The occurrence of gummosis on invasive Acacia decurrens after Mount Merapi eruption in Yogyakarta, Indonesia

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Abstract

1. Gummosis on Acacia decurrens, an invasive tree species, that got established in Merapi Volcano National Park (MVNP) after the eruption of Mount Merapi in 2010 was studied to i) identify the causal organism of the disease, ii) analyze disease symptoms, iii) understand the spatio-temporal distribution of gummosis in the tree population and iv) examine how the disease affects the anatomy of tree wood. 2. Pathological, morphological and molecular studies were used in this studies. 3. Ceratocystis fimbriata was proved to be the causal organism of the disease. The disease spread was probably aided by the ambrosia beetle (Euwallacea sp.) which bores holes on the stem. 4. The disease is noted to spread from the base of the trees, where the ambrosia beetle bores holes first, to the upper part. 5. The number of parenchyma cells in the infected stem was significantly more than in the healthy stem which apparently facilitated water and nutrition transport within the tree helping it to grow normally despite serious gummosis. 6. The management of invasion by A. decurrens in the MVNP area poses a serious challenge due its success as an invader in the volcano impacted area and the threat of the gummosis pathogen spreading to other species both of which will affect the regeneration and establishment of native species and recuperation of the ecosystem.

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Introduction

Large, but infrequent, volcanic eruptions, such as the 2010 eruption of Mount (*Gunung*) Merapi in Yogyakarta, Indonesia, caused significant changes in ecosystem composition and vegetation structure in surrounding areas of the volcano (Anon 2012, unpublished data). Similar ecological impacts were observed in Washington, USA when Mount St. Helens erupted in 1980. The eruption of Mount St. Helens produced a complex of disturbance agents, such as pyroclastic flow, debris avalanche, mud flow, ash deposits, blow down, and several other agents which interacted at specific sites exacerbating the degree of damage and delay in recovery (Adams et al., 1980; Dale et al., 1998; Franklin et al., 1985; del Moral 1993). Moreover, combinations of post-disturbance site characteristics influenced the composition of the biota. The pyroclastic flow from Mount Merapi apparently destroyed all flora and fauna that it contacted in the surrounding area of the volcano.

In a period of 2 yrs following the eruption, *Acacia decurrens*(Wendl.) Willd. (green wattle) was the first tree species to appear, establish, and dominate large areas impacted by the pyroclastic flow. Data collected

by Merapi Volcano National Park (MVNP) in 2012 showed that the density of *A. decurrens* averaged 16167 seedlings ha^{-1} and 11814 saplings ha^{-1} (Anon 2012, unpublished data). Native to Australia, *A. decurrens* is introduced and grown in plantations in several countries in the tropics and subtropics. It is an important invasive species especially in Africa and Oceania where it spreads rapidly via seed and root suckers (CABI, 2018). The tree can invade grass lands, roadsides, savannah and riverine habitats developing dense thickets impacting on native biodiversity and obstructing water flow (Boucher 1978). The rapid establishment of *A. decurrens* in the MVNP area poses a serious threat since it will affect the recuperation of ecosystems and regeneration of native flora impacted by the volcanic eruption.

A preliminary survey conducted in 2014 by MVNP on the health of *A. decurrens* stands showed that the ambrosia beetle, bagworm, *Ceratocystis* sp., *Uromycladium falcatarium* , and *Ganoderma* sp. occur in the stands. Rahayu et al. (2015) have reported that over 50% of *A. decurrens* stands in the region showed gummosis and symptoms of stem canker associated with *Ceratocystis* sp. However, these symptoms were not visible on trees within the area around Mount Merapi before eruption. Gummosis of *A. decurrens* and *Acacia mearnisii* caused by *Ceratocystis fimbriata* Ellis and Halsted was recorded from Brazil and South Africa, respectively by Ribeiro et al. (1988) and Morris et al. (1993). The symptoms described on *A. mearnsii* were similar to those observed on *A. decurrens* in MVNP. The genus *Certocystis* includes some well-known pathogens of trees responsible for a wide range of diseases including stem cankers, vascular wilts and root diseases (Kile, 1993) For e.g., *C. fimbriata* is known to cause diseases of several vegetable crops, fruit trees and forest trees (CABI, 2014). Another species of *Ceratocystis, C. albifundus* occur on *Acacia mearnsii* plantations in southern and eastern Africa causing gum exudation, wood-discoloration, stem cankers, rapid wilting and tree death (Morris et al., 1993; Roux et al. 1999). It is also reported that wounds on trees are a predisposing factor for *C . fimbriata* sensu lato (*s.l .*) to cause infection. These wounds can result from wind and hail damage, growth cracks, insect and animal damage as well as activities such as grafting and pruning.

