



Short communication

Cristulariella moricola associated with foliar blight of Camden white gum (*Eucalyptus benthamii*), a bioenergy crop



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ABSTRACT

We report the association of *Cristulariella moricola* (Hino) Redhead with a zonate leaf spot on *Eucalyptus benthamii* (Myrtaceae). We observed the disease outbreak across a series of experimental *E. benthamii* plantings in Barnwell County, South Carolina, USA in October 2015. The disease caused nearly complete foliar necrosis of most trees in one experimental planting, but disease incidence remained at low levels (<10% of foliage affected in most trees) in other plantings. Disease development coincided with periods of extended rainfall, which has also been observed in outbreaks of zonate leaf spot caused by *C. moricola* in other tree species.

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1. Introduction

Eucalyptus is a large genus in the Myrtaceae family that includes more than 700 species, almost all of which are endemic to the Australian continent [1,2]. Many species of *Eucalyptus* have been planted extensively for timber and pulp production in tropical and subtropical areas outside their natural range [3]. *Eucalyptus benthamii*, commonly known as Camden white gum, has exhibited sufficient cold-hardiness to potentially grow in the southeastern USA, where it is generally considered the best candidate *Eucalyptus* species for bioenergy crop production [4]. Maximal production rates for *Eucalyptus* spp. in the southeastern U.S. can be 250% greater than maximal rates for loblolly pine (*Pinus taeda*), and what are considered low rates of growth for *Eucalyptus* spp. in the southeast can be twice the average rate for loblolly pine [5]. However, potential losses due to disease have not been extensively studied for *Eucalyptus* spp. in the Southeast.

Cristulariella moricola (Hino) Redhead (= *Cristulariella pyramidalis* Waterman & Marshall) is the cause of bull's eye or zonate leaf spot on a wide range of woody and herbaceous plant species [6]. The fungus was first recorded in Japan in 1929 [6] and has subsequently been associated with foliage diseases in several Asian countries [7]. The fungus was first observed in the USA in the 1940s, when it was primarily associated with leaf spots on *Acer* spp. [8]. Foliage diseases caused by *C. moricola* have been responsible for economically significant damage in forest, ornamental nursery, and agricultural crops throughout much of the eastern half of the USA [7,9,10]. The disease can develop very rapidly during periods of extended rainfall and can have devastating effects on susceptible plant populations [10,11].

Here, we report *C. moricola* to be associated with a zonate leaf spot of *E. benthamii*. We describe the damage associated with the disease outbreak in experimental research plantings at a South Carolina location; the weather conditions associated with the outbreak; and discuss how this disease may impact the production of *E. benthamii* in the southeastern US.

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2. Materials and methods

A series of six experimental plantings of *E. benthamii*, each initially consisting of 120 individuals, were established in October 2013 within a single watershed. The greatest distance between any two plantings was less than 2.5 km. All of the experimental *E. benthamii* plantings were nested within a three-year-old operational loblolly pine planting, which was surrounded primarily by loblolly pine plantations with some hardwoods interspersed, particularly near riparian zones. In mid-October 2015, zonate leaf spotting (Fig. 1a), along with occasional foliage blight (Fig. 1b) and leaf mortality was observed in a large number of individuals in one planting (33.260084, -81.620444). By the second week of November, widespread foliar blight had affected 60–95% of the foliage in every tree within that planting (Fig. 2). Trees in the other five plantings experienced only a low incidence of zonate leaf spot and associated leaf mortality (<10%). Disease development coincided with periods of extended rainfall, high relative humidity, and moderate temperatures (Fig. 3).

Samples of symptomatic leaves from *E. benthamii* were collected throughout the planting most affected by the disease. In the lab, samples of leaves were wrapped loosely with moist, sterile paper towels, placed in plastic bags and incubated at 20 °C for approximately 18 h, and subsequently observed with a dissecting microscope. Large fungal fruiting structures with a pyramidal shape and numerous, small conidia were consistently observed on the upper surface of necrotic areas on infected leaves. Isolates of the fungus were obtained by transferring conidia to malt extract agar (MEA) and potato dextrose agar (PDA) and incubating plates at 25 °C. The identity of the fungus was subsequently determined based on conidial morphology and DNA sequencing.

Extraction of DNA from fungal cultures on MEA used PrepMan Ultra (Applied Biosystems, Foster City, CA). The ITS region of three isolates was amplified using previously described primer and PCR conditions [12]. The PCR products were sequenced with ITS1-F at the Iowa State University DNA Facility.

