Short communication

First Report of *Botryosphaeria dothidea* (Moug. : Fr.) Ces. & De Not. (Anamorph: *Fusicoccum aesculi* Corda) on *Jatropha curcas* L. in China

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Abstract

In Luodian County of Guizhou Province, P.R. China, the biofuel crop *Jatropha curcas* L. is prone to many diseases, of which stem and branch canker is one of the most serious diseases. In the field, the disease shows different symptoms and usually results in the death of seedlings, or death of branches and stems resulting in tree decline. Most fungal isolates from the infected stems have similar colonial characteristics and microscopic features, and are pathogenic to *J. curcas* as validated by the pathogenicity test. Based on the results of morphological study and current taxonomic status of the related fungal group, the fungus that causes the canker disease of *J. curcas* in Luodian county of China is identified as *Botryosphaeria dothidea* (Moug. : Fr.) Ces. & De Not., and the anamorph is *Fusicoccum aesculi* Corda.

Keywords: Biofuel crop; Canker disease; Pathogenicity; Causal agent

Physic nut or jatropha (*Jatropha curcas* L.) is a multipurpose plant valued traditionally for its medicinal properties and, recently, as a potential biofuel crop with large-scale plantations being created in India, South-East Asia, West and South Africa, Central and South America. Traditionally, jatropha was believed to be very resistant to pests and diseases; however, when the crop was planted as large-scale monocultures, the species turned out to be susceptible to many pests and diseases. Over the past years, we had investigated and found numerous harmful organisms in the jatropha plantations of Luodian County, which is one of the main jatropha production areas of China. Among them, branch and stem canker is one of the most serious diseases, since it occurs widely and usually results in the death of the seedlings, branches, stems or even the whole plant, and thereby results in great economic losses.

Outbreak of the disease was first noticed during the period of March to April in 2009, after a long period of drought. It occurred all over the jatropha growing areas in Luodian, including nurseries and plantations (authors' observations). Young seedlings, newly transplanted seedlings and stressed trees were mainly affected. Incidences of the disease varied from 15.4 to 94.8% according to locations and crop age. For young seedlings in some locations where water shortage was severe, the disease incidence was 85.6 to 95.4%, most of which was in the symptomatic types of sunken lesions or tip blight. In March, 2011, after a long period of freezing, we carried out an investigation on the jatropha plantations and observed widespread occurrence of branch and stem blistering type of canker disease, with incidence up to 92.3%.

The pathogen infected the seedlings or stressed trees

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mostly through wounds, terminal buds, lateral buds, leaf scars, branch collar ridges, lenticels, etc. Symptoms appeared first as gummy exudations (bleeding) from the infection point, which quickly turned to reddish brown or blood-red and finally turning to dark brown or even black. With the development of the disease, infected tissues became darkened, rotted, and finally dried and sank, with the old lesions sometimes cracking. Fruitbodies occurred in different forms: some were scattered small herpes (still under the epidermis), some were numerous black dots (erupted from the epidermis); usually, numerous fruitbodies converged and appeared in the form of long rough ridges developing diffusely under the bark and later erupted through the bark. In the field, four typical types of symptoms could be distinguished, based on different initial infection modes: *tip blight*: pathogen infected the plant through the terminal buds and developed downward, resulting in the death of tips or upper part of the stems or branches (Fig. 1A); *sunken lesion:* pathogen infected the plant through the buds on the middle part of stems and developed outwards and inwards, finally forming a sunken lesions on the stem; usually, the lesions would completely encircle the stem or branch and kill the plant parts above (Fig. 1B); *neck rot*: the pathogen infected the plant through the weak points on the branch collar or branch bark ridge, resulting in the death of two or more branches at the same time (Fig. 1C); *blistering*: the pathogen infected the plant through the weak point of the wounded bark (usually because of frost injury), resulting in the appearance of blistering all around the stems or branches, which becomes finally rotten and shrunk, with bands of wide rough ridges (Fig. 1D). Microscopic examination of the specimens revealed that most of them had similar structures and were the products of the same fungus.