Recent studies have shown that artificially induced wounds on trees are infected by *Ceratocystis* spp. (Barnes et al, 2003; Roux et al, 2004; Rodas et al, 2008). Success of infection is dependent on a number of physical and environmental factors. For e.g., *C. fimbriata* species complex are able to infect their hosts when viable fungal propagules are deposited onto bark wounds (DeVay et al., 1968). Other *Ceratocystis* spp., such as *C. fagacearum* (Bretz) Hunt, can only infect if viable fungal propagules are deposited onto freshly exposed wood of the host (Kuntz and Drake 1957). Temporal factors also affect the success of infection by *Ceratocystis* spp. For example, Kuntz and Drake (1957) showed that *C. fagacearum* could not cause infection when wounds were older than 24 h. Climatic factors such as temperature and relative humidity have also been shown to influence germination of spores and infection by *Ceratocystis* spp. (Cole and Fergus 1956). So, it needs to be examined whether tree wounds caused by insects was one of the reasons for the occurrence of gummosis on *A. decurrens* in the Mount Merapi region and whether the spread of the disease was assisted by the dominance (monoculture) of *A. decurrens* trees in the MVNP area.

Against this background, the present study aims to i) identify the causal organism associated with the disease, ii) analyze the disease symptoms and predisposing factors for infection, iii) understand the spatio-temporal distribution of gummosis in the tree population and iv) examine the anatomy of the infected wood. This information would help developing methods to manage *A.decurrens* and control the spread of the gummosis pathogen to native plants.

Materials and Methods

Isolation of the causal organism, identification and characterization

The study was conducted at Merapi Volcano National Park (MVNP), which is located in two Indonesian provinces (Yogyakarta and Central Java Provinces) and with geographic coordinates between 110˚15'00" – 110˚37'30" E and 07˚22'30" - 07˚52'30" S, within a 8.4 ha restoration plot. The study area has been occupied and dominated by *A. decurrens* and the trees were approx. 4 yrs old when the study was conducted.

Ten *A. decurrens* trees with gummosis symptoms on the stem were selected randomly and sampled from

the study area. The discolored wood and bark samples were drawn from the leading edge of the gummosis after clearing the gummosis. These excised sections were then wrapped in newspaper to maintain moisture, and they were subsequently transferred to the laboratory for further processing. Carrot slices were used as a bait to isolate *Ceratocystis* from the disease samples (Moller and de Vay 1968). Isolations from stem samples and pathogenicity tests were conducted using standard methodology (Waller et al., 2002). All isolates were maintained on potato dextrose agar (PDA) in the Laboratory of Forests Health and Protection, Faculty of Forestry, University Gadjah Mada, Indonesia. Morphological characteristics of the isolates were studied to identify the species.

DNA extraction from the fungus and amplification of rDNA ITS were conducted in the Molecular Genetics Laboratory, Ministry of Environment and Forestry, Yogyakarta. Sequencing of ITS fragments was conducted in 1st Base Singapore. DNA were extracted using SDS buffer (200mM Tris HCl pH 8.5; 250mM NaCl; 25mM EDTA and 0.5% SDS) (Raeder and Broda 1985) as modified by Glen et al. (2002). The primer pairs used to amplify the rDNA ITS are ITS4 (TCC TCC GCT TAT TGA TAT GC) (White et al. 1990) and ITS1-F (CTT GGT CAT TTA GAG GAA GTA A) (Gardes and Bruns 1993). PCR products were sent to 1st Base (Singapore) for sequencing. DNA sequence chromatograms were viewed in Chromas version 2.6.5 (Technelysium Pty Ltd) software and edited to remove poor quality sequences at each end. Searching of public DNA databases, GenBank (Benson et al. 2017) was conducted to retrieved sequences of high similarity using BLAST (Basic Local Alignment Search Tool) (Altschul et al. 1997). Phylogenetic analysis to confirm the identification of the isolates was conducted using Mega7 (Kumar et al. 2016). Sequences were aligned using Clustal W (Larkin et al. 2007) in BioEdit 7.0.9.0 (Hall 1999) with full multiple alignments prior to phylogenetic analysis. Sequences with high similarity retrieved from GenBank were included in this analysis as references and one sequence from a more distantly related taxon was included as an out group. The phylogenetic tree was constructed using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei 1993).