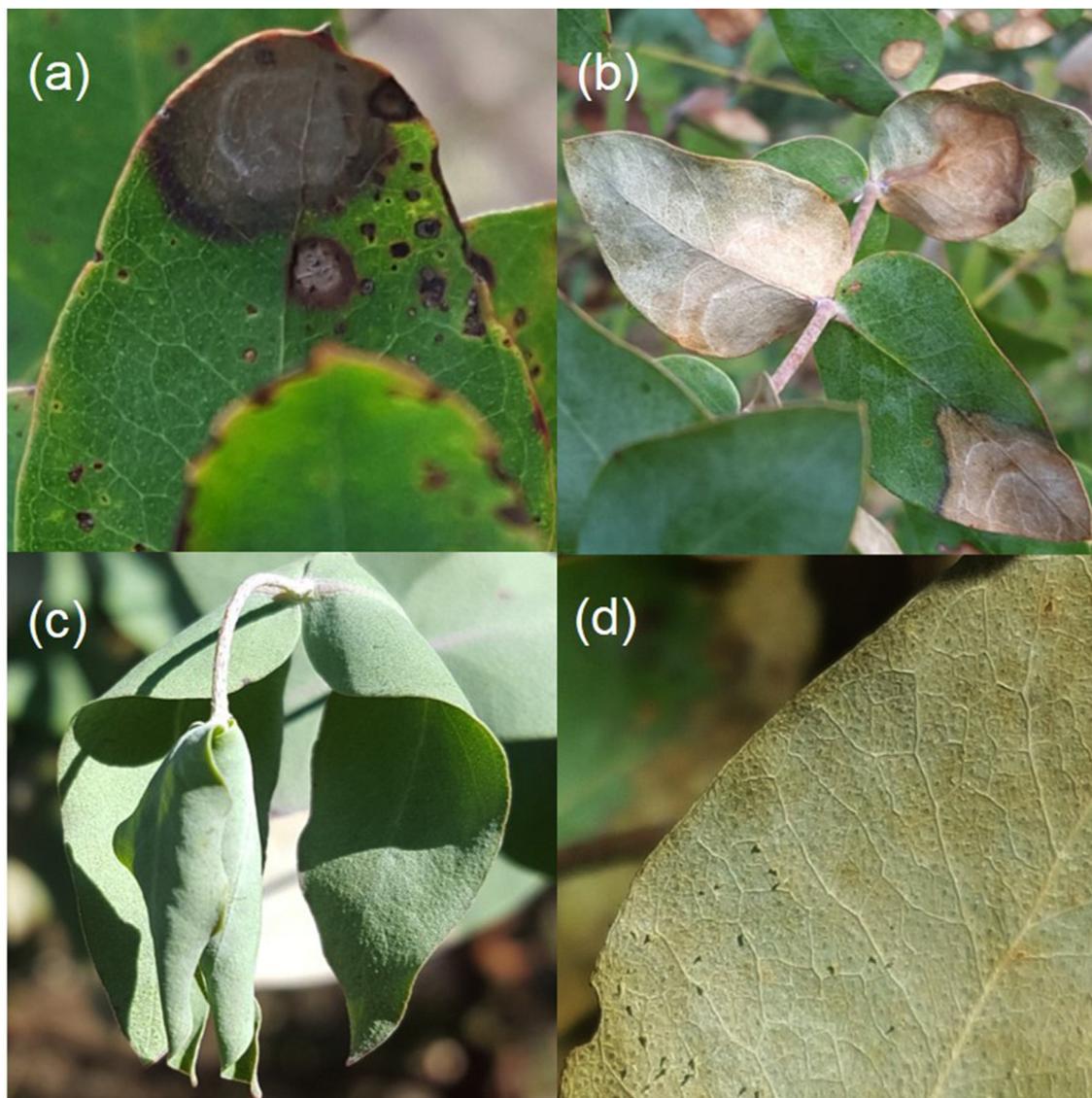


Fig. 1. (a) Zonate leaf spot on *Eucalyptus benthamii* leaf. (b) Growth of individual leaf spots and coalescence of multiple leaf spots, resulting in foliar necrosis and blight. (c) Withering of juvenile foliage in response to death of leaves on the same shoot proximal to the stem. (d) Reproductive structures on surface of infected leaf. Images from November 11, 2015; DOY = 315.



Fig. 2. Foliar necrosis associated with zonate leaf spot on *Eucalyptus benthamii*. The leaf spotting and foliar necrosis begins at the bottom of the tree and progresses upward. The juvenile foliage at the top of the tree remains generally unaffected. Image from November 11, 2015; DOY = 315.

