; Masyahit *et al.*, 2009). Research on dragon fruit in Sri Lanka has been concentrated mainly on developing cultivation technologies and hence information on diseases of dragon fruit in Sri Lanka is scarce. Identification of pathogens is the first step in developing control measures. Therefore, the objective of the study was to determine the incidence and severity of the diseases associated with dragon fruit cultivation, to identify the causal organisms and to confirm the pathogenicity of stem spots on dragon fruit.

MATERIALS AND METHODS

Isolation and Identification of Pathogens

Disease symptoms were recorded from the infected fields at Matale, Minuwangoda and Kurunegala areas in Sri Lanka and also from samples received to the laboratory of Horticultural Crops Research and Development Institute (HORDI), Gannoruwa from Wadduwa, Galaha and Kirindiwela areas of the country during December 2010 to March 2011. Stem parts and fruits of the infected plants of both red-fleshed and white-fleshed species exhibiting different symptoms were collected for pathogens isolation. The fungal pathogens were isolated from lesions of stem and fruits. A small piece of tissues were surface sterilized in 70 % ethyl alcohol for 2-3 min and placed on potato dextrose agar (PDA) containing streptomycin 0.1 % w/v and were incubated at room temperature 26±2°C. Within one to two days, sub-cultures were made to obtain the pure culture and incubated for 5- 7 days at 26±2°C.

Isolations were also made from tissues taken from the xylem and pith of affected stems to determine if the pathogens have invaded systemically. The mycelial growth and conidia development on PDA were observed. Fungal colonies, which were suspected to be from different genera of fungi, were re-isolated on PDA and single spore inoculants of fungi genera were made. The isolated pathogens were then identified based on microscopic observations of conidia and culture characters on PDA.

Pathogenicity test

The inoculation techniques were carried out according to the methods explained by Michailides (1991). Both red-fleshed and white-fleshed varieties were used in the experiments. Two healthy plants of 15-20 cm tall from each variety raised in pots containing autoclaved soil were used to confirm the pathogenicity. The inoculum was prepared from single spore culture on PDA, spores were suspended in sterile distilled water and the concentration of the collected spore suspension was adjusted by heamocytometer to obtain a spore concentration of 10^5 conidia/ml. Each fungal pathogen was wound inoculated into stems of healthy dragon fruit (red-fleshed and white-fleshed) plants. The control plants were inoculated with sterile distilled water. Each inoculated plant was covered with a moist polythene bag for 72 hrs to create a high humid condition and kept in the greenhouse for 3 - 4 days. Plants were kept in moist environment for 2 weeks to observe the development of disease symptoms.

Assessment of disease occurrence

Age of the plants of the inspected cultivations was about 1–2 years and was at vegetative to reproductive stage at the time of observation. The disease assessment was taken from five locations from the infected fields at Matale, Minuwangoda and Kurunegala areas in Sri Lanka. Fifty dragon fruit plants from each species were randomly sampled from each location to assess the disease occurrence. Disease Incidence (DI) was calculated by using the following equation.

$$DI = \frac{\text{No. of infected plant units}}{\text{Total no. of plant units assessed}} \times 100$$

The Disease Severity (DS) was determined according to the alternative rating scale proposed by Brown and Britton (1986) using the scale 0 = no spots, 1 = 0-20% , 2 = 20-40%, 3 = 40-60%, 4 = 60-80% and 5 = 80-100% stem affected, respectively. Additional information such as management practices was also recorded.

RESULTS AND DISCUSSION

Disease symptoms

Two major diseases symptoms, stem spot and anthracnose were observed during the investigation period. Stem spots first appeared as small, round, chlorotic spots, which enlarged to a maximum of 0.5 cm in diameter

and later become reddish-brown. On the fruit, lesions began with small brown spots, slightly raised. The fungus-infected fruits could not be sold as fresh fruits due to their poor appearance. The lesions coalesced and made large yellowing areas followed by rotting and formed cankers, and the stems died in the later stage of the disease development. Numerous black pycnidia, which are globose in shape, were found on the cankers (Figure 1).