Assessment of gummosis symptom

To analyze disease incidence and intensity, a 10 m wide transect was laid along a river in the study site and nine 100 x10 m rectangular plots were marked to record observations. Each plot contained 6-10 trees and the total number of trees measured was 79. The first plot was 10 m away from the boundary of the restoration plot and distance between each plot was a minimum of 20 m. The symptoms of gummosis were evaluated on each tree by marking three segments on the stem viz., 1) lower stem (L) which includes the base of the stem up to 137 cm in height (height at which dbh is measured); 2) upper stem (U) - area between 137.1 cm to the base of the first branch and 3) stem covered by the crown (S) (Fig.2)

The number of wounds with new or old gummosis in each stem segment was recorded. Disease severity was calculated based on the number of wounds with gummosis occurring on each tree. The progress of gummosis along the stem was made by observing occurrence of gummosis on each segment on individual stems. Number of wounds with gummosis in each tree segment (L, U, and S), and distance between asymptomatic and symptomatic trees were recorded bimonthly between February and August 2015. Tree positions were recorded using GPS (Global Positioning System) and computed by ArcGIS 10.1.

Mean disease severity (%) on individual tree was calculated using the following formula (Cooke (1998).

Mean of Disease Severity (%) on individual tree (Eq.1)

$$\frac{(z1 + z2 + z3)}{3} \times 100\%$$

Disease incidence was calculated based on the number of trees showing gummosis out of total number of trees observed.

Disease incidence (DI) (Eq.2)

$$\frac{n}{N} \times 100\%$$

Where

n = Number of trees with new (oozing at the time of observation) or old gummosis

N = Total number of trees assessed

z_1 = Number of gummosis on the lower stem

z_2 = Number of gummosis on the upper stem

z_3 = Number of gummosis on the stem covered by crown

'3' represents 3 segments of the stem observed for gummosis

The horizontal pattern of distribution was arrived at by measuring the shortest distance between trees with gummosis symptoms which was then grouped for each measurement occasion.

Anatomy of gummosis affected stem

To analyze changes in anatomy of the wood, 1 cm^3cuboid wood samples were drawn from each of 10 diseased and healthy trees selected at random. Wood close to the infected area from the diseased tree and that from healthy trees (of similar stem diameter and at the same height) was sampled and stored. Transverse, radial and tangential sections of the stem (10-15 µm thick) were cut using a sledge microtome. The sections were then soaked in lactophenol cotton blue for 1 hr, washed in distilled water, dried and added with a drop of xylol. The sections were then gently warmed to remove water and xylol and mounted on slides using Canada balsam or Entellan. The sections were observed under a light microscope (BX51, Olympus Corporation, Japan), photographed using a digital camera (DP 70, Olympus Corporation, Japan) and the dimensions of fibers and parenchyma cells measured using digital images.

Analysis of data

The qualitative data were presented on a qualitative scale and quantitative data were subjected to an analysis of trends to determine the status of incidence and severity of gummosis. The spatial distribution of gummosis symptoms was analyzed using ArcGIS 10.1 software.

Results

Characterization and identification of the causal organism of gummosis

A total of two *Ceratocystis* isolates (UGM AD 1 and UGM AD 2) were obtained from diseased *A. decurrens* at MVNP. Mature ascomata were produced in culture during a 2-week incubation on PDA. The ascomata had black, globose to sub globose bases (Fig. 3a) and long necks with ostiolar divergent hyphae (Fig 3b) exuding hat-shaped ascospores from their tips (Fig. 3c). Ascomata varied in size, the neck and base were 0.2-1.3 and 0.2-0.6 mm long, respectively. Chlamydospores and both barrel-shaped and cylindrical conidia were produced in culture. (Fig. 3d, e). Based on ITS sequence BLAST on the GenBank database (https://blast.ncbi.nlm.nih.gov), isolates (AD 1 and AD 7) from *A. decurens* showed the closest similarity to *Certocystis fimbriata* (Fig. 4).

Molecular analysis involved 15 nucleotide sequences with *Ceratocystis fimbriatomima* (MH863157) as an out group. There were a total of 172 positions in the final dataset Evolutionary analyses were conducted in MEGA7.

Symptoms of gummosis

Gummosis is evidenced by exudation of resin or gum from the wood which gets deposited on tree bark. The gum is produced in response to wounds on the stem due to insect attack, infection by plant pathogens or physical injury. On *A. decurrens* at MVNP, gum exudation was mainly due to wounds made by insects which were proved by boring dust and pitch tubes occurring outside the bark (Fig. 5a) and characteristic galleries under the bark. Our observations showed that most of the holes in the bark were caused by ambrosia beetles (i.e. *Euwallacea* sp.) which can bore into the xylem of the diseased trunk producing copious amounts of frass (Fig 5b). These beetles are known vectors of*Ceratocystis* spp.