3. Results and discussion

The fungus associated with the leaf disease was tentatively identified as *Cristulariella moricola* based on the presence of distinct pyramidal shaped fruiting structures that occurred in necrotic areas of leaf spots and blighted leaves of *E. benthamii* (Figs. 1d and 4). The generated ITS rDNA sequences of the three isolates were identical (GENBANK accession number KY434080) and matched most closely (448 out of 451 bp) to the sequences of *Cristularia moricola* (JQ036181, KC460209) and *Grovesinia pyramidalis* (Z81433) in BLASTn searches (National Center for Biotechnology Information, U.S. National Library of Medicine, Bethesda, Maryland). Based on the morphology of fruiting structures and DNA sequences, we concluded that *C. moricola* was responsible for the widespread blight observed in *E. benthamii* plantings.

The development of zonate leaf spot followed a consistent pattern within individual plants. Specifically, leaf spots and subsequent blight began at the bottom of trees and progressed upward (Fig. 4). Foliage at the top of the trees remained generally unaffected. One possible explanation for the observed pattern was that

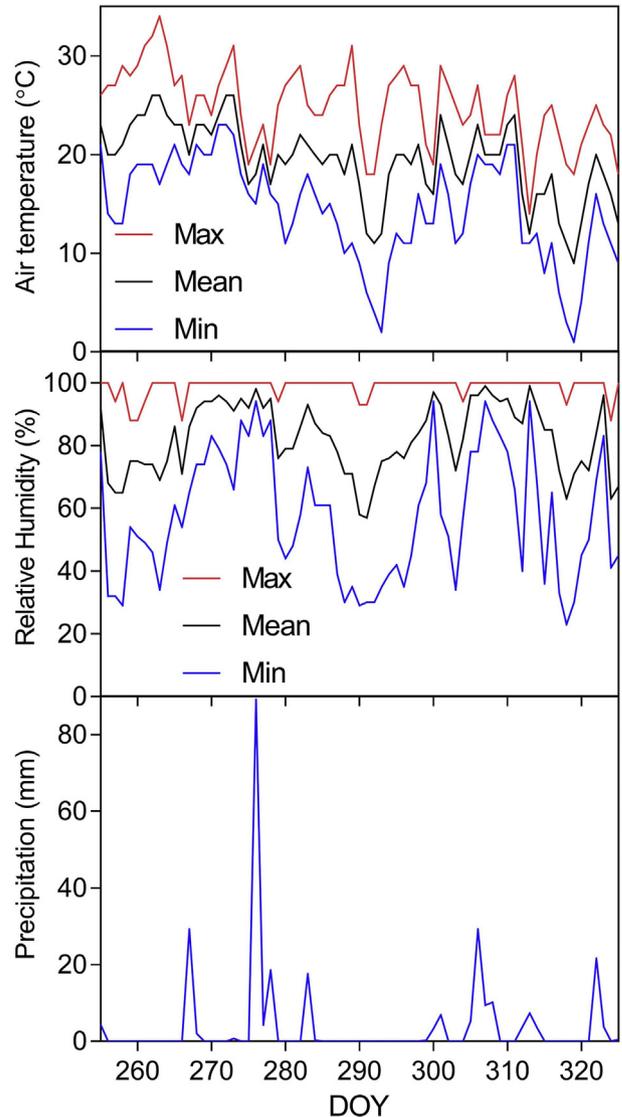


Fig. 3. Air temperature (a), relative humidity (b), and precipitation (c) for the period leading up to and through the observation of zonate leaf spot and blight. Note the numerous precipitation events that occurred from late September through early November which kept the foliage wet and maintained high relative humidity for most of the time period. Micrometeorological data were summarized from Weather Underground, Inc.

the upper canopy was not very dense and allowed better air flow and rapid drying of leaf surfaces, which could impede fungal colonization of host tissues. Another possible explanation for the observed pattern of infection may be related to leaf morphology. *Eucalyptus benthamii* exhibits morphologically distinct juvenile and adult foliage that is spatially discrete in that adult foliage begins to appear in upper portions of the canopy and juvenile foliage is restricted to the lower branches. Juvenile foliage appeared much more severely affected by the disease than did mature foliage (Fig. 5), and this observation appears independent of air movement as some trees had rather dense sections of adult foliage that remained symptomless. Zonate leaf spot will cause infected foliage to prematurely abscise in many deciduous hosts [6,13], but in *E. benthamii*, which is an evergreen species, much of the necrotic foliage failed to abscise (Fig. 6) and generally remained on trees through the winter months.