Several fungi were observed and identified tentatively by direct examination of the fruitbodies (Table 1). However, most of them were not consistently observed, and thus were not considered as the main causal agents of the canker disease. Only one fungal identity, with the typical features of the genus *Botryosphaeria*, which was thus tentatively identified as *B.* sp.1, had a frequency of 77.8%. Its morphological characteristics are multi-layer-walled fruiting bodies (stroma) initially formed under and later erupted through the bark, usually in clusters (Fig. 1E). Ascomata are separate or grouped in complex multilocular stromata, becoming erumpent through the epidermis and opening through a well-developed ostiole; outer wall are dark, thick-walled, becoming paler and thinner towards the interior; contents conspicuously white when dry (Fig. 1G). Asci develop amongst the pseudoparaphyses which are hyaline, septate, branched, 2 to 3.5 µm wide; asci are bitunicate, clavate, hyaline, stipitate, each containing eight spores. Ascospores are unicellular, hyaline, ovate-oblong or broadly fusoid, smooth, thin-walled, unicellular, sometimes with tapered ends giving a spindle-shaped appearance: 7.71–11.90×16.65–25.75 µm (Fig. 1I). Conidiomata often develop on the same stroma as the ascomata (Fig. 1H) structurally resembling the latter. Pycnidia are ostiolate, subglobose, globose to pyriform.

Table 1. Fungi observed from the stem and branch canker lesions of *Jatropha curcas*

Species	Observed times	Observed frequency (%)	Remarks
Botryosphaeria sp.1	35	77.8	Consistently isolated and having similar microscopic features
Botryosphaeria sp. 2	2	4.4	Occurred mixing with *B.* sp.1
Botryosphaeria sp. 3	1	2.2	Occurred mixing with *B.* sp.1
Lasiodiplodia sp.	3	6.7	Observed from the rotted collar
Pestalotiopsis sp.	1	2.2	Observed from the blighted top of a greenhouse seedling
Septoria sp.	1	2.2	Occurred mixing with *B.* sp.1
Unidentified	2	4.4	Occurred mixing with *B.* sp.1

Conidiophores are simple, hyaline, cylindrical, smooth, 0 to 1 septate, producing one or more conidia apically. Conidia are hyaline, aseptate, straight, narrowly fusiform or irregularly fusiform with subobtuse apex and subtruncate to bluntly rounded bases, 4.8–8.1×15.8–29.7μm (Fig. 1J), and they are not becoming septate or darker with age. In older specimens, spermogonia are usually found in the same stroma (Fig. 1K), and spermatia are hyaline, aseptate, rod-shaped, 1.25–1.78×2.54–5.17 μm (Fig. 1L).

Fungal isolates were mainly cultured from diseased jatropha branches and stems with different symptoms, while several isolates were cultured directly from the fresh fruitbodies formed on the canker lesions. Pure cultures were obtained by mycelium tip culture and stored in refrigerator at 4°C. Most of the isolates showed similar colonial characteristics and microscopic features; for example, on PDA medium at 28°C, the cultures were initially white with abundant aerial mycelium, gradually becoming grey to dark grey (without any pigment), while