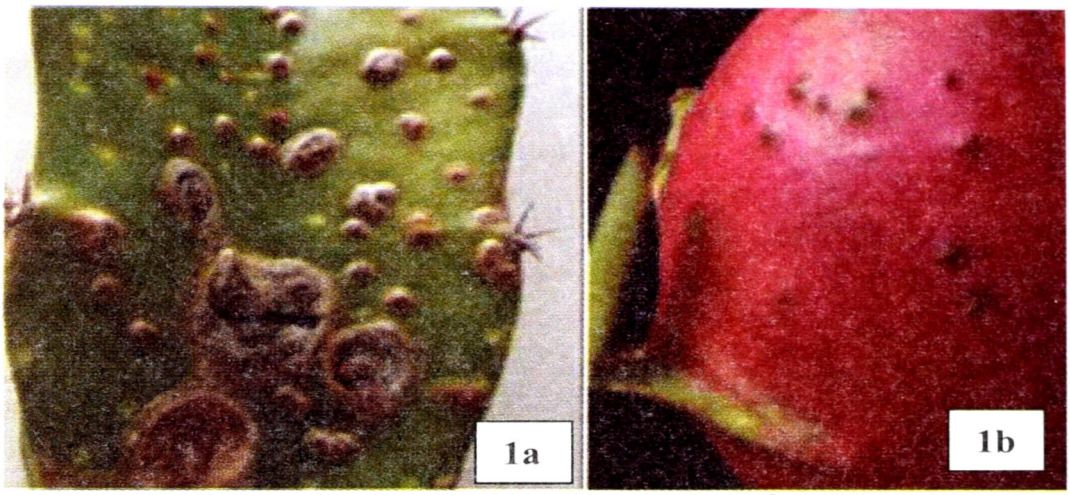

Figure 1. Stem spot symptoms on dragon fruit; 1a- Stem spot initially yellow, become red and finally brown. 1b- Symptoms on fruit

Anthracnose symptoms appeared initially as reddish brown lesions with chlorotic haloes near ribs of the stem and on fruit, and then coalesced to rot (Figure 2). Later, black, minute, slightly raised acervuli (fruiting bodies) were developed as the dark concentric rings on the lesions and then coalesced to rot. Pink masses of conidia developed from the acervuli under high humid conditions. Sometimes mixed infections with stem spot could be seen in red-fleshed variety.

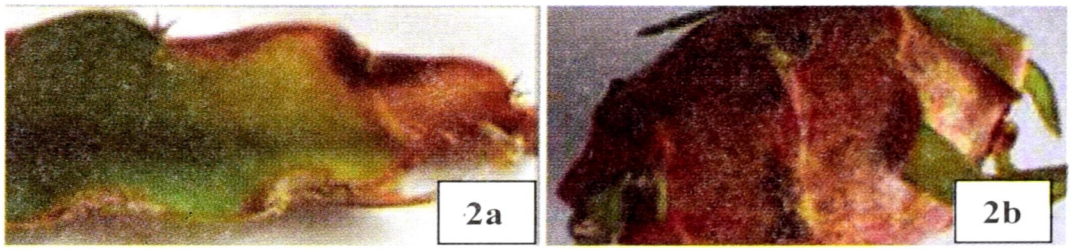

Figure 2. Anthracnose symptoms on dragon fruit; 2a. brown lesions near to rib edge and Lesions coalesced to rot, 2b. Symptoms on fruit

Isolation and identification of pathogen

Two different fungi were isolated, namely *Botryosphaeria* sp. and *Colletotrichum* sp. The *Botryosphaeria* sp. was isolated from white spot and brown spot lesions on stem, in the xylem and in pith tissues, thus indicating that this fungus has potentail to spread throughout the plant.

Cultures of *Botryosphaeria* sp., initially white to olive green in both sides, but after 2-3 days become gray to black and a consistently fast-growing, wasisolated from these diseased stems and fruits. The conidia observed in these bodies were one-celled, hyaline and ellipsoidal to fusoid with truncate bases. The isolated fungus similar to the anamorphic *Fusicoccum* state of *B. dothidea* (Moug.:Fr) Ces & De Not. The teleomorph was never observed in culture, but was seen on infected plants.

Colletotrichum sp. was isolated from the reddish-brown sunken lesions near ribs of affected plant stems. The fungus had whitish orange colony and turn dark grey when older. Reverse of colony grey, black with age and formed perithecia. Conidia are hyaline, aseptate, unicellular and cylindrical with obtuse ends. Isolates of both fungi were then identified by comparison of their colony characters and morphology on PDA and microscopic observations of fruiting bodies and conidia with the published data (Mordue, 1971; Valencia *et al.*, 2003b; Brooks and Ferrin, 1994). Based on above literature, *Botryosphaeria* species was identified as *B. dothidea* (Moug: Fr) Ces and De Not. (Anamorph: *Fusicoccum aesculi*) and *Colletotrichum* sp. was identified as *C. gloeosporioides* (Penz.) Sacc.