Although zonate leaf spot did not appear to cause direct tree

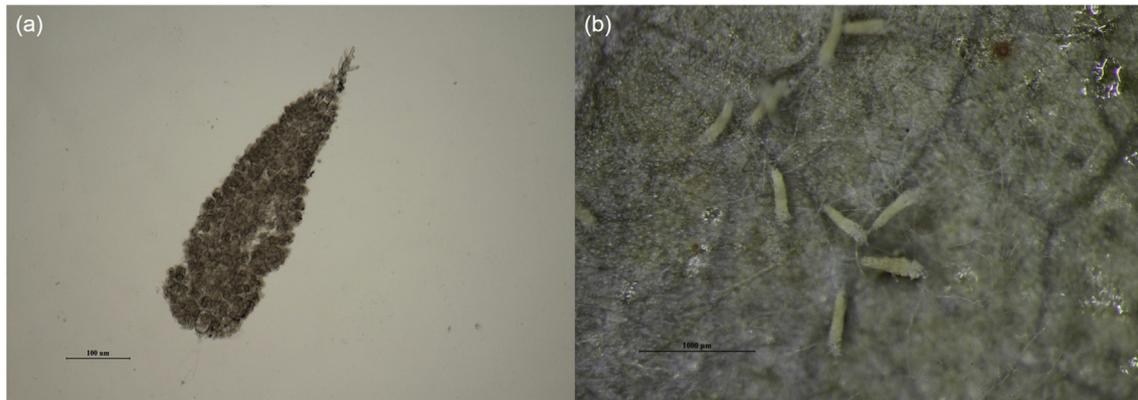


Fig. 4. (a) Reproductive structure of *Cristulariella moricola*. (b) Pyramidal shaped conidiomata of *Cristulariella moricola* on necrotic area of *Eucalyptus benthamii* leaf with zonate leaf spot.



Fig. 5. Zonate leaf spot symptoms and associated tree mortality in a planting of *Eucalyptus benthamii*. Leaf spot and foliar necrosis begin at the bottom of the trees and progresses upward. The tree in the center of the photo shows a transition from juvenile to adult foliage in the upper crown, and the adult foliage remained generally unaffected. Image from November 11, 2015; DOY = 315.



Fig. 6. Newly formed shoot with developing leaves after the defoliation event. Unfortunately, most of the newly expanded leaves were lost to freeze events over the following winter months. Image from December 3, 2015; DOY = 337. Note areas affected by zonate leaf spot on older dead foliage.

mortality on *E. benthamii*, the disease resulted in nearly complete foliar necrosis of most trees on one planting site, and such severe defoliation could affect their growth. Furthermore, new flushes of shoots and leaves began shortly after the blight subsided on some trees (Fig. 6), but many of these succulent, developing shoots and leaves were subsequently killed by freezing temperatures. Ultimately, many of the *E. benthamii* trees that experienced blight due to zonate leaf spot were top-killed during the following winter. Most of the top-killed trees resprouted from the root collar or from epicormic buds on the stem during the following spring; however, a few individual trees died, presumably due to depletion of carbohydrate reserves necessary for refoliation.

We remain uncertain as to why only a single *E. benthamii* planting experienced widespread infection of *C. moricola*, particularly considering the relatively close proximity of plantings. However, there are a few characteristics of the infected plantings that differ slightly from the others and may have facilitated infection of leaves by *C. moricola*. In particular, the planting that experienced the highest incidence of disease occupied the lowest elevation of any of the plantings, was the only planting located on the south side of a riparian zone, and was located within 20 m of the riparian vegetation. There were two other plantings located a similar distance away and only 5–6 m higher in elevation, but on the north side of the same riparian zone. The other three plantings were located at similar or slightly higher (+13 m) elevations and slightly, but not substantially, further from riparian zones (+10 m). Given the relatively small differences in elevation across these plantings, that alone does not seem capable of explaining our observed differences in disease. However, subtle differences in microclimate associated with an interaction between topography and surrounding vegetation may be associated with the observed variation in the incidence of the foliar disease among the plantings. In fact, leaf disease development by *C. moricola* is strongly influenced by temperature and moisture conditions [14]. Development of leaf spots generally occurs when relative humidity (RH) is above 94% and when temperatures range from 18 to 24 °C [14]. Some conidial production can occur when RH is as low as 96%, but optimal development of leaf spots and conidia occurs as RH approaches 100% and when leaf surfaces are wet [14]. The extended period of precipitation that coincided with the observed blight in *E. benthamii* would have maintained leaf wetness and provided environmental conditions similar to those described previously as optimal for zonate spot on other hosts (i.e., high RH and moderate temperatures (Fig. 3). Moreover, we cannot rule out the possibility that microsite differences in temperature may have affected juvenile foliage in the infected planting to a greater degree than in other plantings during frost events. Indeed, the spatial positioning at the south side of a riparian zone would make this particular planting site more prone to frost. Thus, it remains plausible that a frost event preceding the observation of the disease outbreak could have been more pronounced in this particular planting and left the foliage more vulnerable to infection by *C. moricola*.