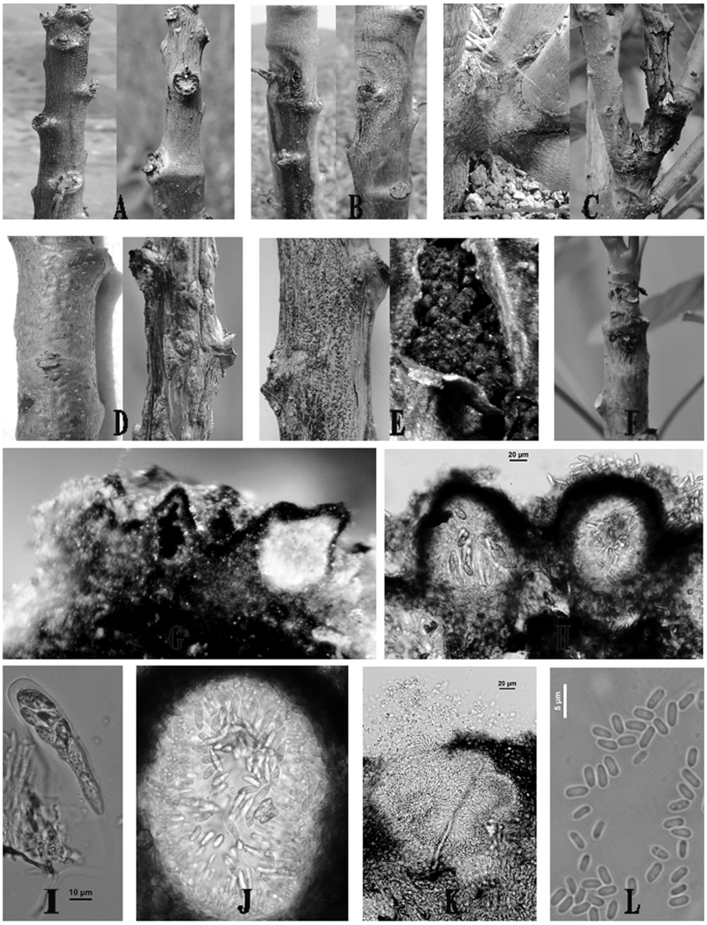

Figure 1. Stem and branch canker disease of *Jatropha curcas* L. and the pathogen *Botryosphaeria dothidea* (Moug. : Fr.) Ces. & De Not. A-D. Different symptom types of the canker diseases including the early stage (left) and the later stage (right) (A. *tip blight*; B. *sunken lesion*; C. *neck rot*; D. *blistering*); E. Fruitbodies on the stem, showing the overall appearance (left) and the close-up of the converged fruit bodies erupting through the bark (right); F. Pathogenecity test in greenhouse, showing a healthy seedling was infected by the pathogen though the leaf scar; G. Transverse section of the stroma; H. Close-up of the stroma showing that the conidiomata usually develop together with the Ascomata; I. One ascus of the pathogen; J. Conidiomata of the pathogen; K. Spermogonia of the pathogen; L. Spermatia of the pathogen.

the reverse side of the colonies was at first white and finally black. After 3 to 4 weeks, rough fruitbodies formed abundantly among the hyphal layer and covered with olive green hyphae. The fruitbodies had similar appearance and inner structures with those observed from the natural fruitbodies, except that the ascomata and the microconidia were rarely found on the artificial cultures. Five isolates with most typical features were selected for pathogenicity test (Table 2), of which fresh and mature fruitbodies were collected and were mashed and, after adding distilled water and being stirred, spore suspensions were produced, with concentration of 3.61×10^4 to 5.58×10^5 spores/ml, respectively. For each isolate, 15 healthy, 2-year-old container seedlings were used for the pathogenicity test, and they were divided into three groups, each containing five seedlings: Group 1: stem of the seedling were slightly wounded by a knife after which they were sprayed with spore suspension; Group 2: sprayed with spore suspension directly without any wounds; Group 3: sprayed with distilled water without any wounds (control). All of the inoculated seedlings were kept in the greenhouse at 20 to 32°C with regular watering, and each treatment was set at a distance of 2 m from others. After 1 month of growing in the greenhouse, all treated seedlings (25) showed typical canker lesions in the wounds. Only one non-wounded seedling treated with one isolate (isolate no.1) showed an obvious canker lesion around the point of a leaf scar (Fig. 1F), while all other seedlings with or without spore suspension treatment showed no signs of canker disease. Besides, the development of the canker (indicated as the size of lesion) was generally much slower than in the field condition. This might be due to better growth in greenhouse, which resulted in higher vigour of the seedlings, which enhanced their resistance to pathogen infection. The re-isolated fungal isolates from the artificially infected stem lesions showed the same morphological characters as the inoculated isolates, fulfilling Koch's postulates. The pathogenicity tests also showed that the pathogen infected the stem mainly through the wounds.

Botryosphaeria is a species-rich genus with a cosmopolitan distribution, commonly associated with dieback and cankers of woody plants (Denman et al., 2000; Crous et al., 2006). Taxonomically, it can be defined as fungus forming uni-to multi-locular ascomata with multi-layered walls, occurring singly or in clusters and usually being intermixed with conidiomata, and the asci are bitunicate, with a well-developed apical chamber (Crous et al., 2006; Zhao, 2007). However, species differences in this genus are manifested in the anamorph than in the teleomorph, thus identification of *Botryosphaeria* species is based mainly on the anamorphic characters (Denman et al., 2000). With the progress of molecular biology, the entire taxonomy of the genus *Botryosphaeria* has undergone changes in the past few years, and an anamorph-based taxonomic system of *Botryosphaeria* is beginning to be established (Denman et al., 2000; Crous et al., 2006; Zhao, 2007). According to this new taxonomic standard for *Botryosphaeria*, combining with the above observed morphological characteristics, the pathogen causing stem and branch canker of *Jatropha curcas* in Luodian is identified as *B. dothidea* (Moug.: Fr.) Ces. & De Not., and the

