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**Ceratocystis sp. CAUSES CROWN WILT OF Acacia spp. PLANTED IN SOME ECOLOGICAL ZONES OF VIETNAM**

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**Abstract**

The plantation area in Vietnam of *Acacia auriculiformis*, *A. mangium* and their hybrid has expanded greatly in the last decade. Recently, a new stem canker disease causing symptoms of crown wilt, followed by wood discoloration then death of infected trees has occurred in many ecological zones. Ascomata were obtained by incubating discolored wood pieces in moist chambers or by carrot baiting. Isolates of fungi were obtained on PDA medium by taking spores emerging from the tips of ascomata necks. *Ceratocystis* was identified based on ascospore morphology and conidial types. Twenty six isolates of *Ceratocystis* were used for pathogenicity assessment on 8-month old seedlings of *A. mangium* in a nursery, with 5 seedlings per isolate. Stems were inoculated by inserting an 8 mm diameter PDA plug covered with 15-day old mycelia onto the cambium about 50 cm above the ground. Five seedlings were inoculated with sterile PDA plugs to serve as the control. The wounds and plugs were sealed with parafilm to protect them against desiccation and rain. After 60 days of inoculation, based on lesion development and tree death, the pathogenicity of the isolates were identified: 2 isolates (AA8, AMH12) nil, 4 isolates (AAHX1, AMH40, AMD26, AHDL1) low, 4 isolates (AA22, AMH9, AMMB7, AHXL3) moderate, 3 isolates (AMBL3, AMPL2, AMH5) high, and 13 isolates (AA54, AA62, AMH24, AMH26, AMH41, AMHX1, AMQN1, AMBL4, AHBB1, AHB1, AHBP1, AHXL1 and AHXL2) very high level of pathogenicity causing plant death. This is the first record of *Ceratocystis* causing damage to *Acacia* plantations in Vietnam. The origin of the pathogen is unknown. Work is progressing to determine whether the species is the same as that known to cause damage to *A. mangium* plantations in Indonesia.

Keywords: *Acacia auriculiformis*, *Acacia mangium*, acacia hybrid, *Ceratocystis*, crown wilt, new disease record, pathogenicity

**Introduction**

There are more than 1.1 million ha of acacia plantations in Vietnam, providing raw materials for the pulp, chip, board and other industries. Acacias were introduced to Vietnam in the 1960s and *Acacia auriculiformis* was chosen for large scale plantings in many locations, mostly in southern provinces (Turnbull et al., 1998). Later, *A. mangium* and *A. auriculiformis* were selected for planting in the north-east, centre and south-east of Vietnam. In 1991, naturally occurring *A. mangium* and *A. auriculiformis* hybrids were observed growing at Ba Vi research station, near Hanoi city. Since then, hundreds of clones of natural and artificial *Acacia* hybrids have been placed in trials in plantations. Industrial acacia plantations are widespread in Vietnam especially in Quang Ninh, Tuyen Quang, Phu Tho, Thai Nguyen, Thua Thien Hue and Dong Nai provinces. From health surveys conducted 1-2 times a year in commercial plantations and genetic trials, the main pathogens associated with *A. mangium*, *A. auriculiformis* and acacia hybrids were
shown to be *Oidium* spp. causing white mildew disease to seedlings in nursery and young plantations, *Meliola* spp. associated with black mildew disease on mature leaves in the lower part of the crown in high density plantations and in hedge orchards, and stem cankers infected by *Corticium salmonicolor*, which has caused serious problems in hybrid plantations of susceptible clones at 3 years of age (Old et al., 2000). Recently, a new disease of trees in acacia plantations in Vietnam has emerged, associated with crown wilt and stem canker followed by wood discoloration. These symptoms were observed for the first time in Quang Ninh province in 2007 in an acacia hybrid at 3 years of age. Since then, the acacia wilt problem has become a serious issue in Vietnam. In the same period, mortality of *A. mangium* in plantations in Indonesia has been associated with the incidence of a virulent species of *Ceratocystis* (Tarigan et al., 2010). Our objectives in this study were to determine if *Ceratocystis* is associated with the new wilt decline of acacia plantations in Vietnam and to test the virulence of the isolates on *A. mangium*. This is the first report of *Ceratocystis* associated with acacia plantations in Vietnam.