Gum exuded from the stem turned dark or black in color after >1-week exposure (Fig. 6a) compared to the newly exuded gum (Fig. 6b). In contrast, gum exuded from the young branches, especially green branches, tended to be clearer in color (Fig. 6c). Most borer holes occurred on the stem, particularly on woody/ older stems, whereas, only limited holes were present on young branches.

Occurrence of symptoms of gummosis on A. decurrens

The first survey (Feb. 2015) indicated incidence of gummosis on 80 % of the trees surveyed. By Aug. 2015 (fourth survey), all the trees (100%) were found infested which indicated an increase of 20% of incidence in six months. Based on the occurrence of gummosis on different segments of the stem, the highest was on the lower stem (L) compared to the upper part (U) and stem covered by crown (S) (Figs. 7 a & b).

Over time, the mean severity of gummosis increased significantly ($R^2 = 0.98$) from the bottom to the top of the tree which indicated that the bark beetles got settled, increased in number and they actively moved from the lower part to the upper part of the stem.

Spatio-temporal distribution of gummosis

Measurement of the nearest distance among symptomatic trees was used as a variable to assess disease spread given that *Ceratocystis* and other disease inducing factors got spread from trees to trees via active movement of the insect vector or passive movement of the wind. Based on the number of trees which showed gummosis from a distance of 1 m to >50 m, it was interpreted that the inoculum was present within 1 to 5 m distance from the healthy trees. The highest number of trees with the incidence of gummosis was observed at 5 m but decreased thereafter without a definite pattern with increasing distance (Fig. 8a). The highest percentage of gummosis incidence also followed the same pattern (Fig. 8b).

Anatomy of the infected stem

It was observed that the percentage of parenchyma cells in infected wood was higher than that on healthy wood (Figs 9). However, the percentage of fiber cell, fiber diameter and length were significantly more on healthy wood compared to that on infected wood (Fig. 10)

Discussion

The occurrence and spread of *A. decurrens* as an invasive species in all ecosystems in MNVP after the eruption of Mount Merapi have been supported by several researchers (Lymberty et al., 2014 and Okoli et al., 2017). However, the ecological impact of the species on the unique successional processes in the post-eruption ecosystems around Mount Merapi is only poorly known.

Ceratocystis fimbriata is proved to be the pathogen associated with gummosis in *A. decurrens.* Ambrosia beetles (*Euwallacea* sp.*)* which bore holes in the stem facilitate infection by *C. fimbriata.* As already discussed, *C. fimbriata* is a pathogen of several crop plants and it is widely distributed in the tropics and subtropics (CABI, 2014). It has been recorded on *A. decurrens* in Brazil (Ribeiro et al. (1988). The occurrence of the pathogen on *A. decurrens* in MNVP poses threat to several crop plants in Indonesia.

In MNVP, although most trees displayed severe gummosis with 3 to 30 wounds per individual stem, the trees appeared healthy, with straight stems, green canopy, and good performance in terms of height and diameter compared to uninfected trees. The normal diameter of 5- yr-old of healthy *A. decurrens* ranged from 9.2 –

(Okoli et al. 2017), while in MVNP the mean diameter of gummosis affected trees was already at 4 years. This indicates that the gummosis had no impact on the diameter growth of *A. decurrens* at MVNP.

The increased number of parenchyma cells in the infected wood and the healthy growth of trees despite gummosis indicate that the parenchyma cells aid in storage, conversion, and active transport of nutrients in the gummosis affected trees (Schwarze et al., 2004). In addition, parenchyma cells can also maintain meristematic activity such as wound healing and regeneration of the young cells. However, *A. decurrens* trees infected by *C.fimbriata* in the Capão Bonito region, Brazil exhibited wilting, branch drying, wood splitting and gum exudation which resulted in tree mortality (Ribeiro et al., 1988). The difference here is that while *A. decurrens* trees in Brazil were cultivated in plantations, trees in MVNP were naturally regenerated under nutrient poor soil and adverse climatic conditions. Thus, the trees in MNVP may have attained resistance towards insect pests and pathogens. The monoculture of *A. decurrens* in plantations also promotes disease severity and spread. However, strength properties of infected trees in MNVP are significantly poor compared to healthy trees since infection affects fiber quality.