Table 2. Five isolates used in the pathogenicity test

Isolates	Origins (in Luodian)	Disease symptoms (spores/ml)	Tissues isolated	Concentration of the spore suspension
1	Gaolan	tip blight	advancing edges of the blight	1.54×10^5
2	Gaolan	sunken lesion	advancing edges of the lesion	3.23×10^5
3	Moyang	blistering	fresh blistering tissue	5.58×10^5
4	Moyang	neck rot	mature fruit bodies	2.44×10^5
5	Moyang	sunken lesion	fresh fruit bodies	3.61×10^4

anamorph as *Fusicoccum aesculi* Corda. The available literature revealed that, this is the first report of *B. dothidea* causing branch and stem canker of *Jatropha curcas* in China.

Results of molecular biological studies show that some fungi have close relationships with *B. dothidea*, and they are *B. cortices, B. mamane, F. dimidiatum* (Alves et al., 2004; Farr et al., 2005; Phillips et al., 2006). However, they can be differentiated from each other by their anamorphs. *B. cortices* mainly infects *Vaccinium* plants and has a bigger size of conidia than that of *B. dothidea* (Phillips et al., 2006); *B. mamane* has conidia longer than 30 µm (Zhao, 2007); *F. dimidiatum* has even more obvious difference with *B. dothidea* since the former's conidia usually have two septa with darker central cell, and because of this, it currently has been treated under a new genus *Neoscytalidium* with a new name of *N. dimidiatum* (Crous et al., 2006).

Similar diseases were reported earlier in other parts of China and in other counties. For instance, stem rot and collar rot caused by *Dothiorella gregaria* Sacc. and *Diplodia sp.*, respectively in Yongren County of Yunnan, China (Wang et al., 2009); bark rot disease caused by *Phoma* sp. in Sichuan (Zhou et al., 2008); root and collar rot disease caused by *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. from India (Latha et al., 2009) and Brazil (Pereira et al., 2009); while in China and Malaysia, the same fungus caused stem canker (Fu et al., 2011; Sulaiman et al., 2012). *Pestalotiopsis mangiferae* (Henn.) Steyaert was also reported causing stem canker disease of *J. curcas*, resulting in a large-scale mortality of young plants in India (Pandey et al., 2006). In Yunnan Province of China, canker disease of cuttings was caused by *B. fuliginosa* (Mongeot & Nestler) Ellis. & Ev. (Wu et al., 2008). Recently, a black rot disease on *J. curcas* also caused by *B. dothidea* was reported in India (Srinivasa et al., 2011), but it showed the symptoms of shrivelling and discoloration of stems at the base of the plants, which is more like the symptoms of root and collar rot disease caused by *L. theobromae* (Latha et al., 2009; Pereira et al., 2009). We have observed that *L. theobromae* mainly infects the collar rather than the upper parts of the stem, which are more likely infected by *B. dothidea.*

Similar diseases having different pathogen easily make us confused. Several reasons may account for this situation. Firstly, stem or branch of *J. curcas* is actually prone to be infected by many different pathogens, and as a result, different pathogens are identified from the diseased stems or branches. Secondly, different pathogens have different infection mechanisms and thus have different infection sites, and as a result, isolates from different parts of the diseased plants may be different, making the researcher confused with mistaken identification. Thirdly, several pathogens can co-infect the plants at the same locations, and in this case, careful analysis and evaluation are needed with the true causal agent discriminated and determined.

In Luodian, occurrence and spread of this disease are mostly observed in March to April when drought conditions prevail and when plants have not fully recovered from the winter freezing injury. This study is in accordance with many previous studies showing that *B. dothidea* is an opportunistic pathogen that becomes more of a problem to stressed plants. Accordingly, management of the disease is more important than control, e.g., to enhance the vigor of the trees by adequate watering and fertilizing, and, if necessary, pro-treating the trees by spraying chemicals to prevent the initial infection of pathogen spores. At the early development stage of the disease, chemical treatments may control the disease to some extent; but when severely infected, it is strongly suggested to remove and destroy the diseased branches or stems or the dead trees.

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