Pathogenicity test

Typical symptoms of stem spots and anthracnose were developed on the stems of both dragon fruit varieties within 3-4 and 5-7days after inoculation, respectively. Both pathogens were re-isolated from artificially inoculated plats satisfying the Koch's postulate. All of the control plants inoculated with distilled water and maintained under same conditions remained healthy.

Based on disease symptoms of the infected plants, pathogenicity test, colony and morphological characters of fruiting bodies and conidia, and microscopic observations, two pathogens were identified as *B. dothidea* (Mong. Fr.) Ces and De Not and *C. gloeosporioides* (Penz.) Sacc. causing stem spots and anthracnose, respectively. Infections of stem spot disease and anthracnose in fruits reduce yield and quality on susceptible varieties under favorable environmental conditions. This pathogen can survive in infected crop debris and can also be transmitted through planting materials. Heavy

rains that prevailed during the early *maha* season coupled with susceptible dragon fruit variety seemed to have favored the development and spread of the disease. Destruction of crop debris will reduce the infection while the use of healthy planting material is important to control the disease.

Stem spot caused by *B. dothidea* has been previously reported to cause panicle and shoot blight and canker diseases of pistachio, peach, apple and fruit rot of avocado, guava, mango and forest trees, chaparral bushes (Brooks and Ferrin, 1994) and many other plant species. The disease has also been recognized as one of the quarantine pests in the world (Brown and Britton, 1986; Michailides, 1991; Menge and Ploetz, 2003; Ploetz, 2003; Jayawardena and Silva, 2010). Hence, it has the potential to cause serious economic losses in dragon fruit and other local fruit crops in the future.

Assessment of disease occurrence

The stem spots symptoms first appeared during the early *maha* season 2010/2011 in all the areas where red-fleshed exotic dragon fruit species was cultivated, but low infections were reported from the white- fleshed species planted adjacent to this red-fleshed variety. Stem spot disease had 100 % incidence and the highest severity at a score scale of 3-4 (40- 80%) in red-fleshed dragon fruit in most of the locations. However, a low incidence (1-5 %) with few spots were found in the white-fleshed species implying that the species is tolerant to the stem spot disease. Anthracnose had relatively lower incidence than stem spot at a range of 1-5 % in both species. Infections of both diseases could not be observed in the dragon fruit cultivation at HORDI research fields (Table 1).

The diseases were low even in red-fleshed dragon fruit variety in one location out of three at Minuwangoda area due to that the crop has been maintained with good management practices (including fertilization, irrigation, weed control) and field sanitation (including pruning of infected stems and destroying them, proper disinfection of pruning equipments), which has helped reducing the disease severity and incidence.

Some farmers had not managed the crop well and the stem spot disease was worse in such cultivations and the affected stems formed cankers due to moisture stress. The stem cuttings used from the infected fields to raise a new crop also found to be infected with stem spots, which clearly indicated that the main method of spread of the stem spot disease is through the diseased planting materials. Even though, the growers sprayed several chemicals available at the market to control the stem spot disease, the disease management has failed. Currently, there is no fungicide recommendation for

dragon fruit cultivation. Therefore, there is a need to identify effective chemicals to control the stem spot disease of dragon fruit.

Table 1. Incidence and severity of stem spot and anthracnose in red-fleshed and white-fleshed varieties of dragon fruit in different locations in Sri Lanka.

District/province	*Red-fleshed*		*White-fleshed*		*Fungi isolated*
	DI (%)	*DS**	*DI (%)*	*DS**	
Matale	100	4	2	0-1	Stem spot /
Kurunegalla	100	3	1	0-1	*Botryosphaeria dothidea*
Minuwangoda 1	20	1	-	-	
Minuwangoda 2	100	3	-	-	
Minuwangoda 3	100	4	5	1	
Research fields/Gannoruwa	-	-	-	-	
Matale	5	1	3	1	Anthracnose / *Colletotrichum gloeosporioides*
Kurunegalla	-	-	1	1	
Minuwangoda 1	-	-	-	-	
Minuwangoda 2	-	-	2	1	
Minuwangoda 3	2	1	5	1-2	
Research fields/Gannoruwa	-	-	-	-	

*DS score: 0= no spots, 1= 0-20%, 2= 20-40%, 3= 40-60%, 4= 60-80%, 5= 80-100% stem affected (Brown and Britton, 1986), - no incidence

Lack of pathogen-free planting materials and knowledge on cultural and disease management practices were identified as the key constraints to dragon fruit cultivation. Awareness programs on the risk of introducing new pathogens through importation of planning materials to the country and to follow the quarantine regulations is a imperativeIt is also important to conduct training programs for growers of dragon fruit on good cultural (weeding, fertilizing, sanitation, etc.) and disease management practices.