**Materials and Methods**

**Stem sampling**
Trees with recent wilt symptoms were located in acacia plantation in Quang Ninh, Phu Tho, Tuyen Quang, Thua Thien Hue, Binh Duong, Binh Phuoc, Lam Dong and Dong Nai provinces in 2008 (Fig. 1). The trees were dissected with handsaws and samples of discolored wood were removed, placed in paper packets and transported to the laboratory for isolation.

**Fungal isolates**
Isolates of *Ceratocystis* were obtained from the germination of single spores. The wood samples were cut into small pieces, some pieces were placed in plastic bags containing moistened tissue paper for 4-10 days to induce sporulation, other pieces were wrapped between carrot slices (that had first been immersed for 1 min in 70% alcohol) and then placed in plastic bags for 3-5 days, or until fruiting bodies were observed (Moller and De Vay, 1968). Single spore drops were collected directly from fungal fruiting bodies onto PDA medium. Isolates collected in this study are maintained in the culture collection of the Forest Protection Research Division, FSIV for further studies.

**Observation and identification**
Two-week-old cultures grown on PDA were used to describe the morphological characteristics of the isolates. Fruiting structures were observed and measured with an Olympus BX50 microscope. Identification to genus was based on the morphology of fruiting structures, ascospores, conidiphores and conidia.

**Pathogenicity tests**
Stems of 18-month-old *A. mangium* seedlings were inoculated 1 m above the ground with mycelium from 20 isolates of *Ceratocystis* (Table 1). A 10 mm diameter cork borer was used to remove a piece of bark from each stem to expose the cambium. A disc of the same size was taken from the edge of a rapidly growing 11-day-old *Ceratocystis* colony and placed into the exposed wound with the mycelium facing the cambium. In order to prevent desiccation, the inoculation sites were covered with tissue paper moistened with sterile water and secured with masking tape. After 10 weeks, the length (L) of the stem lesion was measured and the pathogenicity of the *Ceratocystis* strains ranked on the following scale: 0 no damage, 1) L ≤ 10 cm, 2) 10 cm < L ≤ 20 cm, 3) 20 cm < L ≤ 30 cm and 4) L > 30 cm.
Based on the severity level of every tree, an average disease index (DI) was calculated according to the following formula:

$$DI = \frac{\sum_{i=1}^{4} n_{ivi}}{N}$$

in which, DI is the average disease index, ni the number of trees infected at disease index i, vi the disease index at level i, N number of trees assessed.

**Result**

**Field observations**

Discolored wood samples were collected from wilted *A. mangium*, *A. auriculiformis* and acacia hybrid trees in 8 provinces that had large areas of acacia plantations and covered a wide geographical range from the north to the south of Vietnam (Fig. 1). Wilt (Fig. 2a), crown dieback (Fig. 2b) and canker symptoms were commonly observed on young *A. mangium*, *A. auriculiformis* and acacia hybrid trees, up to 3 years of age, in plantations. The bark and the wood surrounding the cankers were discolored. The discolored wood typically had a streaked appearance, turning a uniform dark brown to dark blue color with age (Fig. 2c). Of the 26 samples that were collected (Table 1), 2 samples of acacia hybrid and 2 samples of *A. mangium* were associated with an ambrosia beetle *Xylosandrus crassiusculus* (Quang Ninh and Phu Tho provinces, respectively), 1 sample of acacia hybrid was associated with pruning wounds (Binh Phuoc province, Fig. 2d) and the remaining samples were not associated with insects or pruning.

![Figure 1. Distribution of Ceratocystis causing wilt in acacia plantations in Vietnam](image)

**Ceratocystis isolates**

A total of 26 *Ceratocystis* isolates were obtained from diseased acacia collected in Dong Nai, Thua Thien Hue, Binh Duong, Binh Phuoc, Tuyen Quang, Quang Ninh and Lam Dong provinces. Within two weeks incubation in the laboratory, mature ascomata were produced.
Based on the severity level of every tree, an average disease index (DI) was calculated according to the following formula:

\[ DI = \frac{\sum_{i=1}^{n} n_i v_i}{N} \]

in which, DI is the average disease index, \( n_i \) the number of trees infected at disease index \( i \), \( v_i \) the disease index at level \( i \), and \( N \) number of trees assessed.