Mitchell et al. (2010) have reported that species with high populations, such as invasive species, are expected to exhibit greater accumulation of pathogens over time in comparison to species with thin populations. And, over time, pathogen accumulation may have little or no effect on invasive species due to tolerance, compensation, or phenotypic plasticity (Gilbert and Parker 2006; Alexander 2010). In certain other cases, many invasive species exhibit substantial phenotypic plasticity such that a reduction in population density has little effect on biomass or seed production per unit area. Given the theoretical and empirical demonstrations of the negative effects of pathogen build-up (Clay and Kover 1996; Mordecai 2011), this outcome may seem unlikely, although it is possible.

The vertical position of gummosis symptoms, which were abundant on the lower stem and comparatively less on the upper stem and stem surrounded by the crown, indicate that formation of the wound and subsequent infection by *Ceratocystis* typically initiated from the lower part of the trees. This observation suggests that ambrosia beetles (e.g.,*Euwallacea* sp.) which bore in to the xylem of trees can serve as a vector for *Ceratocystis* spp. (Somasekhara, 1999). The frass which cling close to the holes or accumulate on the bark and/ or at the base of the tree may help spread of the pathogen (Paine et al. 1997; Six 2003; Harrington 2005).

According to Halloin (2003) and Lieutier (2004), bark beetles generally oviposit at a location about 90 cm above ground (on pine trees) which show that boring by the beetle tends to occur near the bottom of the trees or the lower part of stem. Also, bark beetles locate mates and attract or repel other individuals of the same species by emitting species-specific pheromones (Sanborn (1996). Halloin (2003) observed that when the beetles find a suitable host tree, they will release aggregating pheromones to attract other beetles enabling a "mass attack" that can overwhelm defenses of an otherwise healthy tree. These observations will explain the more clustered spatial distribution of the trees affected by gummosis in this study. Also, the study has shown that the number of trees with gummosis symptoms decreases with increasing distance between infected trees. The infection becomes successful when the beetles releases the pheromones and along with it introduces*Ceratocystis* which blocks the sapwood and weakens the tree. The stress caused to trees caused by the pyroclastic flow can be another reason for the susceptibility of the trees to *Ceratocystis.*

Although *C. fimbriata* does not impact growth of the invasive*A. decurrens* at MVNP, the fungal inoculum poses a threat to other plants within successional processes of the recovering ecosystem. Invasive species are known to possess certain chemical weapons that provide a selective advantage over their competitors (Callaway and Ridenour 2004). It thus appears that the tolerance of *A. decurrens* to *C. fimbriata* allow it to compete more successfully as invasive species within MVNP ecosystem.

To conclude, the challenges here are complex, on the one hand the growth and spread of *A. decurrens* is to be managed and on the other, growth of native species needs to be promoted in the MVNP area by protecting them from the invasive plant and the pathogen which occur on it. Considering the highly successful establishment and spread of*A. decurrens* , attempts to manage it and reclaim the land in the MVNP area for regeneration of native species will be an onerous task unless supported by the Government, land managers

and the public, alike.

Conclusion

Ceratocystis fimbriata was proved to be the causal organism of gummosis on *A. decurrens* trees that invaded MVNP ecosystems following eruption of the Mount Merapi volcano. Four year after eruption, the incidence of gummosis on the trees increased from 80 to 100% within a period of 6 months, with most symptoms located on the lower part of the stem, followed by the upper part and on stem around the crown. This positioning of infection indicated that the vector of*C. fimbriata* , viz., *Euwallacea* sp. (ambrosia beetle), initiated boring the tree at its base resulting in initiation of infection there. Although all the *A. decurrens* tree stems exhibited gummosis with number of gummosis wounds ranging from 3 to 30 per tree, most trees remained healthy and continued to grow. The tree survival and growth is apparently due an increase in the number of parenchyma cells in the wood which aided transport of nutrients within the tree. The mean distance between infected trees in the study plots, over a 6-month period, was , but decreased 1- with the disease progress and spread. Management of invasion and spread by *A. decurrens*poses a challenge to all concerned due to the unparalleled invasion success of the species and the threat from by its gummosis pathogen to native species.

Authors' contributions

SR set up the research methodology and led the writing of the manuscript; RGP and JM collected the data and analyzed the data, MAI and WDN contribute to the writing of the manuscript. All authors contributed critically to the draft and gave final approval for the publication.

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Data Accessibility:

- DNA sequences: GenBank accession MH863157
- Other data uploaded as online

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