CONCLUSIONS

Stem spots and Anthracnose on red-fleshed dragon fruit have been identified as fungal diseases caused by *Botryosphaeria dothidea* (Moug. Fr.) Ces and De Not. (Anamorph: *Fusicoccum aesculi*), a quarantine pathogen and *Colletotrichum gloeosporioides* (Penz) Sacc., respectively. Both diseases can be transmitted through planting material. Further studies on identification on

new pre- and post-harvest pathogens of dragon fruit and their economic impact and screening of new chemicals are needed.

REFERENCES

Brooks, F.E. and D.M. Ferrin, 1994. Branch dieback of southern California Chaparral vegetation caused by *Botryosphaeria dothidea*. Phytopathology. 84:78-83.

Brown, F.E., D.M. Britton. 1986. *Botryosphaeria* diseases of apple and peach In the Southeastern United Status. Plant Disease. 70: 480-484.

Gunasena, H.P.M. and D.K.N.G. Pushpakumara. 2006. Dragon fruit- *Hylocereus undatus* (Haw.) Britton and Rose: a fruit for the future. Sri Lanka Council for Agricultural Policy, Wijerama Mawatha, Colombo 7, Sri Lanka.

Jayawardena, D.T.P. and R.S.Y. De Silva. 2010. Pest risk associated with importation of dragon fruit. Paper presented in International Symposium 2010. University of Ruhuna. Sri Lanka.

Masanto, M. 2009. The first report of the occurrence of anthracnose disease caused by *Colletrotrichum gloeosporides* on dragon fruit (*Hylocereus spp.)* In peninsular Malaysia. American Journal of Applied Science 6:902 - 912.

Masyahit, M., K. Sijam., Y. Awang and M.G.M. Satar. 2009. *In vitro* Assay of Factors affecting the growth of pathogens Associated with diseases on Dragonfruit (*Hylocereus spp.)* In peninsular Malaysia. Plant Pathology Journal. 8 (4):144– 151.

Menge, J.A. and R.C. Ploetz. 2003. Diseases of Avocado. In Diseases of Tropical Fruit crops, Eds. R.C. Ploetz. Pp 35-72. CABI publishing. UK.

Michailides,T.J. 1991. Pathogenicity, distribution, sources of inoculums and Infection courts of *Botryosphaeria dothidea* on pistachio. Phytopathology 81: 566-573.

Mizrahi, Y and A. Nerd.1999. Climbing and columnar cacti: New arid Land crops. In Perspective on new crops and new uses. Eds. J. Janick. Pp 358-366. ASHS press, American Society of Horticultural Science, Alexandra, Virginia.

Mordue, J.E. 1971. (*Glomerella cingulata*). CMI description of pathogenic fungi and Bacteria. No. 315. The common wealth Mycological Institute. Kew,Surrey, England. Ploetz, R.C. 2003. Disease of Tropical Fruit Crops. Diseases of Mango. CABI Publishing. 327p.

Ploetz, R.C. 2003. Diseases of Mango. In Ploetz (ed) Diseases of Tropical Fruit Crops. CAB International Wallingford, Oxon, UK.

Pushpakumara, D.K.N.G., H.P.M. Gunasena and V.P. Singh. 2007. Underutilized fruit trees in Sri Lanka, Volume 1. World Agroforestry Centre. South Asia Reegional Office, New Delhi, India; National Multipurpose Tree Species of Sri Lanka; Sri Lanka Council for Agricultural Policy, Asian Centre for Underutilized Crops, Sri Lanka.fruit trees Wijerama Mawatha, Colombo 7, Sri Lanka.

Valencia, B.A.J., J.S. Sandoval - Islas, E. Cardenas sorians, T.J. Michailides and G.R. Sanchez. 2003a. *Botryosphaeria dothidea* causing stem spots on *Hylocereus undatus* in Mexico. Plant Pathology 52(6): 803.

Valencia, B.A.J., J.S. Sandoval - Islas., E. Cardenas sorians., T.J. Michailides and G.R. Sanchez. 2003b. A New stem spot disease of Pitahaya [*Hylocereus undatus* (Haw) Britton and Rose] caused by Fusicoccum – like anarmoph of *Botryosphaeria dothidea* (Mong.Er) Ces and De Not in Mexico Fitopatologia 22: 140 – 142.