**Result**

**Field observations**

Discolored wood samples were collected from wilted *A. mangium*, *A. auriculiformis* and acacia hybrid trees in 8 provinces that had large areas of acacia plantations and covered a wide geographical range from the north to the south of Vietnam (Fig. 1). Wilt (Fig. 2a), crown dieback (Fig. 2b) and canker symptoms were commonly observed on young *A. mangium*, *A. auriculiformis* and acacia hybrid trees, up to 3 years of age, in plantations. The bark and the wood surrounding the cankers were discolored. The discolored wood typically had a streaked appearance, turning a uniform dark brown to dark blue color with age (Fig. 2c). Of the 26 samples that were collected (Table 1), 2 samples of acacia hybrid and 2 samples of *A. mangium* were associated with an ambrosia beetle *Xylosandrus crassuisculus* (Quang Ninh and Phu Tho provinces, respectively), 1 sample of acacia hybrid was associated with pruning wounds (Binh Phuoc province, Fig. 2d) and the remaining samples were not associated with insects or pruning.

**Figure 1.** Distribution of *Ceratocystis* causing wilt in acacia plantations in Vietnam

**Figure 2.** Disease symptoms caused by *Ceratocystis* in acacia plantations in Vietnam (a-d) a. Wilted *Acacia mangium* in 2-year-old plantation in Phu Tho province, b. severely impacted *Acacia mangium* in 2-year-old plantation in Phu Tho province, c. section of stem showing canker with stained wood below associated with *Xylosandrus crassuisculus* and distal spread, d. stained wood associated with a pruning wound.

**Pathogenicity**

After 10 weeks from inoculation, all of the *Ceratocystis* isolates caused stem cankers in *A. mangium* and the majority were highly virulent. Cankers ranged in length from 3.5 to 40 cm (data not presented) and the disease index ranged from 1.0-4.0 (Table 1).
Discussion

The first death of acacia associated with wilting, canker ing and discolored wood was observed in Quang Ninh province in north Vietnam in 2007. At this site, *Xylosandrus crassiusculus* was present in all the acacia hybrid trees with wilt symptoms and Fig. 2c shows an example of the distribution of blue-green stain in the wood of one tree. In the following year wilt and stem canker symptoms were found in *A. mangium* and an acacia hybrid in Phu Tho province associated both with and without an insect vector. Annual field surveys of plantation health across Vietnam revealed that, between 2008 and 2011, the disease expanded widely in the whole country with mortality rates of 15-20% in 2011 in Thua Thien Hue and Dong Nai provinces. *Ceratocystis* wilt is now the main threat to acacia plantations in Vietnam.

*Ceratocystis* is a worldwide pathogen of woody plants especially in tropical parts of the world (Kile, 1993) and is known to cause wilt and canker in plantation-grown acacias (Roux and Wingfield 2009). In past decades, several species have caused minor damage to acacia plantations in Brazil (Ribeiro et al., 1988; *C. fimbriata s.l.* on *Acacia decurrens* and South Africa (Morris et al., 1993; Roux and Wingfield, 1997; *C. fimbriata* and *C. albifundus* on *A. mearnsii*). More recently, however, *Ceratocystis* has been recognized as an emerging threat to plantations in Asia and Australia (Wingfield et al., 2009). During the course of recent disease surveys in *A. mangium* plantations in Sumatra (Indonesia), significant mortality of young trees showing rapid wilt symptoms was observed and two species of *Ceratocystis* were consistently associated with diseased trees, *C. acaciivora* and *C. manginecans* (Tarigan et al., 2010). Both species produced lesions on inoculation but *C. acaciivora* was the most pathogenic. Similar symptoms to those described by Tarigan et al. (2011) and also in Figure 2 are now present in industrial acacia plantations in Malaysia (David Boden, pers. comm.).

The rapid spread of the disease symptoms in plantations in Vietnam, that are annually monitored for health, suggests that either the pathogen is being vectored or that stands of trees are increasingly becoming stressed and hence are more susceptible to attack by insects and pathogens. Indeed, many of the trees at the time of initial wilting showed symptoms of nutrient imbalance. Whether abiotic stress can lead to bark fracture creating entry wounds for *Ceratocystis* remains to be determined. Furthermore, it is not yet known whether the *Ceratocystis* outbreaks in Vietnam are connected to events occurring elsewhere in SE Asia. Whether the pathogen has been recently introduced or is endemic in the region is yet to be determined. There are unpublished reports of *Ceratocystis* causing canker in some horticultural trees in Vietnam such as *Anacardium occidentale*, *Dimocarpus longan*, *Theobroma cacao* and *Hevea brasiliensis*.

In conclusion, this is the first report of a serious new disease of acacias in Vietnam. Work is progressing to identify resistant acacia clones, the species of *Ceratocystis* that are most virulent and factors that may predispose trees to infection.
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**References**