The most damaging epidemics of zonate leaf spot by *C. moricola* occur during extended periods of rainfall when temperatures are below normal [11]. The outbreak of zonate leaf spot in the *E. benthamii* plantation is very similar to outbreaks of the disease that have occurred in black walnut (*Juglans nigra*) plantations in Illinois [10], where foliar blight caused by *C. moricola* resulted in almost complete defoliation in consecutive years. In each year, the disease outbreak coincided with extended periods of rainfall in the mid to late summer. Severe outbreaks of this disease have also been reported in pecan (*Carya illinoensis*) orchards in Alabama and Georgia [13] and in kenaf (*Hibiscus cannabinus*) plantings in Maryland and Georgia [9], although the disease was not always linked directly with temperature and moisture conditions [9].

The initial source of inoculum of *C. moricola* in the *Eucalyptus* plantation is unknown. *Cristulariella moricola* has a wide host range and can cause foliage disease in numerous tree species indigenous to the southeastern USA, including various maples (*Acer* spp.), pecan, sassafras (*Sassafras albidum*), and hackberry (*Celtis occidentalis*) [13,15]. The fungus also causes disease in common woody shrubs, such as *Vaccinium* spp., and herbaceous plants, including pokeweed (*Phytolacca americana*) [15]. The highest incidence of zonate leaf spot in pecan orchards occurred near hedgerows comprised of native sugarberry (*Celtis laevagata*) trees, which were also highly susceptible to zonate leaf spot [13]. The removal of sugarberry trees near one orchard may have reduced the incidence of the disease in pecans in a subsequent year [13]. The elimination of highly susceptible host trees, establishing large buffers between forests and plantations, or establishing plantations in areas where native susceptible host trees are not present may help to reduce the effects of this disease on *E. benthamii*.

The high incidence of zonate leaf spot in juvenile foliage and the very low incidence of the disease in adult foliage is interesting from a management perspective. This suggests that *E. benthamii* may only be susceptible to blight by *C. moricola* during the first few years of a plantation, and once the stand has transitioned to primarily adult foliage, it may no longer be at risk. However, a coppice approach to *E. benthamii* production where stems are cut every 2–3 years may continually produce juvenile foliage that remains susceptible to *C. moricola*. Although *E. benthamii* is the species of *Eucalyptus* that currently exhibits the best potential for production in the southeast US, other *Eucalyptus* species are also currently being tested, and it is unclear if they would also be susceptible to *C. moricola*.

4. Conclusion

Eucalyptus benthamii is a new host plant record for *Cristulariella moricola*. This fungus is a potential threat to *E. benthamii* cultivation in the southeastern USA, causing major losses in leaf area. Although zonate leaf spot did not appear to cause direct mortality of *E. benthamii* trees, it resulted in nearly complete foliar necrosis of individual trees, which presumably depleted carbohydrate reserves in upper portions of the stems and forced plants to resprout from the root collar or from epicormic buds the following spring. The susceptibility of *E. benthamii* to disease observed in our planting may be related to frost events making foliage more vulnerable to colonization by *C. moricola*, or they may simply be due to prolonged periods of precipitation, high relative humidity, and proximity to other native vegetation that acts as a host to *C. moricola*. As production of *E. benthamii* increases throughout the southeast US, it will be interesting to see if the disease outbreak associated with *C. moricola* is limited to northern plantings in conjunction with cold temperatures or if it also occurs in the absence of frost under prolonged periods of moisture further south. It will also be interesting to see if *E. benthamii* is the only *Eucalyptus* susceptible to this disease in association with *C. moricola* or if other *Eucalyptus* species also exhibit susceptibility. Finally, it may be important to identify native vegetation that hosts *C. moricola* and avoid planting *E. benthamii* nearby.

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